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In-Vitro Digestibility and Gas Production of Wheat Middlings, Solvent Extracted Cottonseed Meal, Soyhulls, and Corn Gluten Feed and the Effects of Monensin on In-Vitro Digestibility and Gas Production

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IN-VITRO DIGESTIBILITY AND GAS PRODUCTION OF WHEAT MIDDLINGS, SOLVENT EXTRACTED COTTONSEED MEAL, SOYHULLS, AND CORN GLUTEN FEED AND THE EFFECTS OF MONENSIN ON *IN-VITRO* DIGESTIBILITY AND GAS PRODUCTION

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By

Dustin Waine DeVore

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ABSTRACT

This study was designed to study the in-vitro digestibility and in-vitro total gas production of corn gluten feed, wheat middlings, solvent extracted cottonseed meal, and soyhulls in multi parous steers. Evaluation of the effects of monensin on in-vitro digestibility and in-vitro total overall gas production of a commercially available complete feed was also studied Feedstuffs were ground through a Wiley mill to ensure uniform samples and were added to digestion jars in a completely randomized design. Rumen fluid was collected from a *Bos taurus* steer via a ruminal cannula. To ensure consistent digesta material the steer was fed a uniform diet two weeks prior to study and was fed and collected at the same time daily. Data was analyzed using Proc GLM of SAS with fixed effects of feedstuff or monensin addition in the model. Digestion of soyhulls resulted in the greatest total gas production, corn gluten feed and wheat middlings were intermediate and cottonseed meal had the least total gas production ($P<0.05$). Digestibility of soyhulls and cottonseed meal was greater than corn gluten feed and wheat middlings. Addition of monensin had no effect on in-vitro digestibility and had no effect on in-vitro total gas production. Results indicate that Monensin has no effect on feed digestibility ($P<0.05$) when incorporated into a commercially available complete feed.

KEYWORDS: methane, propionate, acetate, corn gluten feed, wheat middlings, cottonseed meal, soyhulls, gossypol, Monensin, carbon dioxide

This abstract is approved as to form and content

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Gary W. Webb, PhD
Chairperson, Advisory Committee
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INTRODUCTION

Corn and soybean prices have varied significantly over the last decade (2008-2018) causing producers to incorporate by-product feeds into cattle diets to help stabilize input costs (Buza et al, 2014). With the current (2017) over supply of milk and the increase in corn and soybean production there is an increase in the utilization of by-products in dairy rations by producers trying to cut costs (Martin et al, 2017). By-product feeds can provide producers lower input costs, higher nutrient availability than the original grain, and provide a lower cost alternative to conventional feeds while providing essential nutrients to meet an animals dietary requirements (Wainman et al, 1984). Transportation costs, handling, storage losses, and increased animal waste due to some products being less palatable are potential factors increasing cost that must be considered when determining whether the use of a byproduct feed is beneficial (Bozic et al, 2012).

In the late 2000's, oil price surges led to a major increase in farm input costs due to the increase in fuel prices as well as a large surplus of ethanol co-products that helped to lower the cost of production for livestock producers (Schingoethe et al, 2009). In 2005, the federal government issued the Renewable Fuel Standard, which placed a mandate on the amount of ethanol that must be included in each gallon of gasoline in the United States. For the fiscal year of 2018, the mandate was 73.03 billion liters of ethanol which will primarily come from corn-based ethanol. The principal by-product of ethanol production is distiller's grains (DDG). Livestock producers are developing management programs to utilize by-products more efficiently within their livestock operation (Bozic et al, 2012). In

2017, 40% of U.S. corn crop was utilized for ethanol production resulting in 38.5 million metric tons of DDG (USDA, 2018)

As part of normal rumen function rumen, microbes produce methane (CH_4), carbon dioxide (CO_2), and nitrous oxide (N_2O). Methanogenesis is the process in which ruminal bacteria (specifically the methanogens) utilize hydrogen gas (H_2), formate, and CO_2 to produce into CH_4, and CO_2 (Baldwin, 1983). In the United States, agriculture produces approximately 9% of greenhouse gas emissions per year (EPA, 2016). From 1990-2014, total CH_4 emission declined 5.6% while total greenhouse gas emissions increased 7.4% over the same period. Enteric fermentation produces 164.3 million metric tons (MMT) of CO_2 equivalent and accounts for 22.3% of total CH_4 gas emissions (EPA, 2016). Methane has 25 times the potential to contribute to global warming as CO_2 (Wuebbles and Hayhoe, 2002, EPA, 2016) and an atmospheric life expectancy of 12 years, while the atmospheric life expectancy of CO_2 is 5 years (Archer et al, 2009).

Feed supplementation with ionophores is an effective method to increase feed efficiency and reduce gas emissions (Russell, 1996, Thornton and Owens, 1981). Ionophores are antibiotics that increase the production of propionic acid at the expense of acetic acid, which decreases the overall production of CH_4 as the production of propionic acid directly competes with methanogenesis (Ellis et al, 2012, Baldwin, 1983). Acetic acid production results in the loss of a carbon atom that is then utilized in the production of CH_4. While propionic acid production has no lost carbon molecule that can be utilized for CH_4 production (Hungate, 1966, Christophersen et al, 2008).

Wheat middlings (WM), solvent extracted cottonseed meal (CSM), corn gluten feed (CGF), and soyhulls (SH) are all readily available by-products that can be utilized in

livestock rations as substitutes for more expensive feedstuffs. Wheat middlings are produced as the by-product of flour production; cottonseed meal as the by-product after the de-linting process and the extraction of oil from whole cottonseeds; soyhulls the by-product from the production of soybean meal; corn gluten feed as the by-product of corn starch or corn syrup process.

PROBLEM STATEMENT

The United States utilized 12.54 billion metric tons of corn in 2017, with 5.5 billion metric tons being utilized as feed, the ethanol industry utilized 7.011 billion metric tons, with the remainder of the corn utilized went to food, seed alcohol and other industrial uses (USDA, 2018). By-products produced from ethanol production are important as cost effective alternatives as corn prices are partially driven by ethanol production. Distiller's grains are the principal by-product of ethanol production. Corn accounted for 94% (5.324 billion bushels) of cereal grain utilized as feed or as by-products that are then utilized as feedstuffs (USDA, 2016). In 2015, 32,235 short tons of soybean meal, 122 million bushels of wheat, 43 million bushels of barley, and 70 million bushels of oats were used as livestock feeds (USDA, 2016). The by-products of these grains have not been looked at as extensively as corn by-products. While several corn by-products have been extensively researched over the last decade, by-products of wheat, cotton, and soybeans have not been looked at as extensively. However, there are several by-products available that have not been widely researched that may provide beneficial nutrition at a cost effective price. Wheat middlings, CGF, CSM, and SH are readily available by-products that are utilized in rations to control costs and have not been as widely researched.

Ionophores are used in livestock rations as anticoccidials, bloat prevention aids, and fermentation modifiers. Ionophores increase propionic acid production and dry matter digestibility, decrease feed intake, may help to decrease heat production, and inhibit lactate producing bacteria in the rumen helping to reduce ruminal lactic acidosis

(Horn et al, 1981, Dinius et al, 1976,). Monensin and lasalocid are the most commonly utilized ionophores in cattle diets. Recent regulation has increased interest in determining greenhouse gas emissions of livestock and how to possibly reduce the carbon footprint of livestock (Wuebbles et al, 2002). More research is needed to determine the utilization of these by-products and additives to help in the control of CH_4 and CO_2 production in the future.

REVIEW OF LITERATURE

Ruminants

Ruminants are mammals that are members of the order *Artiodactyla* and suborder *Ruminantia*. Ruminants have a four compartment stomach and an even-toed hoof. The rumen and reticulum are the first two compartments and act as large fermentation vats. The rumen and reticulum (reticulo-rumen) are essentially one compartment only divided by a small fold of muscular tissue. The rumen holds 151.4 liters on average and the reticulum holds 18.92 liters in mature bovine making up approximately 84% of the total volume of the stomachs (Russell et al, 1988). The omasum is the third compartment and holds approximately 7.57 liters. The abomasum is the fourth compartment and it holds approximately 26.5 liters in mature cattle.

The rumen is the primary site for enteric fermentation and the ruminal wall is lined with papillae that increase the surface area to aid in adsorption. The reticulum, also known as the honeycomb, is comprised of a mucosal membrane with intersecting ridges that form compartments trapping foreign objects in mature cattle. The omasum is the third compartment and is believed to aid in decreasing particle size of digesta, absorption of VFAs, and adsorption of some water. The omasum is filled with muscular laminae with papillae and in non-glandular. The laminae have the appearance of a book and are likened to sheets of paper coated with papillae (Umphrey and Staples, 1992). The fourth and final compartment is the abomasum. The abomasum is known as the "true stomach" as it produces hydrochloric acid and digestive enzymes.

Ruminants are born with all four compartments, however; the use of these compartments is limited to the abomasum during the first few months of their lifetimes. Ingested nutrients pass to the abomasum via the esophageal groove. Milk proteins stimulate the esophageal groove to open as well as the animals suckling reflex. As a young ruminant begins to consume forages and grains, the microbial population begins to develop in the rumen and reticulum. Microorganisms begin fermenting these feedstuffs and as fermentation increases, the volume of the rumen and reticulum increase. Fermentation produces SCFAs that are required to promote papillae development in the rumen (Baldwin et al, 2004).

Rumen Microbiome

Ruminant animals are host to several billion microorganisms that act together to create a rather complex microbiome. The reticulo-rumen microbial population is comprised of several species of organisms that break down digesta in the rumen. Cellulolytic and amylolytic bacteria, lactic acid bacteria, protozoa, and fungi are all present in the rumen microbiome. The rumen contains between 10 and 50 billion microorganism per milliliter of rumen fluid depending on pH and fiber content of diet. The majority of the microorganisms in the rumen are bacteria as approximately 10-50 billion bacteria exist in one milliliter of rumen fluid. In comparison one billion protozoa are contained in one milliliter of rumen fluid while the yeasts and fungi are highly variable depending on environment. The environment is influence by the diet of animal and ruminal pH (Hungate, 1966).

Nine different phylum ruminal of bacteria have been detected before 2017 and these phylum are comprised of 88 different genera. *Firmicutes, Proteobacteria, Actinobacteria* and *Bacteroidetes* were the most common representing 82 of the different genera (Creevey et al., 2014). Bacteria in the rumen synthesize amino acids, protein, water-soluble vitamins and vitamin K. Microorganisms and bacteria inside the reticulo-rumen begin to break down cellulose which produces CH_4, N_2O, and CO_2 gases. Ruminal bacteria contain enzymes that break down cellulose and digest starch.

Bacteria in the rumen release volatile fatty acids during the breakdown of carbohydrates. Acetate, propionate, and butyrate are the predominant volatile fatty acids (VFA) released by the rumen bacteria and these acids are absorbed through the rumen wall to be utilized as energy by the host animal. Butyrate is used as the primary energy source for the rumen epithelium. Acetate and propionate pass through the rumen wall into the portal vein which takes them to the liver. Acetate passes through the liver to the hepatic vein and systemic circulation where it is utilized in the animal's body to generate ATP as well as primary precursor for fatty acid synthesis. Propionate is utilized in the liver in gluconeogenesis and is oxidized throughout the tissues for energy (Bergman, 1990).

Protozoa in the rumen predominantly belong to the families *Isotrichidae, Dasytricha,* and *Ophyroscolecidae.* Protozoan synchronize their growth rates with the passage rate of the environments that they thrive (Diaz et al, 2014). *Isotricha* attack starch molecules and *Dasytricha* help break down maltose molecules (Hungate, 1966). Protozoan are highly sensitive to rumen pH and are reduced in number in acidic rumen environments. Protozoan have a symbiotic relationship with the bacteria which produce

CH_4 by disposing of the hydrogen gas produced from their hydrogenosomes (Ushida, 2010). *Methanosphaera, Methanococcus, Methanobrevibacter,* and *Methanobactetriaceae* are methanogens found in the rumen.

A group of anaerobic fungi are found in the rumen microbiome. *Anaeromyces, Caecomyces, Cyllamyces, Neocallimastix, Orpinomyces,* and *Piniomyces* are all fungal species from the family *Neocallimstigaceae* isolated in the rumen (Firkins and Yu, 2015). Anaerobic fungi oxidize pyruvate into acetate, H_2, CO_2 or formate by use of hydrogenosomes. Hydrogenosomes are membrane bound organelles found in the cytoplasm of eukaryotic cells and are producers of energy and hydrogen (Embley et al, 2002).

Several strains of archaea were also isolated from the rumen fluid recently mostly as methanogens. Methanogens are responsible for the conversion of H_2 and CO_2 into CH_4. As part of enteric fermentation ruminant animals produce CH_4, CO_2, and N_2O. The amount of individual gases produced are relative to the ruminal environment. As diet composition changes ruminal environment will change leading to a change in the volume of each gas produced. As a result, ruminants have become more closely scrutinized over the last few years as more people study the causes of climate change.

Gas Production

Methane losses are a potent source of greenhouse gas emissions and a potential source of lost dietary energy. As part of enteric fermentation CH_4, CO2 and N_2O are produced (Bowman et al, 2004). Production of CH_4 is of concern due to a possible link between greenhouse gas emissions and rising average temperatures (Freetly and Brown-

Brandl, 2013). Cattle produce 250 to 500 L of enteric CH_4 per day which has drawn the attention on cattle production by regulatory agencies as well as environmental activists. This level of gas expulsion over 50-100 years is less than 2% of overall greenhouse gas emissions (Johnson and Johnson, 1995).

From 1990 to 2014, overall CH_4 emission fell 5.6% while overall greenhouse gas emissions rose 7.4% (EPA, 2016).The decrease in CH_4 production may be attributed to increased regulation and possible an increase in efficiency of CH_4 producing processes In 2014, 81% of greenhouse gases emissions were CO_2, 11% were CH_4, 6% were N_2O, and 3% were fluorinated gases. In 2014 agriculture accounted for 9% of total overall greenhouse emissions. In 1990 enteric fermentation accounted for 164.2 million metric tons (MMT) of CO_2 equivalent and overall CH_4 production totaled 773.9 MMT CO_2 equivalent. While in 2014, enteric fermentation produced 164.3 MMT of CO_2 equivalent and overall CH_4 production totaled 730.8 MMT CO_2 equivalent (EPA, 2016).

Digestion of cellulose by ruminal bacteria and other microorganisms produce CO_2, CH_4, H_2, and volatile fatty acids (VFA). Measurement of gas produced in vitro provides data about the rate and extent of cellulose digestion in ruminant animals (Schofield et al., 1994). Propionate production is a competitive process to methanogenesis while acetate and butyrate production promote methanogenesis (Moss et al, 2000, Nelson et al, 1958, Prange et al, 1978). Propionate production via rumen fermentation requires hydrogen. Hydrogen is left in the in the rumen by ferredoxin remaining from phosphoroclastic reactions being broken down by hydrogenase enzymes (Hegarty and Gerdes, 1999). Due to this reaction the acetate: propionate ratio in the rumen will inversely affect methanogenesis (Christopherson et al., 2008). Methane

emission is decreased by the inclusion of rumen undegradable protein shifting site of digestion from the rumen to the intestines (Beauchemin and McGinn, 2005).

Production efficiency can be improved by using different compounds that modify the ruminal environment; thereby, influencing rumen fermentation (Horton, 1980, Markantonatos and Varga, 2017). In Holstein steers, reduction in ruminal pH decreased the concentration of CH_4 and ammonia leading the authors to believe that pH potentially could increase feed efficiency in ruminant animals fed soybean meal (Lana et al., 1998). However NDF digestibility from decreased 32.5% to 8.1% when rumen pH decreased from 6.0 to 5.8. Further reduction of pH to 4.5 to 5.0 resulted in complete interruption of fiber digestion. (Hoover, 1986).

Feed Efficiency

Feed efficiency varies among cattle with one hypothesized source of reduction in efficiency being the ability of different animals to more efficiently minimize loss of CH_4. Cattle on average lose about 6% of ingested energy as CH_4, while energy loss in cattle as enteric CH_4 ranges from 2-12% of gross energy uptake (Johnson et al., 2000). Ranges in energy loss are mainly due to DMI, and feed composition (Beauchemin and McGinn, 2005). Freetley and Brown-Brandl (2013) studied the effects of feed efficiency on CH_4 production in ruminants. Methane released as a percentage of intake energy was variable. Feed efficiency can be increased as CH_4 production is decreased. Effects of diet on enteric CH_4 production were studied in relation to forage and grain source. Both decreasing quantity of forage consumed and increasing barley or corn in feedlot diets decreased enteric CH_4 production which was associated with a decrease in acetic acid

production as well as an increase in the levels of propionic acid (Beauchemin and McGinn, 2005). Diets high in grain or other readily fermentable CHO's decrease ruminal pH and decrease enteric CH_4 production.

Feedstuffs

In 2015, corn accounted for 94% or 5.324 billion bushels of cereal grain utilized as feed or as feed residue fed to livestock Along with 32,235 thousand short tons of soybean meal, 122 million bushels of wheat, 43 million bushels of barley, and 70 million bushels of oats that were used in livestock feeds in 2015 (USDA, 2016). Demand for cheaper alternative fuel sources has increased the production of ethanol, soybean oil, corn meal, biodiesel, flour, and many other products. Increases in production of these products will increase the availability of by-product from those industries. By-products that are readily available, beneficial for livestock use, and are currently being utilized are WM, CSM, SH, and CGF.

Corn Gluten Feed

Current expansions of the ethanol and corn syrup industries have increased access to corn by-product feeds by livestock producers. Corn gluten feed is a by-product of wet milling corn for cornstarch or corn syrup and is a blend of hulls, steep water, and corn germ meal (Figure 1). Corn kernels are soaked in water and sulfur dioxide to cause the kernels to swell. Corn germ meal also known as corn gluten meal is the remaining residue after oil extraction. Steep water is the recovered condensed soluble from the initial soak of the corn kernels (Hussein and Bergen, 1995) and the germ, starch, and gluten are then

removed and the remaining bran is mixed with the remaining steep water and sold as wet corn gluten feed (WCGF). Corn gluten feed is often mistaken for corn gluten meal but corn gluten feed contains less bypass protein, lower protein values, and usually costs less. Wet corn gluten feed usually contains between 40 to 45% dry matter. When WCGF is flash dried, moisture levels drop to approximately 10% turning the WCGF into CGF. Wet corn gluten feed is higher in rumen degradable protein and contains more digestible fiber than dried CGF however storage and shipping become problems due to high freight costs and the high water content of wet corn gluten feed. Mildew and molds are issues when wet corn gluten feed are stored for long periods of time (Armentano and Dentine, 1988)

Corn gluten feed is utilized as an alternative source of dietary protein in ruminants (Kampman and Loerch, 1989). Wet corn gluten feed is utilized in dairy cattle diets as a substitute for corn, soybean meal, corn silage, corn grain, and\or alfalfa hay. Corn gluten feed has a moderate protein level (23.8% CP) averaging about three times as high as corn grain with a fiber content (35.5% NDF) that is highly digestible (NRC, 2001). Corn gluten feed has an estimated energy level of which is 10 % less than corn due to CGF's higher content of neutral digestible fiber (NDF) that is fermented slower than the starch contained in corn (Hussein et al., 1995). Slower fermented starch makes CGF a useful protein or energy supplement for beef cows consuming low-quality forage in order to increase total energy intake (Fleck et al., 1988). Corn gluten meal (CGM) is often mistaken for corn gluten feed. Corn gluten meal contains 65% crude protein but is deficient in concentration of the essential amino acid lysine. Corn gluten meal will contain 89% RUP which makes CGM less desirable for use in ruminants (Titgemeyer et al, 1989).

Corn gluten feed used as a supplemental protein can alter ruminal fermentation increasing acid detergent fiber and dry matter digestion in cattle (Fleck et al., 1988). Corn gluten feed may be a viable replacement for corn + urea or corn + SBM in high-energy diets for ruminants (Bowman and Paterson, 1988). However, replacing corn and\or soybean meal (SBM) in high-concentrate finishing diets with over 50% dry CGF will reduce feed efficiency due to a decrease in net energy gain in the total ration (Firkins et al., 1985).

Wet Corn Gluten Feed

Wet corn gluten feed in forage based cattle diets will decrease ruminal pH due to a decrease in feed particle size (Sullivan et al., 2012). When WCGF particle size was increased the ruminal pH was not affected because of a higher rate of fermentation. However, DMI and milk yield were increased over control diet with the maximal response at 24.5% WCGF in the diet. If particle size is maintained while increasing WCGF in the ration, ruminal pH will remain between 6.00 and 6.13 while DMI and milk production increase (Sullivan et al., 2012). Feeding CGF at 30% of a forage based versus a starch based diet resulted in an improvement in ADG and DMI in crossbred steer calves (Loza et al., 2010).

Replacing steam-flaked corn in finishing diets with wet CGF increased ruminal pH and total digestibility of organic matter, NDF, and starch (Montgomery et al., 2004). In dairy rations optimum level of WCGF included in rations for maximum milk yield was 18.6% of the diet dry matter (DM). When increasing WCGF in dairy rations variations of

dry matter intake (DMI) becomes a factor due to the variation in the moisture content of WCGF (Schroeder, 2003).

Soyhulls

Soyhulls (SH) consist primarily of the outer coating of the soybean seed that is left behind following extraction of the soybean meal and removal of the soybean oil. Approximately 5% of the original weight of soybeans is SH (Blasi et al., 2000). An increase in soybean production over the last few years has increased the availability of soyhulls. Pellets are the form SH are utilized in order to increase density and allow for more cost-effective transportation. Soyhulls are commonly used as a source of fiber in ruminant diets and are utilized as an alternative to higher starch supplemental feeds for grazing ruminants to increase or maintain organic dry matter intake while not affecting ruminal pH (Ipharraguerre et al., 2002).

Soyhulls have a fast passage rate out of the rumen due to their high specific gravity when hydrated and small particle size (Ipharraguerre and Clark, 2003). Martin and Hibberd (1990) determined that SH should be used as a supplement for forages, not as a replacement as forage intake will increase or remain the same. However soyhulls will adequately supply energy and improve the utilization of the forages consumed by cattle. Degradable fiber concentrations of soyhulls will cause a reduced effect on ruminal pH (6.0) versus the effects of cereal grain supplementation (5.6); (Galloway et al, 1993). Data suggests that SH are fermented extensively in the rumen by microorganisms. The low (1.4%-3.9%) content of lignin and the small particle size (1.2 - 1.4 specific gravity) allows for rapid fermentation (Ipharraguerre and Clark, 2003). Passage rate of soyhulls in

cattle increased 8% when compared to a diet with no SH and SH were included at 48% of DM. When soyhulls were included at 25% and 48% of a forage based diet, the passage rate for SH was almost double that of forages (Nakamura and Owens, 1989).

Studies have indicated that feeding SH in place of other concentrates resulted in an average pH of 6.0. Ruminal pH of 6.0 seems to support the ruminal microflora. Concentration of VFAs also increased by replacing corn in cattle diets with soyhulls. The acetate to propionate ration was also increase with an increase in acetate production (Highfill et al., 1987; Anderson et al., 1988; Galloway et al., 1993; Mansfield and Stern, 1994; Elliot et al., 1995). However it is important to maintain a pH of 5.6 -6.0 to prevent acute acidosis or subacute ruminal acidosis (SARA). Subacute ruminal acidosis is repetitively dropping the ruminal pH and then increasing in a cyclistic manner. Acute acidosis is the dropping of rumen pH and maintaining for over 24 hours. Both SARA and acute acidosis are caused by an accumulation of lactic acid in the rumen which causes the drop in pH before the acids can be absorbed into the bloodstream. Both forms of acidosis will cause reduced feed intake, diarrhea, reduced milk production, or death (Plaizier et al, 2008)

When soyhulls are used to replace corn in finishing diets, VFA concentrations in the rumen increase while microbial protein synthesis was not affected, this indicates that organic matter of SH supports fermentation in the rumen at a rate similar to corn (Mansfield et al., 1994). While replacing large amounts of corn with soyhulls may affect the site of organic matter digestion, it does not decrease the amount of organic matter digested in the entire gastrointestinal tract (Ipharraguerre et al., 2002). Soyhulls were the equivalent of corn as an energy supplement in grazing cattle and as a supplement in high

forage diets. In addition, the negative effect on rumen pH was reduced when compared to the effect of corn due to lower starch level of soybean hulls (Anderson et al., 1988). When ground corn was replaced by SH in high concentrate supplements fed to grazing cattle the risk of acidosis was reduced and there was little effect on digestibility of nutrients (Anderson et al., 1988 a). Also DMI increased with the inclusion of SH in the diet.

Cottonseed Meal

Cottonseed meal (CSM) has a protein content of approximately 45 percent; therefore making CSM a viable alternative to soybean meal as a protein supplement. Cottonseed meal is the by-product that remains following oil extraction from the cottonseed. The type of extraction process must be determined to effectively utilize CSM in cattle diets. Three principal methods are used to extrude oil from cottonseed; direct solvent extraction, prepress followed by solvent extraction, and expeller process. During the extrusion process CSM will reach temperature of 120^{o} C. These temperatures potentially reduce the degradability of protein in the rumen and increase the protein in the small intestine (Winterholler et al., 2009). Extruder expelled CSM inadequately protects proteins from degrading in the rumen making extruder expelled CSM a poor source of rumen undegradable protein (Meyer et al., 2001).

When feeding CSM, the cost of elimination of excess nitrogen must be taken into account. Supplementing ruminant animals with CSM elevates ammonia and nitrogen concentrations in the rumen (Judkins et al., 1987). Cattle fed prairie grass hay and supplemented with CSM had higher level of ruminal ammonia, increased propionate:

acetate ratio, and the in-vitro dry matter disappearance of prairie grass hay was greater at 6, 12, 18, and 24 hours. Steers supplemented with CSM consumed more prairie grass hay than steers not supplemented with CSM (McCollum and Galyean, 1984). Ruminal ammonia concentration increases with a decrease in available CHOs. When CHOs are limited in the rumen the end products of protein degradation are ammonia and VFAs (Russell and Strobel, 1987)

When fed at 16% of diet dry matter, the net energy for gain of CSM is 1.24 Mcal/kg and the net energy for maintenance is 1.88 Mcal/kg for cattle. Organic matter digestibility decreased in the rumen but starch and nitrogen digestion were not changed. When fed levels higher than 16% of diet DM, weight gain was depressed and feed efficiency was lowered. Therefore the amount of CSM should not exceed 16% of diet DM in feedlot cattle (Zinn et al., 1994). The reduced feed efficiency and weight gain may have been due to excessive nitrogen intake as well as high intake of gossypol, a toxic compound found in cottonseed (Zinn et al, 1994).

Gossypol is a yellow polyphenolic compound produced by the pigment glands of the cotton plant that can cause anorexia, decreased growth rate, labored breathing, dyspnea, or death (Randel et al, 1992). Gossypol toxicity also can cause dilation and hypertrophy of the heart muscles and degeneration of the muscle fibers (Rogers et al. 1975). Gossypol is contained in the roots, leaves, stems, and seeds of the cotton plant and the production of this phenol provides the plant with insect resistance. Gossypol is also found in the endosperm of cottonseed and is increased in concentration when the seed is dehulled to produce CSM.

Monogastric species and immature ruminants are susceptible to gossypol poisoning as they lack the ability to detoxify the gossypol in the gastrointestinal tract as gossypol is bound by microbes in the rumen of ruminant species. Postmortem evaluation of afflicted animals indicates fluid accumulation in the animal's body cavities and congestion and edema in liver, lungs, and spleen. Mature ruminants detoxify the free gossypol in the rumen by binding it to soluble proteins turning the gossypol into bound gossypol a physiologically inactive form (Randel et al., 1992).

When gossypol is bound it is considered to be nontoxic as it cannot be broken down in the lower gastrointestinal tract (Mena et al, 2004). However, high concentrations of gossypol in the rumen may escape detoxification by rumen microbes leading to poisoning. Bound gossypol is produced from the formation of covalent bonds between gossypol and free epsilon-amino acid groups from lysine and arginine. Heating CSM promotes the reaction of gossypol with proteins (Broderick et al., 2013), lowering the concentration of gossypol. When gossypol was ingested at .225% of cattle diet, the plasma concentrations of free gossypol was elevated compared to cows fed soybean meal (Lindsey et al., 1980). Female ruminants are thought to be relatively insensitive to high levels of dietary gossypol, while males exhibit damage to testes (Randel et al, 1992).

Wheat Middlings

Wheat middlings (WM) are a by-product of the wheat milling industry (Figure 2). After wheat is milled into flour, the leftover bran (cuticle, pericarp, and seedcoat), shorts (fine bran, germ, and endosperm), germ (embryonic axis and scutellum), red dog shorts (aleurone layer between bran and endosperm), and tailings (amylodextrin) are sold as

wheat middlings. After milling, approximately 25% of initial wheat remains as WM (Sunvold et al., 1991). Wheat middlings are usually between 14 and 18 % crude protein on a DM basis. Wheat middlings contain approximately 40 % NDF which is highly digestible in the rumen. Protein levels in WM are highly degradable in the rumen and the energy available from digestible fiber is similar to the energy level from the starch in corn when included in forage based diets (Bernard, 1991).

Wheat middling gas production is similar to that of wheat bran and less than half of wheat flour (Jha et al., 2012). Meat goats fed a wheat middling diet demonstrated a lower rumen pH (6.23) than goats consuming soyhulls (6.41), CGF (6.35), or hay (6.52); (Moore et al., 2002). Goats also demonstrate higher acetate to propionate ratios for hay and soyhulls than the WM and CGF but no differences in total ruminal VFA were found (Moore et al., 2002). Cattle supplemented high levels (2 times CP of low diet) of WM had a lower total digestible DMI than cattle fed the low level of WM supplementation. Weight gains also increased as the level of WM were increased (Heldt et al., 1998).

Wheat middlings can replace only up to 5% of corn in cattle finishing diets without decreasing efficiency and digestibility. However replacement of alfalfa hay with WM had no effect on feedlot performance (Dalke et al., 1997). In one trial two groups of cattle fed WM at different (25% of corn/SBM replaced with WM and WM restricted to 75% of ad libitum intake) levels. Both supplemented groups had increase in ruminal ammonia concentration. The results of this study suggest that supplementing poor quality forages with WM at 20% protein concentration or higher will increase rate of digestion. Diets containing 25% WM fed at 75% of intake showed no impact on ruminal digestibility (Hermesmeyer et al., 2002). Ruminal pH was significantly decreased by WM

supplementation however not enough to significantly affect fiber digestion in cattle (Sunvold et al., 1991). Jha et al. (2012) studied the degradation and fermentation characteristics of wheat by-products in-vitro inoculated with swine feces. The results of the study suggest that fiber concentration in the diets of swine is associated with the degradability and fermentation of an increase in wheat by-products added to the ration. Total gas produced ranged from 101 to 148mL/g DM, VFA production ranged from 2.0 to 3.0 mmol/g and the fractional rate of degradation ranged from 0.08 to 0.11/h.

Ionophores

Ionophores (carboxylic polyether ionophore antibiotics) are biologically active molecules produced from a strain of *Streptomyces cinnamonesis* that aid in transporting cations across membranes (Fuller and Johnson, 1981). The benefits from ionophores are divided into three key areas; increased efficiency of energy metabolism of rumen bacteria and (or) the animal, improved nitrogen metabolism of rumen bacteria and (or) the animal, and retardation of digestive disorders resulting from abnormal rumen fermentation (Bergen and Bates, 1984).

Ionophores help reduce volume of excreta and gas emissions, reduce morbidity and mortality, and therefore have a positive effect on the environment (Bergen and Bates, 1984). Ionophores are toxic to many bacteria, protozoa, and fungi due to the lipophillic properties of the compound (Russell, 1996). Ionophores inhibit CH_4 production by decreasing the primary substrates hydrogen and formate, utilized by gram positive bacteria during methanogenesis (McGuffey et al., 2001).

Gram positive bacteria are surrounded by a peptidoglycan layer that is highly porous allowing the lipophillic ionophores to pass through the membrane (Callaway et al, 2003). Reticulo-ruminal bacterium utilize an environment that is high in sodium and low in potassium. Intracellular potassium is high in the ruminal environment. Ionophores exchange hydrogen for sodium or potassium and move this ion across the cellular membrane. The movement of ions across the cell membranes leads to a change in the acidity in the cells (Pressman, 1976). This acidification of the cellular environment leads the cell to activate a reversible ATPase to decrease this acidification. Hydrolysis of ATP is severed from the growth of the cell and lowers the internal ATP pool which leads to cellular death (Russell and Strobel, 1989, Callaway, 2003)

Ionophores are non-essential antibiotics increase feed efficiency in ruminant animals (Callaway et al., 2003). Ionophores commonly used in livestock production today are monensin, lasalocid, and laidlomycin. Ionophores have oxygen molecules in their chemical structure. These oxygen molecules make a cavity in the molecular structure which allows the ionophore to bind and carry a molecule across a cell membrane (Bergen and Bates, 1983). Ionophores differ in their cation affinity due to the size of the cavity formed by the oxygen molecule in the chemical structure. Monensin has an affinity for sodium due to the small size of its cavity. Lasalocid has an affinity for potassium and laidlomycin has an affinity for sodium (Callaway, 2003)

Monensin

Monensin is an anticoccidial, bloat prevention aid, and used to increase levels of propionic acid in the rumen. Feeding monensin lowers the digestion of starch in the

rumen. However post ruminal digestion of starch is increased to compensate (McGuffey et al., 2000). Monensin theoretically increases concentration of metabolizable energy by increasing the propionate to acetate ratio in the rumen. Due to the increase in this ratio, hydrogen is diverted from CH_4 to propionate (Lemenager et al., 1978). Monensin also helps to increase dry matter digestibility and decreases feed intake. Lactate producing bacteria are inhibited by monensin in the rumen helping reduce lactic acid production and thereby preventing a drop in ruminal pH and acidosis from a high starch diet (Goodrich et al., 1984).

Monensin is widely utilized by the livestock industry to increase feed efficiency and is usually fed as a premix in the animal's diet (Goodrich et al., 1984). Fuller and Johnson (1981) suggest that monensin added to ruminant diets will lower CH_4 energy losses by an average of 3% in corn based finishing diets and decreased the amount of ammonia produced 63% compared to the finishing diet. This suggests that monensin will decrease CH_4 production, and shift VFA fermentation patterns. Feed efficiency is increased by the 10% decrease in CH_4 production and 5% increase in metabolizable energy in the feeding trials (Thornton and Owens, 1981). Monensin will have no effect on milk production and no effect on VFA's or ammonia in rumen fluid. The acetate to propionate ratio decreased in rumen fluid of steers fed forage based diets (Grainger et al., 2010). Acetic acid levels decreased 66.7% to 61.3% and propionic acid levels increased from 20.1% to 26.1% when including monensin in the diet (Dinus et al., 1976).

Monensin will inhibit CH_4 production in steers but the reduction occurs over 24 hours after inclusion of monensin into the diet. Monensin decreased CH_4 production by 16% for a low roughage diet (12.6% CP, 12% ADF) and 24 percent for a high roughage

diet (14.4% CP, 40% ADF). The reduction in CH_4 matched the increase in intraruminal propionate due to the use of hydrogen gas to produce propionate (Thornton and Owens, 1981). Adding 200 mg of monensin to the ration of lactating dairy cows grazing low quality winter forage will reduce forage intake 19.6% and grazing time was reduced 14.6%. Adding monensin can help to increase efficiency of grazing animals and reduce CH_4 emissions. Ruminal acetate decreased and propionate increased when 200 mg of monensin ADFI was added to the ration resulting from an increase in ruminal efficiency in mature Hereford cows fed native grass pasture (Lemenager et al., 1978). Monensin addition to the diet reduced the loss of dietary energy by 9% and monensin lowered CH_4 production by 8.6% but had no effect on tract digestibility of nutrients in cattle (McGinn et al., 2004). High concentrations (200 mg/head daily) vs low concentrations (50 mg/head daily) of monesin yielded similar feed efficiency and feed intake in feedlot steers (Goodrich et al., 1984).

The effects of monensin on the growth performance of cattle was studied in twenty-four trials (Potter et al., 1986). In one trial, 200 mg of monensin was added to concentrates used supplement grazing cattle. The addition of monensin to the ration increased ADG by .09 kg/d. In addition monensin reduced feed intake by 3.1%, and improved feed efficiency by 15.3%. In two separate trials over a two year period 125 heifers were fed 200 mg\hd\d of monensin. Heifers gained 0.08 kg/day more than heifers not fed monensin (Horn et al., 1981). Adding monensin fed in diets with high levels of fermentable carbohydrates increases feed efficiency and reduces feed intake. When fed forages monensin does not reduce intake, feed efficiency is improved, and weight gain is enhanced (Bergen and Bates, 1984).

Conclusion

From the early 2000's through today livestock producers have utilized several by-products that are becoming more widely available as grain production continues to increase. The utilization of by-products continues to be fueled by the demand for a cost effective alternative to the most commonly utilized feedstuffs. The institution of a mandate for ethanol production has created a surplus of by-products. Current instabilities in the global markets and unpredictable weather patterns worldwide often resulting in dramatic fluctuations in grain prices that can result in product shortages. Use of by-products may help to minimize the cost fluctuations associated with the instability in the grain markets.

Producers may also utilize additives that allow for more efficient use of supplements. Feed efficiency needs to be maximized and cost of gain must be minimized for a producer to realize maximum return on investment. With the implementation of more stringent regulation by governing bodies, agricultural producers must actively engage in research and development of methods to effectively limit greenhouse gas emissions. Achieving these goals may be realized through increasing feed efficiencies, utilizing ionophores, and controlling the ruminal environment to reduce gas production.

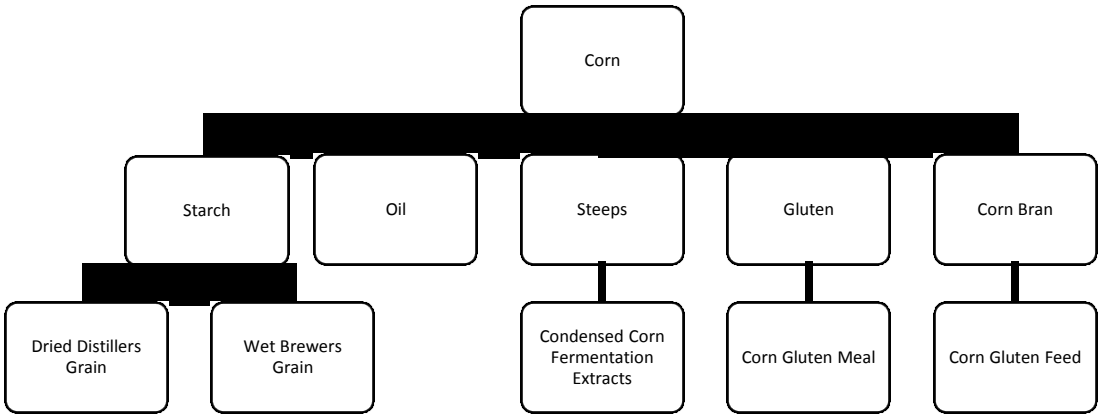

Figure 1. Overview of by-products formed as a result of the Corn Wet Milling Process (Dien et al, 2004).

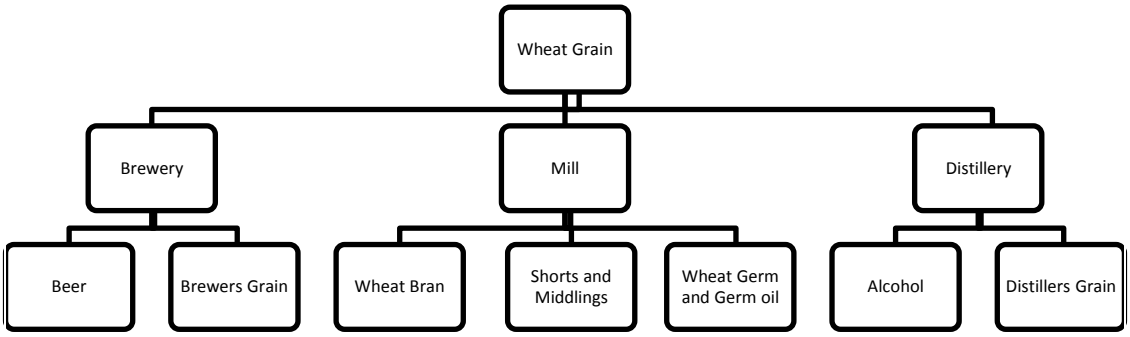

Figure 2. Products produced from wheat production processes (Dziki and Laskowski, 2005).

OBJECTIVE AND NULL HYPOTHESIS

The objectives of this study are to provide further information into the nutritional benefits of wheat middlings, corn gluten feed, cottonseed meal, and soyhulls by determining rumen digestibility and ruminal production of overall gas in-vitro. Evaluation of these feeds will determine the benefits of feeding by-products on increasing ruminal efficiency and reducing greenhouse gas emissions. The effect of monensin on total ruminal gas production and rumen digestibility of a commercially available feed will be examined to determine if the addition of monensin will affect ruminal gas production or feed digestibility.

No difference will be observed in total ruminal gas production and digestibility between wheat middlings, cottonseed meal, corn gluten feed, and soyhulls. Total ruminal gas production and digestibility of feed will not be affected by the addition of monensin.

MATERIALS AND METHODS

In accordance with an approved Missouri State University Animal Care and Use Committee protocol (no. 14-034.0), this study was conducted from September 2013 to October 2013, at the Darr Agriculture Center in Springfield, MO, approximately 6.8km southwest of the Missouri State University campus. Rumen fluid was collected daily from a mature Hereford steer via a ruminal fistula. The steer was housed in a 3.6576m x 7.3152m pen and provided access to ad libitum cool-season grass hay (Table 1), water, and a trace mineral block. The steer was moved into a covered pen and fed a grass hay only diet for 15 days prior to beginning of study.

A standardized method developed by Ankom Technology was followed for collection and preparation of ruminal inoculum (Ciriaco et al, 2016). Briefly, a one liter thermos warmed to 39ºC was used to collect rumen fluid from the steer every 24 hours. The rumen fluid served as the inoculant and digesta media. Under a constant blanket of CO_2 gas, the digesta was poured into a blender that was pre-warmed to 39º C and purged with CO_2. Digesta was blended at 28000RPM in an Oster© blender for 20 to 25 seconds and then strained through four layers of cheesecloth under a blanket of CO_2. A buffer solution was prepared according to procedures developed by Ankom© Technology Corporation, measured into 4000ml beaker before digesta collection to minimize loss and pH adjusted to 6.8.

By Product Feed Gas Production

Gas production of four by-product feedstuffs (SH, CGF, CSM, and WM) was measured utilizing gas production modules (Ankom© Technology Corp., Macedon NY). Average nutrient composition of by-product feeds from Nutrient Requirements of Beef Cattle (NRC, 2016) is presented in Table 2. A standardized method developed by Ankom© Technology was followed for collection and analysis of in-vitro (Ciriaco et al, 2016). Briefly, the feedstuff was ground using a Wiley mill (Thomas Scientific©, Swedesboro, NY) to pass through a 1mm screen. Glass bottles were randomized using a random number generator. Fifteen 250mL bottles were filled with 0.7g of randomly assigned feedstuff (3 of each feedstuff and two controls), along with 100ml of buffer solution. This trial was replicated five times over five days for 15 individual samples. Bottles were filled with 25ml of inoculum then purged for 10 seconds with CO_2 gas. The gas production modules were placed on top of the bottles, purged for 10 seconds with CO_2 gas then placed at random into a pre-warmed water bath and set to incubate for 24 hours at 39ºC. Gas pressure was recorded digitally by a computer every 10 minutes for 24 hours.

By-product Feed In-vitro Digestibility

In-vitro digestibility of four by-product feedstuffs (soyhulls, corn gluten feed, cottonseed meal, and wheat middlings) was determined utilizing a Daisy© 2 incubator (Ankom© Technology Corp., Macedon, NY). A standardized method developed by Ankom© Technology was followed for collection and analysis of in-vitro digestibility data (Krizsan et al, 2013). Briefly, the feedstuff was ground to pass through a 1mm

screen using a Wiley mill. Digestion jars were pre-warmed to 39ºC and 1600ml of buffer solution added. Sample bags (F57; Ankom© Technology Corp., Macedon, NY) were pre-rinsed with an acetone wash for 3 - 5 minutes to remove a surfactant that inhibits microbial activity then allowed to completely air-dry. The filter bags were weighed and 1.0g of each feedstuff was added to two sample bags (each feedstuff being evaluated two times with two controls during five different experimental sets for 10 different samples). Feed samples were randomly placed in sample bags using a random number generator, sealed using a heat sealer and then placed into digestion jars. The digestion jars were purged for 20 seconds with CO_2 gas, 400ml of inoculum was added and then the jar was again purged for 20 seconds with CO_2 gas. The digestion jars were placed in the Daisy 2 incubator (Ankom© Technology Corp., Macedon, NY) for 24 hours, after which the sample bags were removed, and rinsed with a gentle stream of tap water until rinse material was clear. Sample bags were placed into a drying oven at 78ºC for 24 hours to remove all moisture. Samples were reweighed and weights were recorded.

Monensin Effect on Gas Production

Gas production of a commercially available feed (Cattle Charge© R-36, MFA Inc. Columbia MO) with 36g/ton monensin (M+) and the same feed without monensin (M-) was measured utilizing gas production modules (Ankom© Technology Corp., Macedon NY). A standardized method developed by Ankom© Technology was followed for collection and analysis of in-vitro gas production data (Ciriaco et al, 2016). Briefly, the feedstuff was ground using a Wiley mill (Thomas Scientific©, Swedesboro, NY) to pass through a 1mm screen. Glass bottles were randomized using a random number generator

and 0.7g of feedstuff was place into twelve 250ml bottles, along with 100ml of buffer solution. This trial was replicated five different dates with five different samples for 25 individual samples. Bottles were filled with 25ml of inoculum then purged for 10 seconds with CO_2 gas. The gas production modules were placed on top of the bottles, were purged for 10 seconds with CO_2 gas then placed at random into a pre-warmed water bath and set to incubate for 24 hours at 39ºC. Gas pressure was recorded by a computer every 10 minutes for 24 hours.

Monensin Effect on In-vitro Digestibility

In-vitro digestibility of a commercially available feed (Table 3) with 36g/ton monensin and the same feed without monensin was analyzed utilizing a Daisy 2© incubator (Ankom© Technology Corp., Macedon, NY). A standardized method developed by Ankom© Technology was followed for collection and analysis of in-vitro digestibility data (Krizsan et al, 2014). Briefly, the feedstuff was ground using a Wiley mill to pass through a 1mm screen. Digestion jars were pre-warmed to 39ºC and 1600ml of buffer solution added. Sample bags (F57; Ankom© Technology Corp., Macedon, NY) were pre-rinsed with an acetone wash for 3 - 5 minutes to remove a surfactant that inhibits microbial activity then allowed to completely air-dry. The filter bags were weighed and then 1.0g of feedstuff was added to ten sample bags (each feedstuff being evaluated 4 times with 2 controls over 5 different trials for 20 individual samples). Feed samples were randomly placed in sample bags using a random number generator, sealed using a heat sealer and then placed into digestion jars. The digestion jars were purged for 20 seconds with CO_2 gas, 400ml of inoculum was added and then the jar was again

purged for 20 seconds with CO_2 gas. The digestion jars were placed in the Daisy 2© incubator (Ankom© Technology Corp., Macedon, NY) for 24 hours, after which the sample bags were removed, and rinsed with a gentle stream of tap water until rinse material was clear. Sample bags were placed into a drying oven at 78ºC for 24 hours to remove all moisture. Samples were reweighed and weights were recorded.

Table 1. Dry matter, crude protein, acid detergent fiber, neutral detergent fiber, total digestible nutrients, and net energy maintenance of cool-season grass hay*.

DM	CP	ADF	NDF	TDN	NE (maint.)
85.92	15.3	37	63.5	60.4	0.59

*One sample analyzed (Midwest Labs Omaha Nebraska)

Table 2. Nutrient composition of soyhulls (SH), corn gluten feed (CGF), wheat middlings (WM), and solvent extracted cottonseed meal (CSM).

	CP	CF	TDN	RUP	NDF	ADF
SH	10.7**	42.8**	67.30*	70*	60.3*	44.6*
CGF	20.4**	7.91**	82.7**	85	35.5	12.1
WM	21.1**	12.6**	73.3*	90*	36.7*	12.1*
CSM	49.7**	12.1	66.4	92	30.8	19.9

*National Research Council, 2016

** Midwest Labs 2013 DM. CP=crude protein, CF= crude fiber, TDN= total digestible nutrients, RUP= rumen degradable protein, NDF= neutral detergent fiber, ADF= acid detergent fiber

Table 3. Nutrient composition of commercially available feedstuff (Cattle Charge© R-36, MFA Inc., Columbia MO)

	CP	CF	TDN
Cattle Charge	16.8	15.3	77.2

Midwest Labs, Omaha Nebraska, 2013. CP=crude protein, CF= crude fiber, TDN= total digestible nutrients

STATISTICAL ANALYSIS

Gas pressure was converted from pounds per square inch to moles of gas using the ideal gas law.

$$n = p\,(V/RT)$$

Gas produced in moles is represented by n, p is pressure in kilopascals, V is head-space volume in the glass bottle in liters, T is temperature in Kelvin, and R is a gas constant (8.314472 $L \cdot kPa \cdot K^{-1} \cdot mol^{-1}$). Gas in moles was then converted to milliliters (mL) of gas according to Avogadro's law

$$\text{Gas mL} = n \times 22.4 \times 1000$$

Avogadro's law states that at standard temperature and pressure (273.15^{o}K and 101.325kPa, respectively) 1 mole of gas will have a volume of 22.4L.

Gas production data was analyzed as repeated measures using the mixed procedure of SAS (SAS© Inst. Inc., Cary, NC) with fixed effects of time and treatment. Trial was considered a random effect and the repeated measure was time with treatment (trial) as the subject and compound symmetry used for the var-covariance matrix. In-vitro digestibility data were analyzed as a completely randomized design of SAS (SAS© Inst. Inc., Cary, NC) with treatment as fixed effect and trial as random effect. LS means were separated using Fishers LSD and considered significant at $P<0.05$

RESULTS

Gas Production of By-product Feeds

Total gas production over 24 hours of solvent extracted cottonseed meal was lower (Figure 3) than the gas produced by soyhulls, corn gluten feed, and wheat middlings (45.683 vs. 79.735, 73.772, and 93.681mL respectively; $P<0.05$). Corn gluten feed and wheat middlings gas production were not different ($P>0.05$) at 79.735mL and 73.772mL respectively. Soyhulls produced the greatest volume of gas at 93.681mL ($P<0.05$).

In-vitro Digestibility of By-product Feeds

In–vitro digestibility of wheat middlings and corn gluten feed were not different ($P>0.05$) at 62.82% $IVTD_{DM}$ (In-Vitro True Digestibility Dry Matter); and 60.24% $IVTD_{DM}$, respectively (Figure 4). In-vitro digestibility of soyhulls and solvent extracted cottonseed meal were not different ($P>0.05$) at 75.44% $IVTD_{DM}$ and 72.64% IVTD respectively. However, soyhulls and cottonseed meal had greater ($P<0.05$) in-vitro true dry matter digestibility than corn gluten feed and wheat middlings.

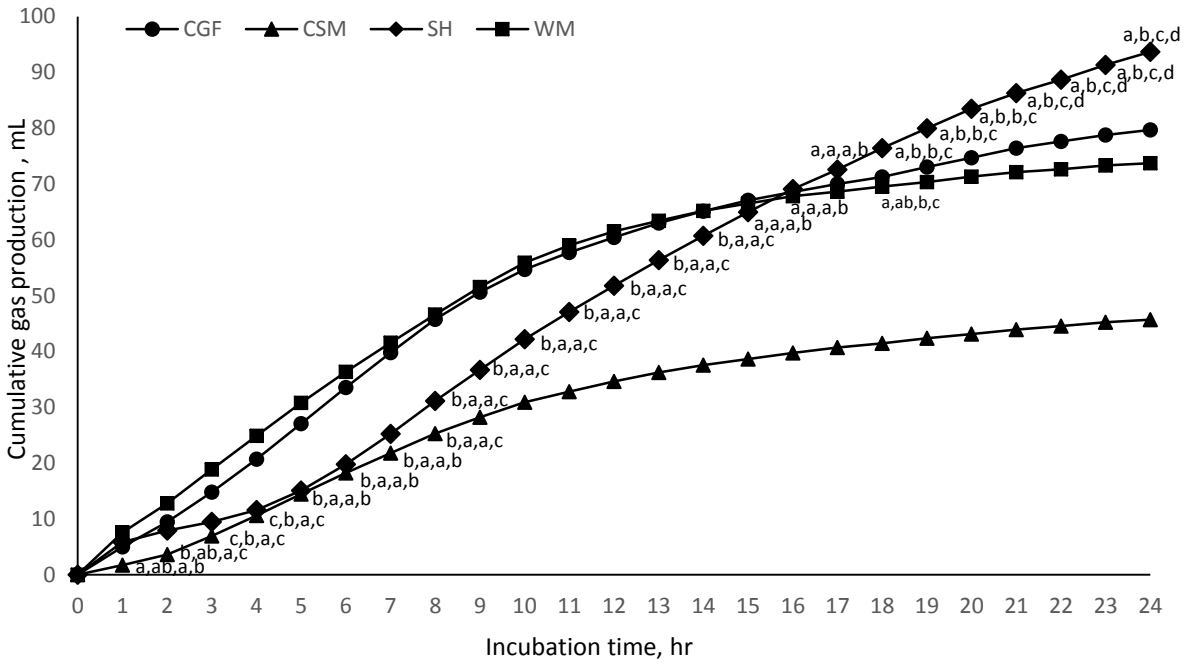

Figure 3. In-vitro gas (n=15) produced (mL) by wheat middlings (WM), corn gluten feed (CGF), soyhulls (SH), and solvent extracted cottonseed meal (CSM) over 24 hour period. [abcd] LSMeans at each time point without a common superscript differ at $P < 0.05$. First superscript corresponds to LSmeans for SH, second superscript corresponds to LSmeans for CGF, third superscript corresponds to LSmeans for WM, and fourth superscript corresponds to LSmean for CSM. Standard error of the mean is 1.5196mL.

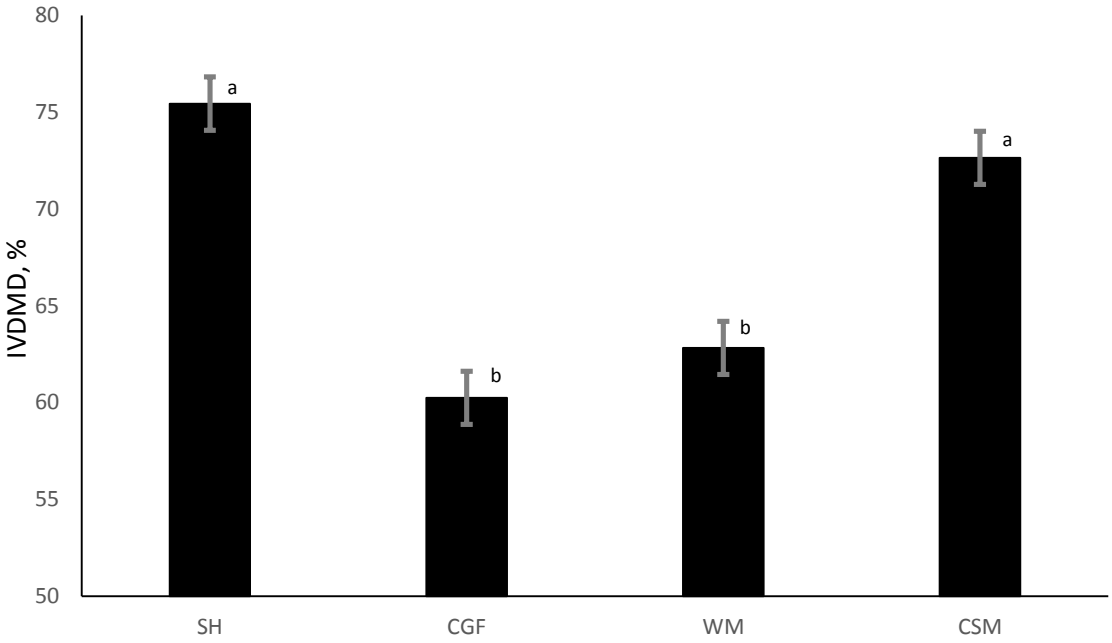

Figure 4. Mean overall percent (n=10) in-vitro digestibility of corn gluten feed (CGF), solvent extracted cottonseed meal (CSM), soyhulls (SH), and wheat middlings (WM).[ab] LS means for each feedstuff without common superscript differ at $P < 0.05$. Standard error of the mean is 0.01373mL.

Gas Production of Monensin

No difference (P>0.05) was observed in in-vitro gas production between a commercially available feed with monensin (M+) and a commercially available feed without monensin (M-). Feedstuff with added monensin produced 89.2652mL of gas compared with 88.1047mL of gas for the feed without monensin (Figure 5).

In-vitro Digestibility of Monensin

No difference (P=0.1281) in in-vitro dry matter digestibility was observed between commercially available feed with added monensin and commercially available feed without monensin. In-vitro dry matter digestibility of the commercially available feedstuff with monensin was 63.06% over 24hr compared with 64.42% for the commercially available feedstuff without monensin (Figure 6).

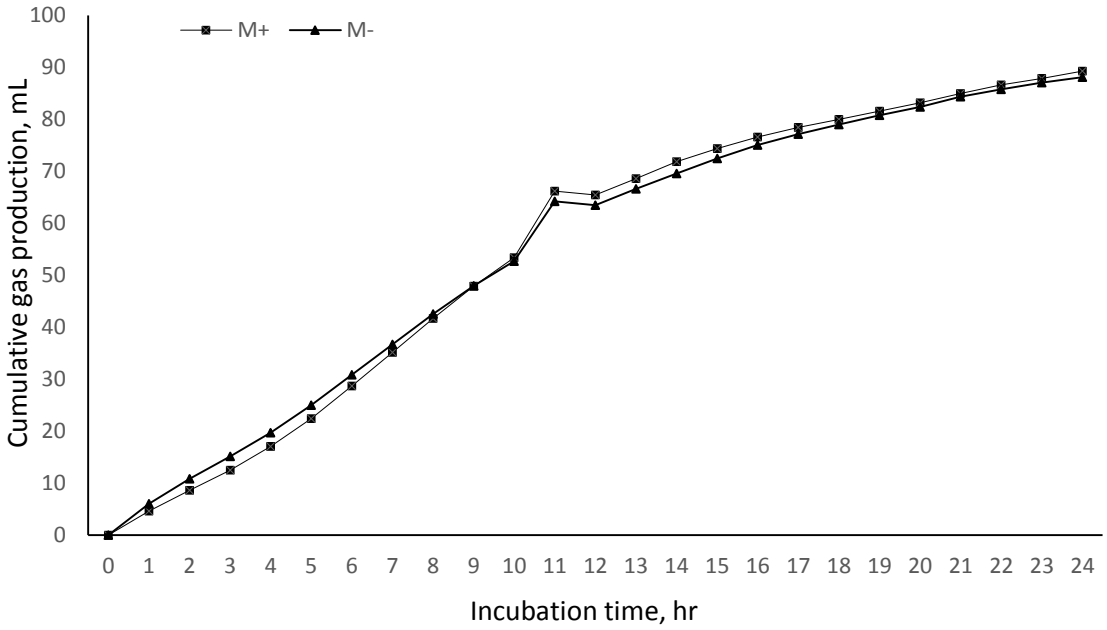

Figure 5. Total volume (n=25)) of gas produced in-vitro from a commercially available complete cattle feedstuff (M-) versus commercially available complete cattle feed with monensin (M+) over a 24 hour period.

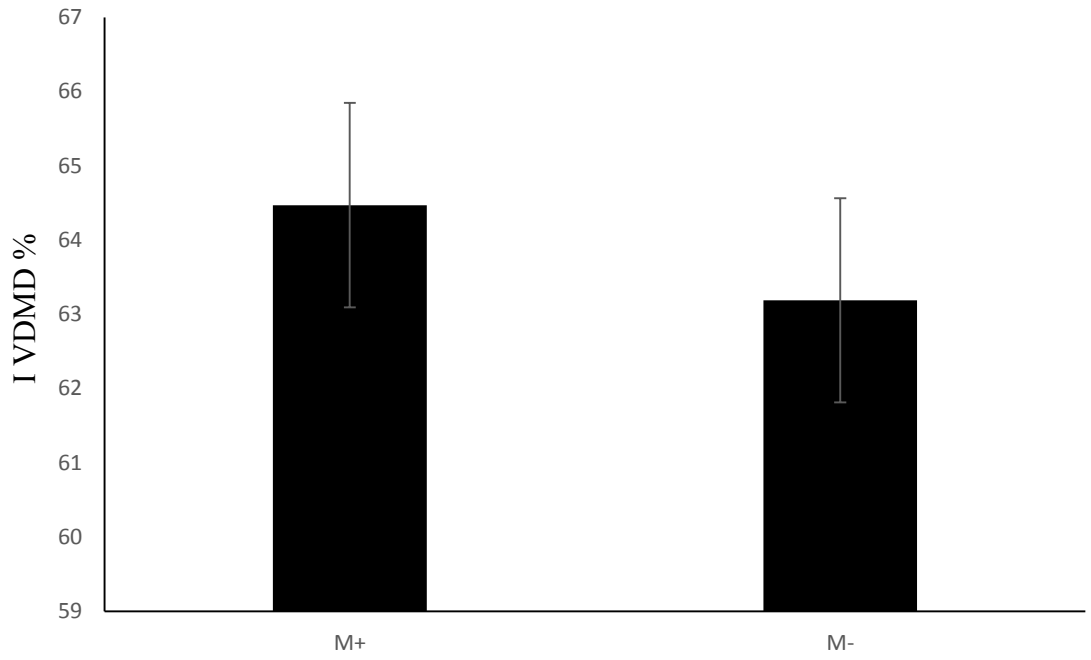

Figure 6. In-vitro dry matter digestibility (n=20) of a commercially available complete feed with monensin (M+) and without monensin (M-) over 24 hours. Standard error of the mean is 0.01376mL.

DISCUSSION

By-product Overall Gas Production.

The results of this study indicate differences in the gas produced of four by-product feeds. In this study soyhulls produced the most volume of gas followed by corn gluten feed and wheat middlings. These findings are consistent with the neutral detergent fiber and acid detergent fiber content of these feedstuffs. Higher fiber concentration of soyhulls lead to an increase in gas production as the higher fiber content possibly lead to an increase in acetate whereas the corn gluten feed and wheat middlings have a higher starch content which would lead to an increase in propionate. Greater propionate production would lead to a decrease in available H_2 and therefore a decrease in CH_4 production.

During in-vitro digestion cottonseed meal produced significantly less gas than soyhulls, corn gluten feed and wheat middlings even though cottonseed meal was higher in fiber. The observed difference in gas produced may be due to condensed tannin that are prevalent in cottonseed meal. Bhatta et al. (2009) found that tannins suppress methanogenesis both directly and indirectly. Tannins were found to indirectly suppress methanogenesis through their anti-protozoal properties and directly due to the anti-methanogenic activity. Condensed tannins are polyphenolic polymers that are found in various legumes cereals and grains. These polymers form complexes with various proteins and are now being used to influence rumen fermentation and inhibit methanogenesis (Patra and Saxena, 2010). Grainger et al. (2010) found that adding

cottonseed to the diet of dairy cows over 12 weeks decreased the CH_4 emissions 3.5g\kg of dry matter intake.

In sheep removing tannins or binding tannins has been shown to increase the degradability of protein increasing the ammonia nitrogen concentration in the rumen (Bhatta et al, 2009). Cottonseed hulls have been shown to significantly lower the nitrogen solubility in the rumen and whole cottonseed has shown minimal effect on ruminal N solubility (Yu et al, 1995). Cottonseed meal that contains hulls may well contain more condensed tannins than cottonseed meal without hulls. Bound condensed tannins have been shown to reduce ruminal fiber digestion (Yu et al, 1995). Fiber digestion by ruminal fibrolytic bacteria depends on ruminal environment, fiber type, as well as species of bacteria (Grant and Weidner, 1991). Condensed tannins have shown to be immobile in the rumen during enteric fermentation locally suppressing fermentation and decreasing gas production (Yu et al, 1995).

By-product Feed Digestibility.

The results of this study indicate the in-vitro dry matter digestibility of cottonseed meal and wheat middlings was not different and the in-vitro dry matter digestibility of corn gluten feed and wheat middlings was not different. The fiber content and starch similarities of the products may have resulted in the two groups of feedstuffs producing similar volume of gas. The acid detergent fiber and neutral detergent fiber of corn gluten feed and wheat middling were similar (Table 1). Also the total digestible nutrients were similar between the two feeds in each group. The result of this study is similar to the

results of a study by Jaworski et al (2015) who found that no difference between the total tract digestibility of feedstuffs with similar carbohydrate content.

Monensin Overall Gas Production.

Monensin addition to a commercially available feed had no effect on overall gas production in this study (Figure 5). A study performed by Guan et al (2006) evaluated the effect of monensin on enteric CH_4 production. The addition of monensin decreased enteric CH_4 production as a percentage of gross energy intake. Clary et al (1993) determined there was no increase in animal performance with the addition of an ionophore. Animals fed a diet low in fiber and high in starch have been less responsive to monensin. High starch diets already promote propionate production over acetate production therefore negatively impacting methanogenesis (Yang et al, 2014). Monensin will decrease dry matter intake of cattle thereby reducing CH_4 production.

Monensin Effect on In-vitro Digestibility.

Addition of a commercially available feedstuff did not have an effect on digestibility when included at 36 g/ton. Addition of antimicrobial in commercial feed may have reduced fermentation in the rumen therefore reducing gas produced in the rumen. Monensin has been shown to slow passage rate of high fiber diets thereby increasing fiber digestion and, therefore, increasing gas production (Bell et al, 2017). Addition of monensin to a commercially available feed had no effect on gas production

due to higher levels of starch as well as digesta utilized in experimental set was extracted from rumen of a steer consuming a forage exclusive diet. Monensin fed for 42 days reduced CH_4 production by 15.8% but dry matter digestibility was not effected (Bell et al, 2017).

CONCLUSION

Previous studies indicate that greater fiber concentration of feedstuffs will increase gas concentration in the rumen. Data presented in this study is in agreement with the previous studies in that soyhulls which has the highest neutral detergent fiber concentration also produced the most gas over a 24 hour period of in-vitro digestibility. Solvent extracted cottonseed meal was the exception to this example. Cottonseed meal contains less neutral detergent fiber than corn gluten feed and wheat middlings, however cottonseed meal does contain more crude fiber. Cottonseed meal contains tannins, which may have an effect on microbial activity. In this study gas production was not different between wheat middlings and corn gluten feed as they have a similar nutrient profile.

Monensin was found to have no effect on total gas production. Additionally, monensin did not have an effect on the digestibility of a commercially available feedstuff. These results may be due to in-vitro incubation as benefits from the addition of monensin may result from effects on in-vivo fermentation kinetics.

IMPLICATIONS

When ruminal environment is manipulated gas production can either be increased or decreased. The data from this study along with other studies indicate that diet fiber concentration has an effect on digestibility and will influence the ruminal environment. Fatty acid production is correlated to ruminal environment. More research is needed into the effects tannins contained in cottonseed may have on ruminal digestion. No difference in volume of gas produced was observed in-vitro when adding monensin to a commercially available feed. More research is needed to explain the benefits of feeding ionophores in high fiber diets vs. high starch diets.

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