Evaluation of grain sorghum hybrids reveals potential for improving ruminal fermentation

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Abstract

In vitro incubations were used to compare fermentation characteristics of corn (uncharacterized hybrid) to 24 sorghum parental lines and hybrids (Clemson University; Richardson Seeds Inc., Lubbock, TX; Scott Seed Company, Hereford, TX). Two *in vitro* experiments were conducted as randomized complete block designs using ruminal contents from two ruminally-fistulated steers (blocks). Grains were ground to similar consistency (1-mm screen) and used as substrates (2 g; DM basis) in laboratory fermenters containing strained ruminal contents and bicarbonate buffer. Fermenters were equipped with pressure-monitoring devices (ANKOM Technology, Macedon, NY) to measure gas production. Gas production, organic acid production, terminal pH of cultures, and dry matter disappearance were used as indicators of microbial digestion. Cultures were incubated for 24 (experiment 1) to 30 hours (experiment 2), with gas production recorded at 15-minute intervals. Experiment 1 compared corn to 23 sorghum cultivars, and experiment 2 compared corn to six sorghum cultivars, five of which were represented in experiment 1, plus one waxy hybrid. Data were analyzed using the MIXED procedure of SAS version 9.1 (SAS Inst. Inc., Cary, NC), with treatment as the fixed effect and block as the random effect. For gas production data, time and the interaction between time and cultivar also were used as fixed effects. For both *in vitro* experiments, there was an interaction between cultivar and time ($P < 0.0001$) for gas production, revealing large differences among cultivars with respect to their relative susceptibilities to microbial digestion. Similarly, DM disappearance; production of acetate, propionate, and butyrate; and acetate:propionate varied substantially among cultivars ($P < 0.01$) and in many cases exceeded measurements obtained with the corn control. A backgrounding study also was conducted to evaluate performance in crossbred steers (n=120, initial BW 273.08 kg ± 2.94) fed diets containing dry-rolled corn, ground 341x120 sorghum hybrid, or ground waxy sorghum diet. Daily feed intakes were monitored for individual animals using the RIC Feeding System (Hokofarm, Netherlands). Feeding ground sorghum-based diets to backgrounding steers increased DMI by 0.51 kg/d ± 0.18 when compared to the dry rolled corn diet. Gain efficiency (G:F; $P = 0.36$) was not influenced by grain source, but ADG ($P \leq 0.005$) was greater for cattle fed waxy sorghum compared with other treatments. Compared to corn, feeding waxy sorghum increased final BW ($P < 0.003$), but BW of cattle fed the 341x120 cultivar was not different from other treatments ($P = 0.12$). Sorghum cultivars used in these experiments revealed substantial heterogeneity with respect to their susceptibilities to digestion

by ruminal microorganisms. Additionally, the backgrounding experiment revealed potential for sorghum cultivars to compete with corn as energy sources in cattle production.

Keywords: *in vitro,* sorghum grain, feedlot, microbial fermentation

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Acknowledgements

I would first like to start with a quote that has pushed me since my freshman year of college which I believe would help many in today's world to understand and follow. Pele said "Success is no accident. It is hard work, perseverance, learning, studying, sacrifice and most of all, love of what you are doing or learning to do".

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Dedication

I would like to dedicate this thesis to my parents, Phil and Nancy Johnson. Thank you for instilling in me my passion for the livestock industry and giving me the opportunity to be a part of a truly special business. You have always pushed us to achieve our best but never expected more of us than what we were truly capable of, and for that I am forever grateful. I was through your support and tutelage that I have reached this important milestone in my life and none of it would have been possible without you.

Chapter 1 – Literature Review: Advantages of Sorghum Grain Genetics and Their Impact on *In Vitro* and Animal Feeding Systems

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Abstract

Sorghum grain is reported to possess nearly 90% of the feeding value of corn (Huck et al., 1999). Sorghum cultivars are found to be advantageous in arid climatic regions as they require less saturation to reach their optimal growth conditions when compared with other grain sources. Sorghum cultivars vary widely in their influence on animal performance, owing to differences in genotype, phenotype as affected by geographical location or agronomic conditions, and processing method (Healy et al., 1993). Phenolic compounds located deep within the pericarp are also of interest, as they are known to affect microbes within the gut, potentially conferring health benefits (Ashley et al., 2019). Kaufman et al. (2013), however, reported that tannins located in the pigmented testa of the grain bound proteins, and thus were negatively correlated with digestibility. Boyles at al. (2016) reported a positive relationship between sorghum yield and starch content, and a negative relationship between yield and fat, crude protein, and gross energy contents of grain. The NRC (1999) assigned steam-flaked sorghum a feeding value that is 92% that of steam-flaked corn (Zinn et al., 2008), though Huck et al. (1999) found that steam-flaking sorghum grains increased feeding value by 15%. Results of previous *in vitro* and *in vivo* research indicated a high degree of variability among sorghum cultivars. Therefore, our objective was to identify cultivars that are best suited for use in beef production systems within arid regions, such as the Southern Plains of the U.S.

Key words: sorghum, feeding value, polyphenols

History

Sorghum, one of the oldest known grains, originates from Africa, and in the U.S. has its greatest production in Kansas. Indigenous to Egypt, sorghum fields were carved on the walls of the tomb of Amenembes, which was built over 2200 years before the Christian era (Vinall et al. 1936). If this carving is accurate, it establishes sorghum grain as an important cereal crop as early as 700 B.C. (Vinall et al., 1936). Domestication of sorghum in Africa came in response to climatic changes during the Holocene era, which was characterized by a surge in agricultural innovation (Smith et al., 2000). Intermating and selection for region-specific advantages resulted in an exponential increase in the variety of domesticated sorghum cultivars (Rooney et al., 2007). In the United States, sorghum use historically has been split into thirds, with its major applications in renewable fuels production, domestic animal feeding, and export markets (Sorghum Checkoff, 2016). Use in consumer goods also is expanding, as sorghum now can be found in over 350 product lines in the United States alone (Sorghum Checkoff, 2016).

Currently sorghum grain is being developed as a bioenergy crop, which was proposed over 20 years ago (Rooney et al., 2007). Improvements in sorghum yield and feeding potential can be achieved by taking traditional cultivars and progeny from crosses, then employing specific traits of exotic cultivars into the breeding (House, 1995). As the world's fifth ranking crop, recent increases in sorghum production have been attributed to yield improvements rather than changes in planted acreage (Maunder, 2002). Progress is limited however, as Frey (1994) reported that research related to sorghum breeding is only a tenth that of corn (Maunder, 2002). Much like the United States, Europe increased use of sorghum in cattle feed through enhanced feed processing technology, making sorghum's most realistic use cattle feed (Berenji et al., 2004). Sorghum in cattle feed takes the form of hay, silage, or grain; however as of 2016

sorghum is still being utilized in minimal amounts in the U.S. cattle feeding systems. According to the Sorghum Checkoff (2016), end uses of sorghum were dispersed across industry with 55% to export markets, 21% ethanol production, and only 15% as livestock and poultry feed.

Introduction

Grain sorghum is known to have significant variability in nutrient content across animal species, but is estimated to have 95% of the DM digestibility of corn (Simpson et al., 1985). Variability can be attributed to environment, processing methods, or genotype among the sorghum (Healy et al., 1993). Concentrations of phenolic acids, flavonoids, and tannins in certain sorghum varieties can impact their susceptibility to microbial fermentation. Sorghum historically has traded at a discount to corn, typically trading at 87% the value of corn according to Huck et al. (1999). Ease of processing and digestibility can be influenced by differences in kernel size; small, compact kernels may take longer to ferment (Goldy et al., 1987). As a result, sorghum often is processed through steam flaking, dry rolling, or roasting, with the aim of increasing its susceptibility to digestion.

Sorghum varieties characterized by low tannin content and, therefore, greater availability of beneficial phenolic compounds is considered ideal. *In vitro* methods have been developed to elucidate the benefits of these phenolic acids and flavonoids from low-tannin varieties. There may be human- or animal-health advantages to feeding these types of sorghums in mixtures with other grain types. Such studies may enhance our understanding of the benefits of phenolic acids and flavonoids.

Polyphenols

In an effort to evaluate fermentation characteristics of sorghum, it is important to identify the individual compounds believed to aid or inhibit the process. For example, phenolic

compounds in sorghum have been associated with health benefits (Ashley et al., 2019). Dykes and Rooney (2007) define a phenolic compound as "any compound containing a benzene ring with one or more hydroxyl groups". Polyphenols are compounds generated by metabolic pathways of many plants and some manifest antimicrobial activity (Ashley et al., 2019). Sorghum cultivar's polyphenol content is dependent on endosperm type. More specifically, varieties such as Sumac are rich in proanthocyanins and black varieties contain 3-deoxyanthocyanins (Ashley et al., 2019). Phenolic compounds also may have an adverse impact on digestion of starch, protein, and minerals (Wu et al., 2016). Uchimiya et al. (2016) pointed out that the United States sorghum reserves are developed to contain low-tannin levels which should allow livestock to consume the product with relatively high efficiency.

Certain polyphenols have been shown to have positive effects on gut microbiota. Ashley et al. (2019) found that sorghum extracts could be utilized to help modulate gut microbiota, increasing presence of certain types of bacteria. The three major chemical classes that constitute polyphenols are phenolic acids, flavonoids, and condensed tannins.

Phenolic Acids

With existence in all cereal grains, phenolic acids are broken into two categories--hydroxybenzoic acids and hydroxycinnamic acids. Of these two divisions, hydroxybenzoic acids are comprised of gallic, p-hydroxybenzoic, vanillic, syringic, and protocatechuic acids (Dykes et al., 2019). The hydroxycinnamic acids are made up of a C3-C6 structure in which coumaric, caffeic, ferulic, and synaptic acids are included (Dykes et al., 2019). Within these two divisions, the phenolic acids also occur in both free or bound forms. "Free phenolic acids are located in the outer layer of the pericarp and can be extracted with organic solvents. Bound phenolic acids are

esterified to cell walls; acid or base hydrolysis is required to release these bound compounds from the cell matrix" (Dykes and Rooney, 2007).

Phenolic acids are known to have antioxidant activities, thus promoting gut health and animal performance. While causing changes in gut health, increasing phenolic acids in sorghum varieties leads to physical differences in grain preparation for processing. It is unknown if germination affects the content of phenolic acids in sorghum grain or their antioxidant levels (Dicko et al., 2005). Dicko et al. (2002) reported that an inter-seasonal variation in concentrations of total phenolic compounds existed among the sorghum varieties tested. Environmental factors, such as precipitation and heat, can play an enormous role on grain composition and resulting animal performance. Genotype, irrigation, and genotype × irrigation influenced antioxidant activities of polyphenols found in sorghum grain. Wu et al. (2017) observed a significant effect ($P \leq 0.05$) of all three. They also can be impacted by biotic and abiotic stresses (Dicko et al., 2005).

There are specific instances when a weaker correlation exists between phenolic levels and antioxidant activity. Dicko et al. (2005) showed that this could occur after germination when the synthesis of other antioxidant compounds, more specifically vitamin C and tocopherols, took place. It has been shown that accumulating phenolic compounds by use of the phenylpropanoid pathway is organized by phenylalanine ammonia-lyase (Wu et al., 2017). Wu et al. (2017) found that phenylalanine ammonia-lyase activity and total phenolics were enhanced under deficit irrigation.

Condensed Tannins

The most frequently studied phytochemical constituents of sorghum are the condensed tannins which make up the pigmented testa layer of certain sorghum cultivars. Condensed

tannins serve as a defense mechanism against pathogens and predators (Kaufman et. al., 2013). They cause an astringency to develop during grain maturation which discourages depredation by animals or insects (Dykes et al., 2019). Condensed tannins bind to proteins *in situ*, which sharply limits their ruminal digestibility. Also referred to as proanthocyanins or procyanidins, they form strong hydrogen bonds with proteins and have hydrophobic interactions that link them to the large proline-rich proteins in feedstuffs (Kaufman et al., 2013). Ritchie et al. (2015) stated that some condensed tannins are non-hydrolysable proanthocyanins, which can polymerize, allowing for more extensive bonding to proteins. Kaufman et al. (2013) found that tannin content of sorghum was negatively correlated with both protein digestibility and positively correlated to antioxidant activity promoted by the grain. It then makes sense to see where limited protein digestibility corresponds to increased antioxidant properties in sorghum. The decrease in extractable proanthocyanins that occurs following germination may be due to leaching of water-soluble proanthocyanins (Dicko et al., 2002).

Sorghum grains contain kafirins, which are the predominant proteins that influence endosperm hardness, Kafirins also can affect lysine content. Kafirins can be either monomeric or polymeric (Boyles et al., 2016). Polymeric kafirins are developed through intermolecular disulphide cross-linkages (Ezeogu et al., 2005). Kaufman et al. (2013) found through chromatographs that increases in tannin content in the bran of samples led to disappearance of soluble y-kafirins, which resulted from binding of the y-kafirins to tannins, making them insoluble.

Differences in sorghum grain endosperm type can influence digestibility and feed value. Differences in endosperm type can influence the behavior of tannin oligomers and polymers, by manipulating their influence on the nutritional value of the cultivar (Kaufman et al., 2013).

Tannins slow the rate of starch hydrolysis by amylases in the sorghum endosperm, likely due to proteins in the endosperm preventing proanthocyanins interaction with starch (Dunn et al., 2015). The starch expands, opening pores in the granule which allow tannins to reach the starch polymers inside the granule (Amoako et al., 2016). Dunn et al. (2015) hypothesized that the proanthocyanins formed cross-linking networks with gluten during mastication, effectively inhibiting access to starch by amylase.

Amoako et al. (2016) observed that the condensed tannins from sorghum varieties have an interaction with amylose, forming amylase-resistant starch, which is a gelatinized dispersed starch that comes from the previously mentioned hydrogen bonding. Furthermore, tannin disappearance in the rumen is poorly understood, but it is believed that condensed tannins disappear in the rumen by bacterial destruction (Streeter et al., 1990).

Flavonoids

Most flavonoids are found in the outer grain layers, which results in their concentrations being cofounded with pericarp color, thickness, and the presence of testa (Wu et al., 2016). Flavonoids are made with a C6-C3-C6 skeleton and two aromatic rings held together with a three-carbon link (Dykes et al., 2019). Flavonoids can be further broken down to include anthocyanins, flavanols, flavones, flavanones, or flavanols (Dykes et al., 2019). Concentrations of flavonoids; free, bound, and total flavonoids, varied with genotypes ($P \leq 0.05$) (Wu et al., 2016). Dykes et al. (2019) reported that anthocyanins were water soluble pigments that create color in most plants. These can be found in the pericarp of numerous grain types. Unlike most flavonoids which easily break down during microbial fermentation, anthocyanins are resistant to acidic conditions (Ritchie et al., 2015).

Perhaps the most important anthocyanins in sorghum are the 3-deoxyanthocyanins, which are resistant to low pH levels due to their lack of a hydroxyl group in the 3 position of the C-ring (Dykes et al., 2019). Bioactive compounds such as these may have the capability to change luminal environment and the microbial population of the gut (Ritchie et al., 2015). Wu et al. (2016) found that free flavonoids in sorghum increased when exposed to deficit irrigation compared with full irrigation, regardless of the cultivar tested. Wu et al. (2016) found that total flavonoid contents in their study were greater than those previously reported from decorticated sorghum flour. Flavonoids have unique antioxidant properties which have therapeutic value for treatment of cancer, inflammation, and gastritis (Dykes et al., 2019).

Genetics and Crop Yield

Sorghum has been selected over time through the utilization of genetics by sorghum breeders trying to improve upon crop yield, tannin content, and antioxidant levels in the crop. Identifying genes that influence expression of valuable phenotypes could be used to improve grain quality and texture to target specific markets and to create new products (Boyles et al., 2016). In order to do so, several region-specific cultivars have been developed. It is understood that desired grain composition needed is determined by end-use. Locating genes which regulate grain quality is fundamentally important in this process (Boyles et al., 2016).

One of the factors that seems to help us define the make-up of specific grains is the endosperm color and texture. Endosperms can be either floury or vitreous. Ezeogu et al. (2005) found that both endosperm types gave similar kinetic constants, suggesting that digestibility differences are attributable to extrinsic factors. Endosperm texture can be broken down into the five specialized cell types: the central starchy endosperm, the subaleurone layer, the aleurone layer, the basal endosperm transfer layer, and the embryo-surrounding region (Kladnik et al.,

2006). Sorghum's aleurone layer is made up of cells which are significantly smaller than those in the rest of the endosperm (Kladnik et al., 2006). Research by Kladnik et al. (2006) showed that the endopolyploidy explained the difference in endosperm cell volume.

Two cellular processes play a significant role in endosperm size; cell division and cell growth. Cell growth is believed to be correlated with endoreduplication of nuclear DNA (Kladnik et al., 2006). Endoreduplication leads to increased cell volume, allowing for greater storage availability for starch in the endosperm (Kladnik et al., 2006). Endopolyploidy cells also aid in the accumulation of starch at the base of the endosperm. Study of sorghum endoreduplication during caryopsis is lacking, making corn the primary source of endosperm information for the industry (Kladnik et al., 2006).

Sorghum grains are known to vary with respect to nutrient densities, including starch, protein, and fat (Boyles et al., 2016). Macronutrients are quantitative traits, which are influenced by a large number of genes. Kladnik et al. (2006) stated that for sorghum cultivars, the pericarp accumulates a major proportion of the starch right after pollination and before it has the opportunity to reach the endosperm. Streeter et al. (1990) found that starch content was greatest in normal sorghum varieties and tended to be less in those which are bird resistant (i.e., with elevated condensed-tannin concentrations).

Ratnavathi et al. (2003) reported that endosperm color was associated with physical characteristics of grain; red varieties are larger in size while those yellow and white in color are smaller. Chemical imbalances can be found when comparing genotypes. Varieties with a red endosperm tended to have lesser starch concentrations than yellow or white genotypes (Ratnavathi et al., 2003). Ratnavathi et al. (2003) found that red endosperm varieties tended also

to have greater concentrations of protein and polyphenols concentrations than yellow or white varieties.

Sorghum can also be classified in accordance with the type of endosperm it contains. For instance, waxy endosperm is typically associated with increased *in vitro* digestibility, likely due to a less peripheral endosperm and less amorphous protein matrix (Streeter et al., 1990). The waxy sorghums are also low in amylose content and, as such, may be more useful than non-waxy sorghum varieties as feedstuffs and for ethanol production (Boyles et al., 2016). Crude protein content of non-waxy sorghums is generally less ($P < 0.01$) than that of the waxy varieties (Streeter et al., 1990). These researchers speculated that the differences in digestibility performance between non-waxy and waxy sorghum varieties attributed to a reduction in acid detergent fiber (ADF) concentration (Streeter et al., 1990).

Endosperm character has the opportunity to influence starch digestion ($P < 0.001$) by amylase (Ezeogu et al., 2005). Ezeogu et al. (2005) found that fecal pH is greater from cattle fed waxy endosperm varieties versus non-waxy varieties. This suggests non-waxy varieties have greater degrees of starch fermentation in the small intestine compared with waxy varieties, thus resulting in less substrate available for fermentation once they reach the large intestine. Digestibilities of organic matter and starch are increased in waxy cultivars compared to non-waxy phenotypes. This may be related to differences in susceptibility of amylopectin and amylose to enzymatic and microbial attack, accompanied by differences in solubility of the protein matrix which surrounds the starch (Streeter et al., 1990). The structure of amylose provides greater surface area for hydrogen bonding compared to highly-branched amylopectin, and this structural difference should then allow tannins to act as cross-linkers within the starch granule (Amoako et al., 2016). Streeter et al. (1990) believed that denaturing the protein matrix

around the starch granules could have an adverse impact on digestion of the peripheral endosperm in the rumen.

Ratnavathi et al. (2003) provided evidence that toxin contamination and infection can be dependent on sorghum genotype. For instance, only 8% contamination exists for white varieties, while some yellow and red varieties can reach 30% contamination (Ratnavathi et al., 2003). Ritchie et al. (2015) reported that black and brown sorghum cultivars had altered short-chain fatty acid contents compared with other cultivars, suggesting possible changes in intestinal microbiota. Tannin presence in brown endosperm sorghum varieties have links to fungal resistance (Ratnavathi et al., 2003); thus, differences in aflatoxin risk exist among sorghum phenotypes (Ratnavathi et al., 2003). Resistance to these fungal infections is largely dependent on grain color, pericarp type, and hardiness (Ratnavathi et al., 2003). As aflatoxin infection increases in sorghum cultivars, starch content decreases, whereas protein content increases.

Boyles et al. (2016) evaluated the impact of crop yield on grain quality. It seems as though crop yield can impact macronutrients of sorghum grains in a variety of ways. Boyles at al. (2016) pointed out that a positive relationship was observed between sorghum yield and starch content, whereas yield had an inverse relationship to fat, crude protein, and gross energy. The high correlation between grain yield and starch content provided explanation for the greater use of sorghum lines high in starch content (Boyles et al., 2016). Boyles et al. (2016) found that with specific varieties (e.g., P850029), a negative correlation existed between grain yield and amylose content, while yield factors were not influenced by amylose in other sorghum cultivars (e.g., BTx642). In fact, the separation of yield in the P850029 variety impacted the association analyses for sorghum quality, which found representation in the cultivar genotype and genetic profile relationship with crop yield and their overall quality (Boyles et al., 2016). Grain yield was

also observed to decrease with the presence of a number of alleles that were associated with increased protein content (Boyles et al., 2016).

There are numerous alleles that are associated with increased crop yield. Boyles et al. (2016) suggested that incorporating these alleles into a superior germplasm could generate sorghum cultivars with elevated crude fat and gross energy levels while not diminishing the performance of the grain's field performance. Sorghum cultivars are made up of large genomic regions which breeders utilize for manipulation of the grains genotype, thus fulfilling needs of specific grain markets (Boyles et al., 2016). Genetic differences in grain sorghum varieties are dependent on the genetic differences in parent lines, which could be pointed out by an increase in the occurrence of polymorphisms and recombination breakpoints (Boyles et al., 2016). Researchers have utilized the observation of quantitative trait loci (QTL) to find specific alleles which could be utilized to improve grain quality and yield performance simultaneously. For instance, Boyles et al. (2016) conducted a study in which they identified a favorable allele at one specific QTL on 57 different genotypes. Those alleles found were classified as both elite or exotic but fell under the five major botanical races of sorghum recognized by the USDA (Boyles et al., 2016).

Quality differences can also result from environmental factors. Boyles et al. (2016) showed a year-to-year impact on grain quality, with a correlation between environment and year that the sample was collected. In fact, the combination of linkage mapping by environment allowed the identification of genetic backgrounds and QTL which were linked to specific environment-based factors (Boyles at al., 2016).

In Vitro

Evaluation of grain cultivars using animal performance studies is time consuming and costly. *In vitro* studies can be valuable as screening tools to assess differences in grain attributes that apply directly to animal feeding, such as starch degradability, protein degradability, and end-product formation through microbial activity. They can also be used to measure fermentative gas production, composition of fermentative gases, pH, and production of organic acids or ammonia as end-products of digestion. Breakdown of the protein matrix of starch granules causes a decrease in the digestion of peripheral endosperm starch (Ritchie et al., 2015).

Sorghum fermentation results in unique profiles of butyrate, and acetate, which could promote changes to the gut microbiota as a result of antioxidant activity (Ritchie et al., 2015). In fact, many researchers have found through the use of *in vitro* studies that varieties containing higher tannin levels can have a significant impact on the amount of bacterial survival and residue expected in the gastrointestinal tract (Ritchie et al., 2015). *In vitro* studies done by Hamaker et al. (1986) showed that heat treatment of sorghum grains can cause a decrease in protein digestibility. It is likely that such action causes the formation of a disulfide-bound protein. Sorghum and corn were found to be less digestible (10%) than barley, wheat, and rice (Hamaker et al., 1987). Osman et al. (1970) pointed out that moist-heat treatment of sorghum can improve *in vitro* fermentation. Greater dry matter disappearance was observed *in vitro,* coinciding with an increase in gas production was found for barley in comparison to sorghum, along with 14% greater increase in volatile fatty acid production before the steam flaking process (Osman et al., 1970). Steam flaking improved dry matter digestibility, gas production, and the total volatile fatty acid profile of sorghum grain through microbial fermentation (Osman et al., 1970).

Grain processing is not the only factor which can impact *in vitro* fermentation and organic acid production from sorghum cultivars. Ashley et al. (2019) compared *in vitro*

fermentation of Sumac and black sorghum varieties to quantify the impact of polyphenol composition. These researchers found that when increasing the fermentation time from 12 to 24 hours, short chain fatty acid yields were decreased (Ashley et al., 2019). Ashley et al. (2019) found when looking at volatile fatty acid content that acetic acid concentrations were greater ($P < 0.05$) for black sorghum varieties compared to Sumac at all time points. Sumac had greater ($P < 0.05$) butyric acid concentrations when compared to black varieties at all time points (Ashley et al., 2019). *In vitro* fermentation allows insight to specific rumen microbiota and the phylum levels influenced by specific cultivars. Ashley et al. (2019) found that from 0 to 24 hours fermentation was supported significantly by *Bacteroidetes* which decreased beyond 24 hours. At 24 hours of *in vitro* fermentation, black sorghum varieties had greater concentrations of *Bacteroidetes* in the culture media (Ashley et al., 2019).

Sorghum used in diets

Rich in starch, sorghum should have greater hind-gut utilization (≥ 70% starch, where 75:25 amylopectin/amylose) (Ezeogu et al., 2005). Utilization of sorghum in the feedlot diets comes with a series of trade-offs. While sorghum costs less to grow and harvest compared with corn, there is an increase in processing cost compared with corn. Sorghum is typically processed by steam flaking, dry rolling, or roasting. During steam flaking, grains are often tempered which is the process where moisture is added to the grain through a chemically-facilitated process (Zinn et al., 2008). Zinn et al. (2008) pointed out that the NRC (1999) gave steam-flaked sorghum 92% of the feeding value found in steam-flaked corn. Tempering of the grain gelatinizes starch granules, allowing for improved utilization by the animal. Feeding value of sorghum grain is increased by roughly 15% when exposed to additional moisture before processing (Huck et al., 1999). Huck et al. (1999) found a positive correlation between gain efficiency and tempering;

however increasing the moisture content of the grain tended to lower dry matter intake in feedlot cattle ($P < 0.10$; Huck et al., 1999). Theurer (1986) observed that steam flaking sorghum elevated grain digestibility, whereas Ezeogu et al. (2005) reported that increasing cooking time of sorghum grain decreased starch digestion. Zinn et al. (2008) observed through a feeding study that steam flaking of sorghum grain decreased DM intake ($P < 0.01$) while improving G:F ($P < 0.01$) of the ration when it is compared to a dry-rolled grain treatment.

When the grain goes through the steam-flaking process the major factor which enhances the feeding value of the grain is the shear of the starch that takes place when grain goes through the rolls of the flaker (Zinn et al., 2008). Increasing pressure from the rolls of the flaker causes a reduction in bulk density and logically would result in an increase in total starch digestibility. From that it was predicted that shear of the starch granules exposes more of the endosperm allowing the exposure of the protein matrix to the post-ruminal proteolytic process, which would allow enhanced availability of starch to the amylolytic process (Zinn et al., 2008). Each flaking increment ($P < 0.05$) improves starch digestion (Osman et al., 1970).

Steaming the sorghum grains for roughly 25 minutes and subsequent flaking improved feed efficiency and overall weight gains when used in study for comparison to dry rolled sorghum (Osman et al., 1970). Further research showed that steam flaking improves digestibility and nitrogen-free extract (Osman et al., 1970). It was also found that the inclusion of moisture through the tempering process was associated with tendencies for greater subcutaneous fat thickness and incidence of liver abscesses ($P < 0.10$; Zinn et al., 2008). Huck et al. (1999) reported that cattle fed sorghum diets with 25-35% added moisture had a tendency ($P < 0.10$) to produce less backfat than those being fed steam-flaked corn diets.

Sudweeks et al. (1998) explained that roasting of sorghum grain offered no advantage in gain or efficiency; however, roasting resulted in elevated ruminal acetate, propionate, and butyrate concentrations (Sudweeks et al., 1998). Streeter et al. (1990) ran a study in which they observed greater chyme flow in bird resistant sorghum varieties than non-bird resistant varieties. Increased chyme flow was deemed indicative of greater ruminal fluid dilution rate, and typically is associated with reduced digestion of organic matter and starch (Streeter et al., 1990).

In an *in vivo* study, Streeter et al. (1990) found that sorghum varieties which possess a non-waxy endosperm were associated with greater amounts of condensed tannins ($P < 0.01$) exiting the ileum when compared with a waxy endosperm sorghum variety. Upon further investigation, these researchers also found that the breakdown of organic matter prior to the cecum had a tendency to be less ($P < 0.11$) for sorghum varieties with non-waxy endosperm than those with waxy endosperm (Streeter et al., 1990). Streeter et al. (1990) found that starch was found to be more digestible ahead of the ileum for waxy varieties than those with a non-waxy endosperm, resulting in less starch reaching the large intestine for those same waxy sorghum types.

Conclusion

Sorghum requires less water consumption compared to other cereal grains to achieve optimal growing conditions (Streeter et al., 1990). With roughly 87% of the value of corn, sorghum cultivars gain popularity through their price structure (Huck et al., 1999).

Sorghum cultivar type has been shown to influence animal growth and efficiency. This can be attributed to differences in genetics (endosperm type, bran color, starch content), crop yield, polyphenolic concentrations, or processing procedures. Phenolic acids and flavonoids are among the polyphenols identified by researchers as supportive to gut health and provide greater

gut utilization of starch. In contrast, condensed tannins have been shown to develop bonds within the protein matrix which produce barriers that interfere with starch digestion. Processing sorghum grain using steam-flaking and other processes could improve grain feeding value to upwards of 15% (Huck et al., 1999).

The impact of grain sorghum on *in vitro* and feedlot performance can be extremely variable. Factoring in growing conditions, processing methods, and grain genotype can fluctuate results within cultivar type.

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Chapter 2 – *In Vitro* Evaluation of Grain Sorghum Hybrids Reveals Potential for Improving Ruminal Fermentation

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Abstract

In vitro incubations were used to compare fermentation characteristics of corn (uncharacterized hybrid) to 24 sorghum parental lines and hybrids (Clemson University; Richardson Seeds Inc., Lubbock, TX; Scott Seed Company, Hereford, TX). Two experiments were conducted as randomized complete block designs using ruminal contents from two ruminally-fistulated steers (blocks). Grains were ground to pass a 1-mm screen and used as substrates (2 g, DM basis) in laboratory fermenters containing strained ruminal contents (10 mL) and bicarbonate buffer (140 mL). Fermenters were equipped with pressure-monitoring devices (ANKOM Technology, Macedon, NY) to quantify gas production as an indicator of microbial digestion. Cultures were incubated for 24 (experiment 1) or 30 hours (experiment 2), and gas production was recorded at 15-minute intervals. Endpoint measurements included culture pH, concentrations of organic acids, and DM disappearance. Experiment 1 compared corn to 23 sorghum cultivars, and experiment 2 compared corn to six sorghum cultivars, five of which were represented in experiment 1, plus one waxy hybrid. Data were analyzed using mixed models with cultivar as a fixed effect and block as a random effect. For gas production data, time and time × cultivar also was used as fixed effects. For both experiments, there was a time × cultivar interaction ($P < 0.0001$) for gas production, revealing large differences among cultivars with respect to their relative susceptibilities to microbial fermentation. Similarly, DM disappearance; production of acetate, propionate, and butyrate; and acetate:propionate varied substantially among sorghum cultivars ($P < 0.01$) and in many cases exceeded corresponding measurements obtained with the corn control. Sorghum cultivars used in these experiments varied widely in their susceptibility to digestion by ruminal microorganisms, revealing potential for development

of hybrids that can compete with corn as energy sources while contributing to improved sustainability of feedlot production.

Introduction

Grain sorghum was introduced to North America in 1757; and the United States now is the leading producer of grain sorghum worldwide (Dahlberg et al., 2011). Over 60 million tons of sorghum grain are produced annually in the United States (Dicko et al., 2005). Sorghum is known to require less water than other cereal crops, allowing it to be grown successfully in arid climates (Streeter et al., 1990). As a C4 plant, sorghum projects downward with a fibrous root system that extracts nutrients from deep within the soil profile (Smith et al., 2000). It is a logical alternative to cereal crops, such as corn, that are characterized by much greater water demand.

Though well-adapted to the arid growing conditions existing in much of Kansas, sorghum grain generally is regarded as being inferior to corn as an energy source for feedlot cattle. Variations in kernel size, challenges associated with processing, and perception of poor digestibility in comparison to corn all are cited as reasons for limited use of sorghum grain in the High Plains feedlots (Healy et al., 1993). Identifying attributes of sorghum that bring value to feedlots is thus a first step toward increasing its use in feedlots.

Sorghum has been selectively bred over time, resulting in wide compositional variation among cultivars. One source of this variation is the relative mixture within specific cultivars of simple phenols, hydroxybenzoic acids, hydroxycinnamic acids, anthocyanins, proanthocyanins, and several other flavonoids (Dicko et al., 2005).

The main protein constituents of sorghum grain, kafirins, exist in both monomeric and polymeric forms. The polymeric kafirins are formed through intermolecular disulphide cross-linkages located typically in the vitreous endosperm fraction (Ezeogu et al., 2005). Differences in sorghum endosperm structure and protein composition may influence starch digestion dynamics (Ezeogu et al., 2005).

It is known that endosperm characteristics and condensed-tannin levels may alter efficiency of feed utilization (Streeter et al., 1990). Proanthocyanins (i.e., condensed tannins) are often regarded as an anti-quality characteristic because they form complexes *in vivo* with proteins and carbohydrates, thereby reducing their fermentability (Dicko et al., 2005). Conversely, they have potential to serve as biological antioxidants (Dicko et al., 2005). Over time, sorghum antioxidant activity has been increased and total condensed tannins decreased through selective breeding (Dykes et al., 2019).

There is a high correlation between total phenolic compounds and antioxidant activity (Dykes et al., 2019). Phenolic acids are derivatives of benzoic and cinnamic acids present in all cereal grains; these are located either in the outer layer of the pericarp (free phenols) or esterified to cell walls (bound phenolic acids).

The rumen of cattle contains a diverse population of microorganisms, including bacteria, protozoa, fungi, and methanogenic archaea (Streeter et al., 1990). Many of these organisms play an essential role in digestion but others produce undesired end products such as methane (a significant energy loss), or may be pathogenic to animals or humans (e.g., *E. coli* O157:H7). Our long-term goal is to identify sorghum grain cultivars, or components thereof, that can stimulate desirable microbial species to enhance efficiency of feed utilization or that can selectively inhibit undesirable microorganisms such as *E. coli* or *Salmonella*.

Objective

These studies were used to evaluate a diverse group of sorghum grains using an *in vitro* fermentation system in which a broth of mixed ruminal microbes was used as microbial inoculum. Total volume of fermentative gas generated by an *in vitro* batch culture system was utilized as an indicator of the susceptibility of the grains to microbial digestion within the rumen,

which is the principal site for starch digestion in cattle. Cultures also were characterized with respect to production of volatile organic acids, which are the principal end-products associated with microbial fermentation of carbohydrates. This profiling was deemed important because the relative proportions of organic acids produced determines energetic value of cereal grains. Finally, *in vitro* cultures were evaluated with respect to total disappearance of DM, which is another means of determining susceptibility of grains to ruminal digestion. These *in vitro* screening methods can be applied to a relatively large number of cultivars using only small amounts of the grain. The goal in this exercise is to identify cultivars that are highly susceptible to digestion by mixed ruminal microbes, thus allowing for a more targeted approach to selection of grains to be used in costlier and time-consuming *in vivo* growth experiments.

Materials and Methods

Grains and Preparation of Materials.

Experiment 1.

Twenty-four samples of grain produced in the United States were collected for comparison. The standardized corn sample was obtained from the Beef Cattle Research Center (Manhattan, KS), eleven sorghum varieties (Richardson Seeds, TX), and 12 sorghum varieties from a diversity panel (Clemson University, SC) comprised the grain sources used in these experiments. Grain samples were milled separately to a common particle size (1-mm) using a Udy Cyclone Sample Mill (Udy Corporation, CO). Thorough cleaning took place after each sample was ground to avoid cross-contamination of samples, thus maintaining properties of each individual grain source.

Experiment 2.

Seven samples of grain produced in the United States were compared. Six non-waxy endosperm sorghum cultivars, five of which were represented in experiment 1, one waxy endosperm sorghum hybrid (Scott Seed Company, TX) and the corn control were subject to analyses. Standardized corn samples were similar to those used in experiment 1. Preparation of the grain samples followed the same procedure as the previous experiment.

DM Content.

All feed ingredients were analyzed in duplicate for DM. In each case, 2-gram samples were prepared in duplicate, placed into an oven at 105°C, and left to dry for 8 to 12 hours. Samples were weighed, and DM of the original sample was calculated as:

$$(\text{Weight of dried sample} \div \text{weight of original sample}) \times 100$$

In Vitro Gas Production.

Experiment 1.

In vitro incubations were conducted in four separate runs, with each run performed on a different day. A full set of samples was prepared for each run, including a blank culture bottle (i.e., no added substrate) that was used to correct for background levels of DM and organic acids contributed by strained ruminal contents.

On each day of an *in vitro* assay, ruminal fluid was collected from two ruminally-cannulated steers fed a diet consisting of 50% roughage and 50% concentrate, strained through 4 layers of cheesecloth, and placed into a prewarmed insulated bottle. Ruminal contents were transported to the Preharvest Food Safety Laboratory. In the laboratory, the fluid was strained again through 8 layers of cheesecloth into a 1-L separatory funnel, oxygen was purged with N_2 gas, the funnel was capped, and then placed into an incubation chamber at 39°C. Within approximately 1-hour, ruminal contents stratified into 3 distinct layers (lower sediment, floating

mat layer, and middle fluid layer). The lower sediment layer was discharged from the funnel and discarded. The middle fluid layer was then collected into a pre-warmed flask for use as microbial inoculum.

Cultures consisted of 3 grams of a given cereal grain as substrate (DM basis) placed into a 250-mL screw-top bottle. To each culture bottle, 140 mL of pre-warmed McDougall's artificial saliva and 10 mL of strained ruminal contents were added. Bottles were flushed with N_2 gas, initial pH was recorded, and bottles were then capped with a pressure-sensing module (ANKOM RF Gas Production System; Ankom Technology, Macedon, NY). Culture bottles then were placed into an incubator at 39°C and agitated continuously for 24 hours. Internal pressure of each vessel was recorded at 15-minute intervals throughout the 24-hour incubation period. Terminal pH was measured after 24 hours of incubation.

Experiment 2.

In vitro incubation took place in the same manner as experiment 1 with small differences. This experiment was performed in a singular run with four replications for each sample. Incubation time was extended to 30 hours, allowing gas production to plateau.

Volatile Fatty Acids (VFA).

To quantify volatile fatty acids (VFA), 4 mL of the fluid layer from each culture bottle were removed and combined with 1 mL of a 25% (w/v) solution of metaphosphoric acid in a scintillation vial. Vials were frozen (-20°C) to facilitate deproteinization. Samples were then thawed and homogenized using a vortex mixer. A 2-mL aliquot of the fluid was transferred by pipette into a 2-mL micro-centrifuge tube and centrifuged for 15 minutes at 14,000 × *g*. Supernatant (1.5 mL) was transferred by pipette to a gas chromatography vial and sealed. Concentrations of volatile fatty acids were determined using an Agilent 7890A gas

chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a flame-ionization detector. A capillary column (Nukol 15mm × 0.5mm × 0.5μm; Supelco Analytical, Bellefonte, PA) was used with hydrogen as the carrier gas. Concentrations of acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, isocaproate, caproate, and heptanoate were measured. Results were expressed as millimoles of acid produced per gram of substrate, using blank tubes to correct for background concentrations of volatile fatty acids contributed by the microbial inoculum.

In Vitro Dry Matter Disappearance (IVDMD).

The remaining contents of each culture bottle were carefully transferred to shallow aluminum pans and weighed. Pans were then placed into an oven at 105°C and left until all moisture evaporated (3 to 4 days). Pans containing dried residues were then weighed; DM disappearance was calculated as:

$$[1 - (\text{dry residue weight} \div \text{dry weight of initial sample})] \times 100$$

Dry residue weight was corrected for background amounts of residue contributed by the microbial inoculum (i.e., weight of dried culture – weight of dried residue in blank).

Statistical Analyses.

For statistical analyses of pH, DM disappearance, and volatile fatty acid production, the MIXED models procedure of the Statistical Analysis System (SAS Inst. Inc., Cary, NC) was used. The fixed effect was cultivar and the random effect was replicate (i.e., run). Least-squares means were separated using the PDIFF function. Cultivar effects were deemed significant at *P*-values less than 0.05.

Model components for gas production included fixed effects of cultivar, time, and time × cultivar. Time was treated as a repeated measure and compound symmetry were used as the

covariance structure. The slice option was used to assess differences among cultivars at 0, 6, 12, 18, and 24 hours. Cultivar effects were deemed significant at *P*-values less than 0.05.

Results and Discussion

In Vitro Gas Production.

Phenolic compounds found with different sorghum cultivars found are of interest for intervarietal comparison with differences in reactivity exist among phenolic compounds of certain classes (Dicko et al., 2005). In experiment 1, total gas production was used to indicate fermentative activity of the culture. Treatment × time was analyzed for 6-hour intervals. Figure 2.1 illustrates cumulative gas production for each of the 24 cereal grains. No significant differences among cultivars were observed after 6 hours of incubation. By hour 12, however, there were measurable differences among cultivars and these widened after 18 or 24 hours of incubation. At 24 hours of incubation (Figures 2.1 and 2.2), there was a significant cultivar × time effect ($P < 0.0001$).

Several cultivars yielded greater amounts of fermentative gases than the corn control, in contrast with previous reports. The major nutritional concern for phenolic acids is their ability to bind to large proteins and proline-rich proteins, causing reductions in total cultivar digestibility (Dicko et al., 2005). Perhaps the reason for greater digestibility than expected was reported by Dicko et al. (2005) where the free-radical scavenging activity of phenolic acids. This capability may have allowed them to degrade in the digestive process into low molecular weight monomers allowing proteins to remain uncomplexed.

Lower values for terminal pH of cultivars should be indicative of increased microbial digestion of grains. Terminal pH ranged from 6.18 for SAP-134 to 6.57 with R.96. As shown in

Figure 2.3, large differences among cultivars were evident ($P < 0.0001$). Overall gas production observed in both experiments also varied substantially among cultivars.

For experiment 2, total gas production for each of the 7 cereal grains is depicted in figure 2.5. No significant differences were found among sorghum varieties after 6 hours of incubation; however, differences among cultivars were evident at later time points. After 30 hours of incubation (Figures 2.4 and 2.5), there was a significant cultivar × time effect ($P < 0.0001$). Greater gas production and starch digestion were noted with waxy endosperm varieties compared with non-waxy endosperm sorghum. This may be due to greater availability of amylopectin than amylose to enzymatic and microbial attack or to a greater solubility of the protein matrix surrounding the waxy starch (Streeter et al., 1990). *In vivo* experiments have provided further evidence of improved digestibility of waxy endosperm varieties, which were observed to have greater fractional starch digestion in the small intestine of heifers (Streeter at al., 1990).

As mentioned before, pH changes support gas production results observed during incubation. In the second experiment, terminal pH ranged from 6.06 for Waxy to 6.30 with Sumac. Figure 2.6 depicts differences ($P < 0.0001$) in terminal pH among sorghum varieties.

It has been found that sorghum prolamins form more intermolecular linkages and complex structures than their corn counterparts (Ezeogu et al., 2005). Increased gas production noted for some sorghum cultivar varieties suggests potential exists for researchers to isolate cultivars with disulphide cross-linkages that reduce significantly over time. This should be associated with improvements in starch digestion. It would make sense that incubation would allow greater starch accessibility to amylase by reducing disulphide-bonded polymerization of the prolamin proteins (Ezeogu et al., 2005).

Volatile Fatty Acid Production.

In experiment 1, we observed that volatile fatty acid production varied markedly among cultivars. Tables 2.1 summarize the VFA profiles resulting from *in vitro* fermentation of 24 different cereal grains. Effects of grain source were noted for acetate, propionate, and total VFA production ($P < 0.01$). We observed no significant effects of cultivar on amounts of minor volatile fatty acids produced (i.e., isobutyrate, isovalerate, valerate, caproate, isocaproate, and heptanoate). Caproate, isocaproate, and heptanoate were not usually present in detectable amounts.

In vitro production of VFAs is the consequence of microbial degradation of carbohydrates; it is a reasonable proxy for the ruminal digestion of grains. Greater *in vitro* production of volatile fatty acids is consistent with greater susceptibility of grains to ruminal digestion. Likewise, extensive ruminal digestion generally is consistent with improved animal performance due to limitation in post-ruminal amylase secretion in ruminants. Consequently, one would anticipate that cultivars with extensive *in vitro* digestion would promote superior animal performance. It is noteworthy that more than half of the sorghum cultivars used in our comparisons had VFA yields that were superior to corn.

The ratio acetate:propionate is a useful measure of energetic efficiency, as metabolic pathways that lead to greater proportions of propionate reflect greater ATP yield. Conversely, fermentations that yield greater amounts of acetate are associated with increased production of methane, a source of energy loss. The cultivars evaluated in this study produced a wide range of A:P ratios. Measurement of methane was outside the scope of this experiment, but follow-up experiments were done to determine if the large differences in A:P ratio observed herein are commensurate with changes in methane production.

Experiment 2 also evaluated volatile fatty acid profile of cultures from the seven grains under investigation. Table 2.2 summarizes VFA profiles for the cultures after 30 hours of *in vitro* incubation. Grain type influenced acetate ($P < 0.0375$), propionate ($P < 0.0007$), butyrate ($P < 0.0001$), and acetate:propionate ($P < 0.0001$); moreover, total VFA production tended ($P < 0.0588$) to be influenced by grain type. No significant effects on concentrations of minor VFAs were observed. Isobutyrate, caproate, isocaproate, and heptanoate were not present in detectable amounts. All sorghum varieties, with the exception of Sumac, had greater VFA production than corn.

High grain diets typically result in greater proportions of propionate at the expense of acetate production (Sudweeks et al., 1998). In our second study, the ratios of acetate to propionate were unexpectedly different from those observed in experiment 1. Perhaps extending the incubation period by 6 hours allowed for metabolism of fibrous grain components that yielded more acetate. Alternatively, portions of the propionate were further metabolized to yield other compounds.

In vitro cultures with ruminal microbes fed waxy, 304x5, 341x120, and 366x58 sorghum varieties had greater ruminal butyric acid concentrations when compared to *in vitro* cultures fed corn. This may be indicative of an increase in formation of soluble proteins during the anaerobic phase of reconstitution (Simpson et al., 1985). The relatively poor performance of Sumac grain in both experiments may be due to greater condensed-tannin content, which is known to depress digestibility, making it an inferior option as an energy source in most cattle feeding systems.

In Vitro Dry Matter Disappearance (IVDMD).

Disappearance of dry matter from culture vessels also can be used as a measure of the susceptibility of grains to microbial digestion. Starches are fermented by ruminal microbes,

yielding volatile organic acids that are removed by evaporation when cultures are oven-dried in the oven at 105°C. Weight of the resulting culture residue consists of microbial cells, the proportion of which is assumed to be constant across culture vessels, and undigested sample residue. Disappearance of DM from the *in vitro* system is a reasonable proxy for *in vivo* ruminal digestion, and normally relates closely to gas production. As fermentation proceeds, fermentative gasses are generated, though not all gasses emitted from the cultures are produced by the microorganisms. Figure 2.7 illustrates that disappearance of DM from cultures varied substantially among the hybrids in experiment 1, again suggesting there is opportunity to identify hybrids that are better suited to cattle feeding than others. Within experiment 1, inconsistencies exist between gas production and DM disappearance. Isolating sorghum varieties in experiment 2 suggest 341x120, 304x5, and waxy cultivars fed to ruminal microbes resulted in greater dry matter disappearance during the incubation period as seen in figure 2.8.

Tannin binding of protein enhances tryptic digestion of specific proteins, presumably by causing conformational changes to denature the protein. Streeter et al. (1990) suggest that denaturation of the protein matrix surrounding sorghum starch granules in the peripheral endosperm may increase digestion of peripheral endosperm starch in the rumen. Couple this with gas production and it appears there are sorghum varieties with fermentative capabilities greater than corn, leaving merit for an *in vivo* trial to further support the findings of these assays.

Conclusion

Based on these *in vitro* studies, it is evident that substantial diversity exists among sorghum cultivars with respect to their susceptibility to digestion by mixed ruminal microbes. In addition, many of the cultivars evaluated had fermentation characteristics that were comparable or superior to those of the corn. Sorghum grain appeared to increase gas production, total volatile

organic acid content, and dry matter disappearance for numerous cultivars when compared to corn. Waxy endosperm sorghum varieties appeared to have superior fermentability in comparison with the other grains evaluated, with further research needed to explain its acetate to propionate ratio. We concluded that potential exists for the development of sorghum hybrids that can compete with corn as energy sources, whilst still contributing toward improved sustainability of agricultural systems in water-stressed environments.

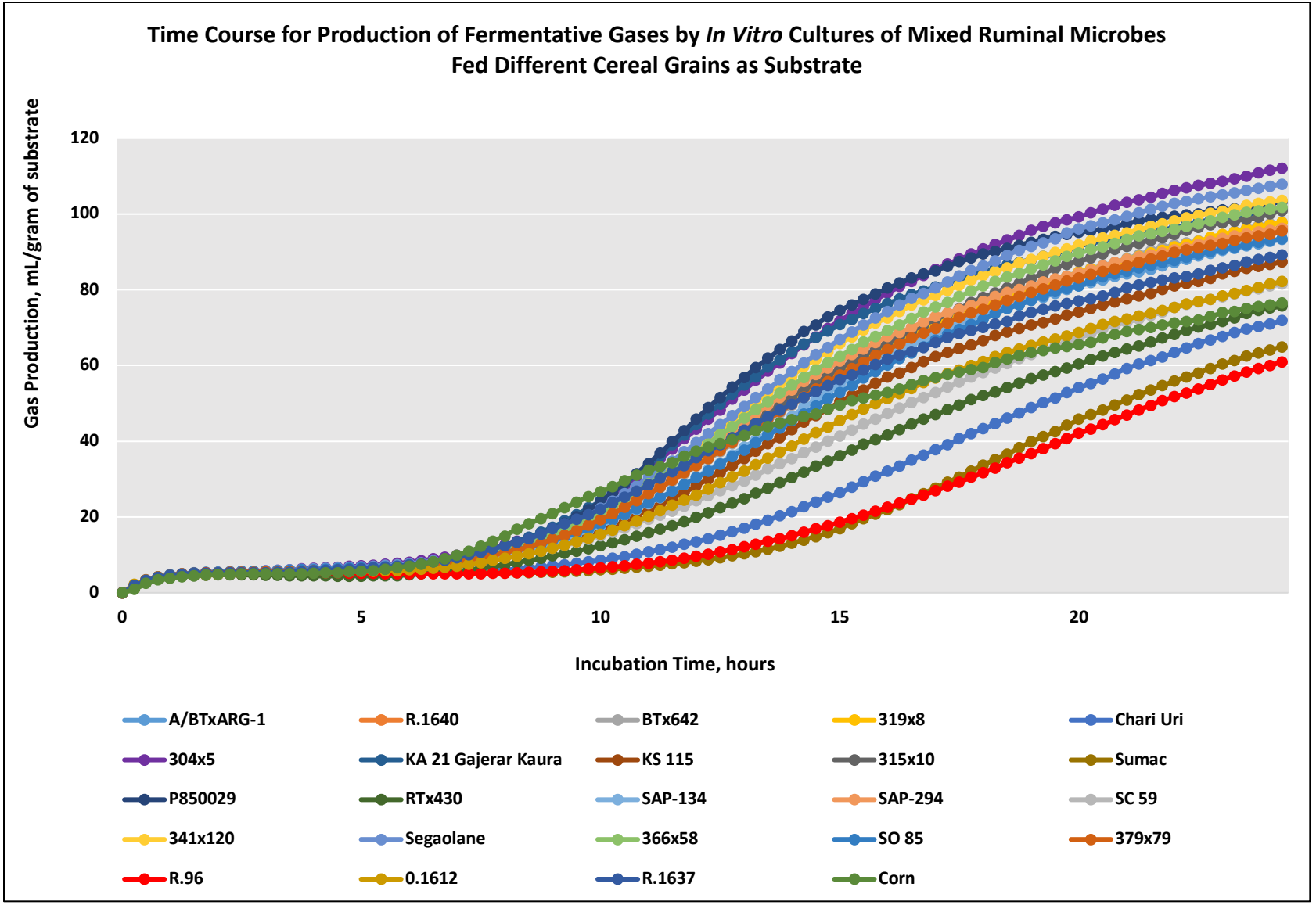

Figure 2.1: Experiment 1 - Effect of cultivar on in vitro gas production by mixed ruminal microbes. SEM: 1.2; P < 0.0001

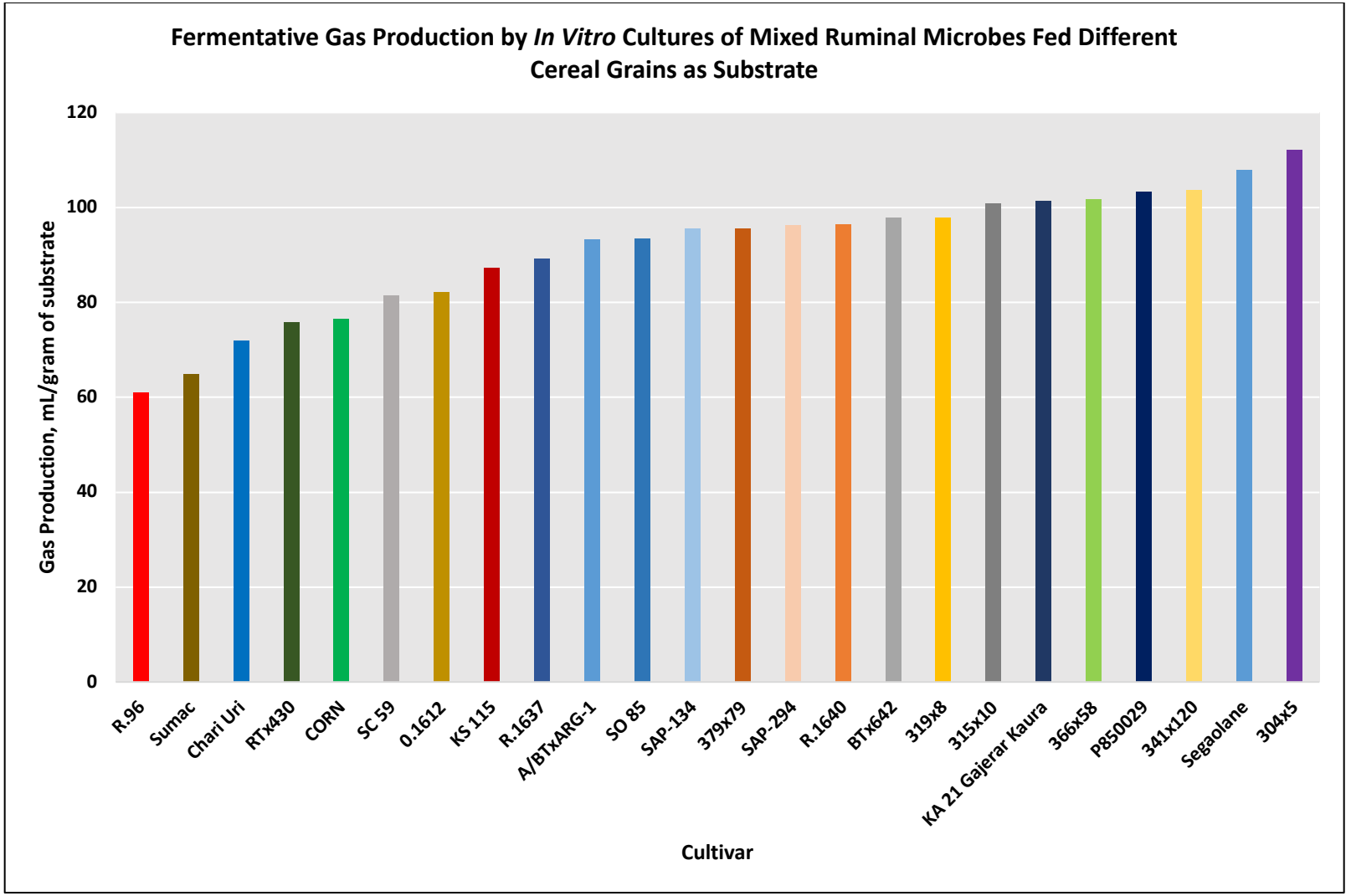

Figure 2.2: Experiment 1 – Total gas production by in vitro cultures of mixed ruminal microbes after 24-hour incubation period. SEM: 1.2; $P < 0.0001$.

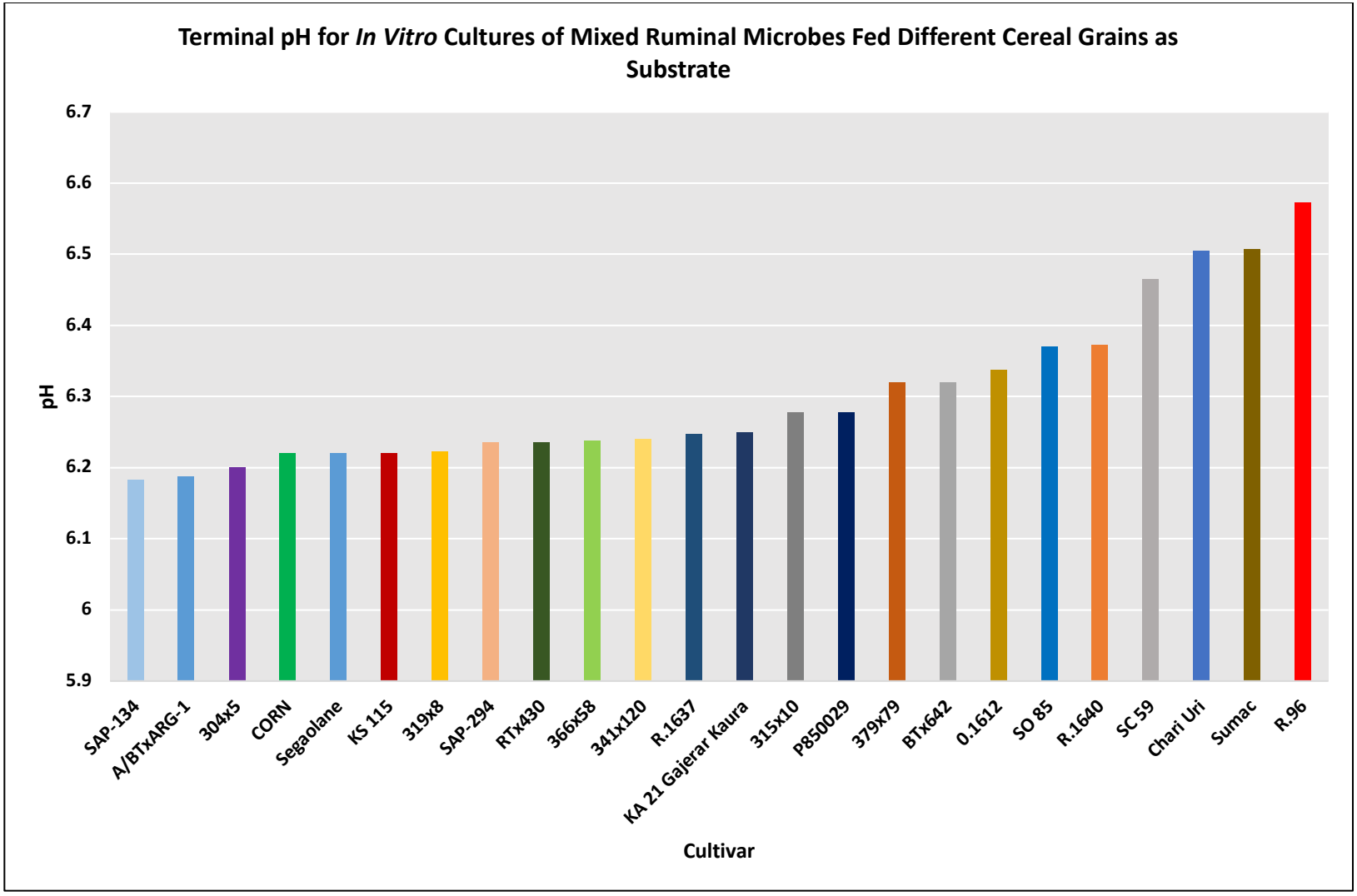

Figure 2.3: pH measurements from in vitro cultures taken after incubating grains with mixed ruminal microbes for 24 hours. SEM: 0.0961; P <0.0001.

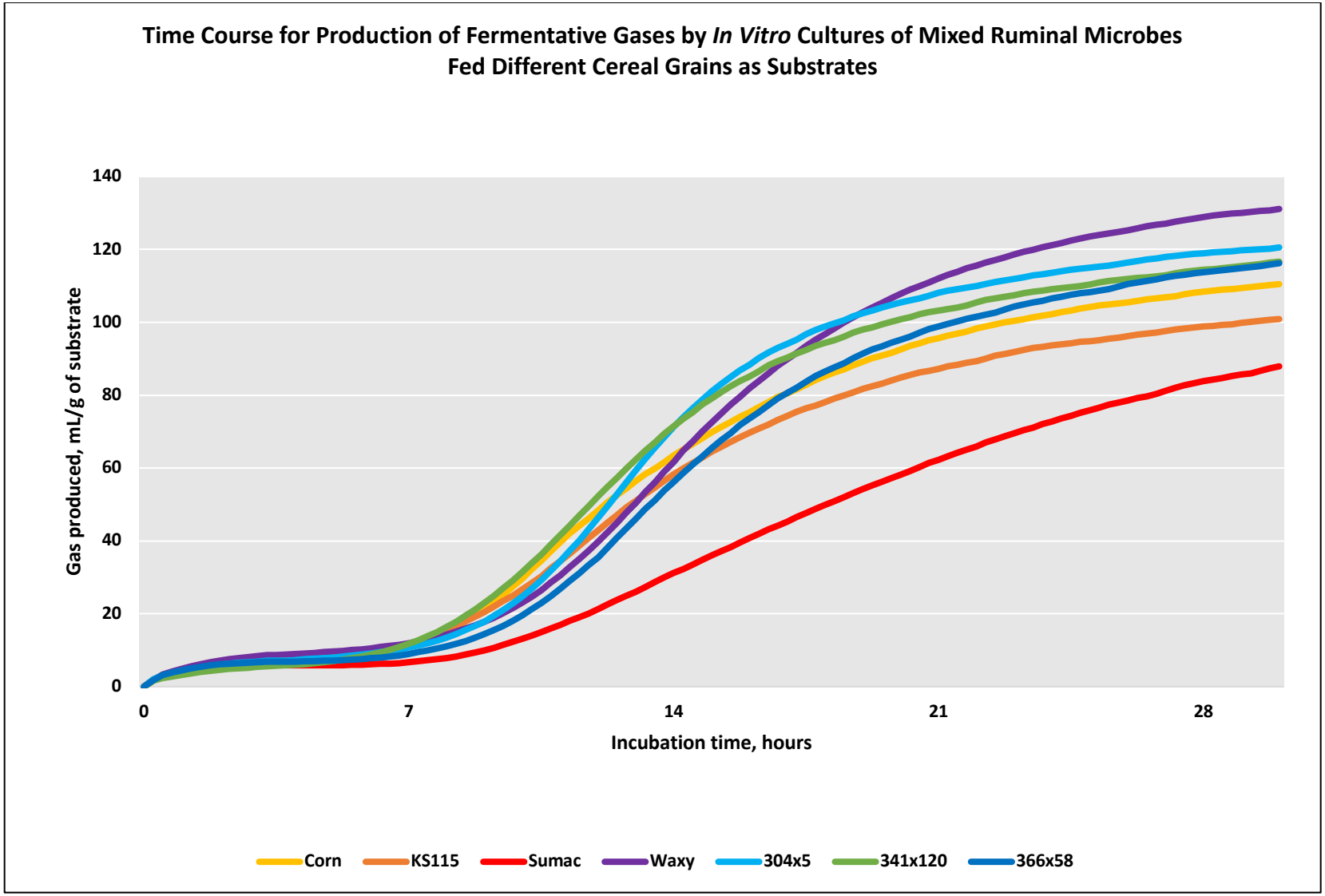

Figure 2.4: Experiment 2 – Effect of cultivar on in vitro gas production by mixed ruminal microbes through 30 hours. SEM: 1.1984; P < 0.0001.

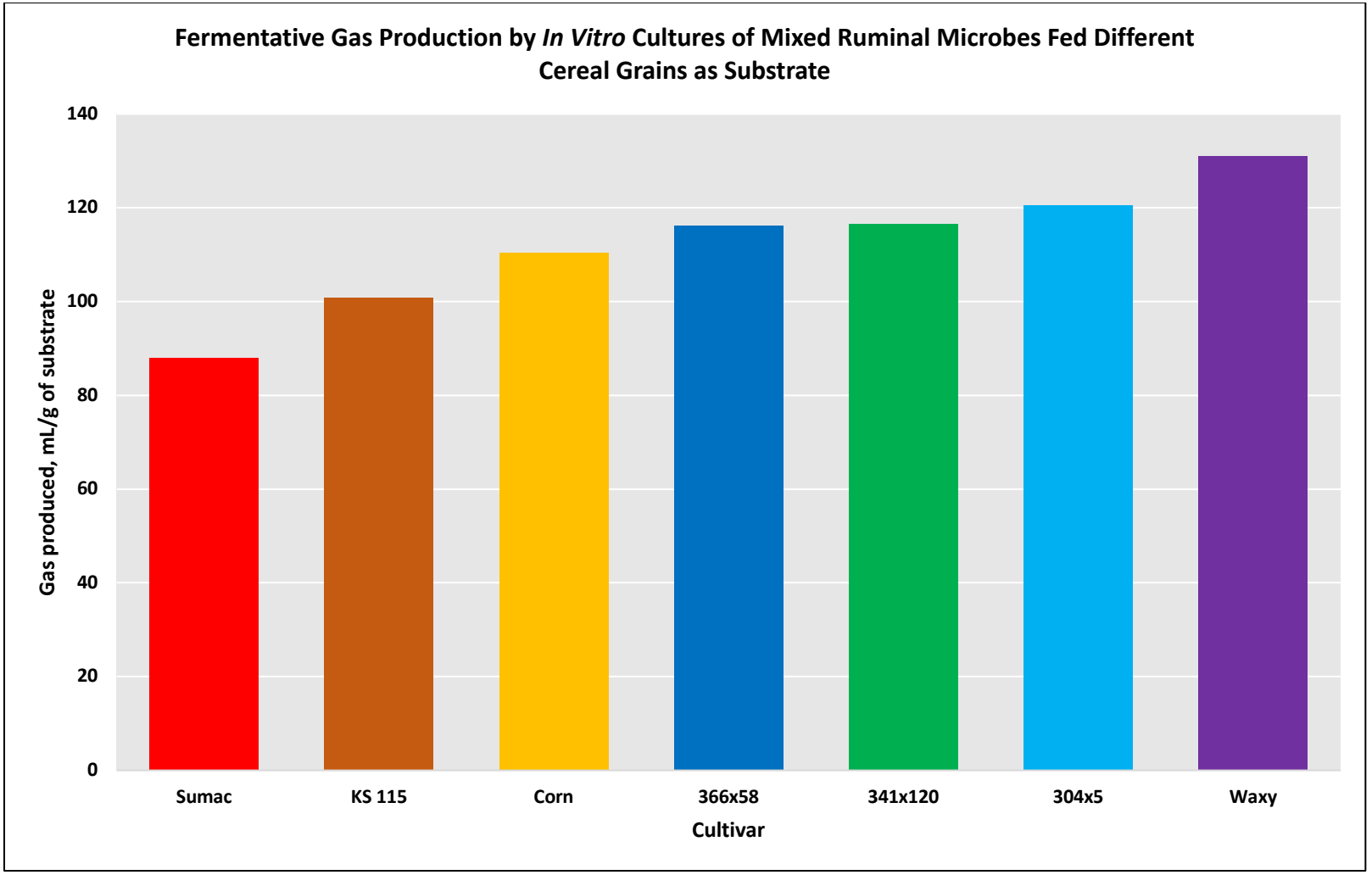

Figure 2.5: Experiment 2 – Total gas production by in vitro cultures of mixed ruminal microbes after 30-hour incubation. SEM: 1.1984; P < 0.0001.

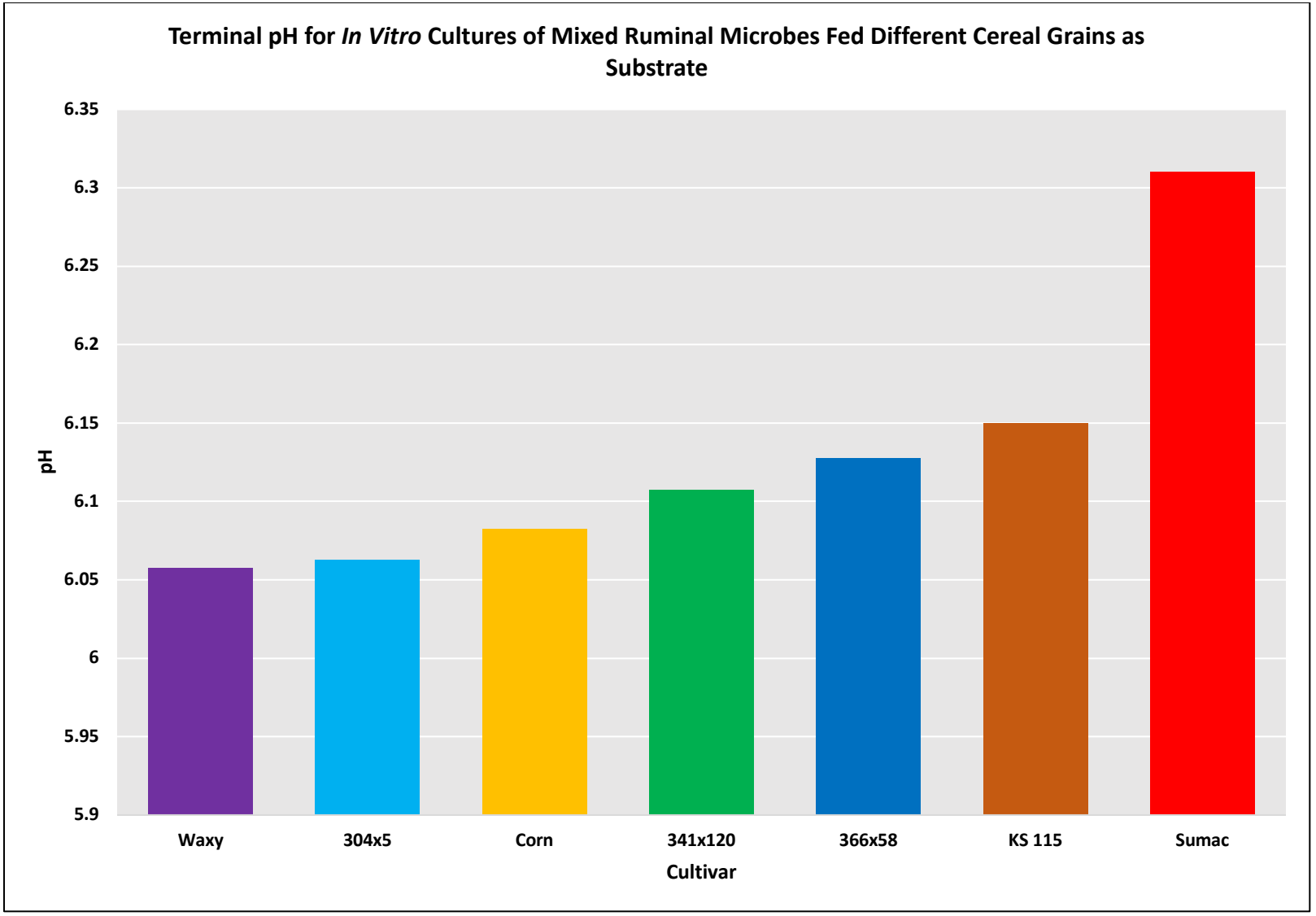

Figure 2.6: Experiment 2 – pH measurements taken from in vitro cultures taken after incubating grains with mixed ruminal microbes for 30 hours. SEM: 0.01357; $P < 0.0001$.

	mmoles VFA/g grain				
Cultivar	**Total VFA**	**Acetate**	**Propionate**	**Butyrate**	**Acet:Prop**
R.96	**2.61**	**1.20**	**1.06**	**0.34**	**1.251**
Sumac	**2.71**	**1.25**	**1.21**	**0.25**	**1.192**
Chari Uri	**3.01**	**1.33**	**1.36**	**0.31**	**1.028**
SC 59	**3.44**	**1.46**	**1.56**	**0.40**	**0.971**
Corn	**3.56**	**1.21**	**2.10**	**0.22**	**0.672**
SO 85	**3.76**	**1.45**	**1.82**	**0.45**	**0.784**
315x10	**3.77**	**1.61**	**1.64**	**0.49**	**0.986**
0.1612	**3.78**	**1.45**	**2.10**	**0.20**	**0.711**
319x8	**3.89**	**1.51**	**2.05**	**0.29**	**0.736**
P850029	**3.94**	**1.54**	**2.04**	**0.33**	**0.711**
BTx642	**4.10**	**1.63**	**1.99**	**0.44**	**0.829**
379x79	**4.16**	**1.67**	**2.02**	**0.43**	**0.846**
341x120	**4.22**	**1.70**	**1.92**	**0.55**	**0.851**
304x5	**4.23**	**1.79**	**1.81**	**0.57**	**1.012**
Segaolane	**4.31**	**1.79**	**1.98**	**0.47**	**0.893**
RTx430	**4.33**	**1.54**	**2.66**	**0.10**	**0.557**
A/BTxARG-1	**4.34**	**1.62**	**2.44**	**0.21**	**0.668**
SAP-294	**4.38**	**1.70**	**2.20**	**0.40**	**0.738**
366x58	**4.39**	**1.87**	**1.98**	**0.49**	**0.958**
R.1637	**4.45**	**1.70**	**2.52**	**0.20**	**0.679**
R.1640	**4.60**	**2.04**	**1.98**	**0.54**	**1.044**
SAP-134	**4.63**	**1.72**	**2.62**	**0.24**	**0.659**
KA 21 Gajerar Kaura	**4.81**	**1.90**	**2.53**	**0.34**	**0.713**
KS 115	**4.88**	**1.79**	**2.85**	**0.18**	**0.635**
SEM	**0.839**	**0.488**	**0.439**	**0.088**	**0.175**
***P* <**	**0.0001**	**0.1039**	**0.0001**	**0.0001**	**0.0001**

Table 2.1: Experiment 1 - Effect of sorghum variety on volatile fatty acid production by *in vitro* cultures of mixed ruminal microbes

	mmoles VFA/g grain				
Cultivar	**Total VFA**	**Acetate**	**Propionate**	**Butyrate**	**Acet:Prop**
Sumac	**11.62**	**5.93**	**5.14**	**0.55**	**1.148**
Corn	**12.37**	**5.52**	**5.75**	**1.05**	**0.954**
366x58	**13.68**	**6.32**	**5.77**	**1.59**	**1.083**
304x5	**13.98**	**6.56**	**5.44**	**1.94**	**1.201**
Waxy	**14.84**	**8.18**	**4.82**	**1.77**	**1.693**
341x120	**15.32**	**7.43**	**6.10**	**1.72**	**1.215**
KS115	**15.81**	**7.24**	**7.56**	**0.89**	**0.957**
SEM	**1.012**	**0.578**	**0.357**	**0.123**	**0.037**
***P* <**	**0.0588**	**0.0375**	**0.0007**	**0.0001**	**0.0001**

Table 2.2: Experiment 2 – Effect of sorghum variety on volatile fatty acid production by in vitro cultures of mixed ruminal microbes

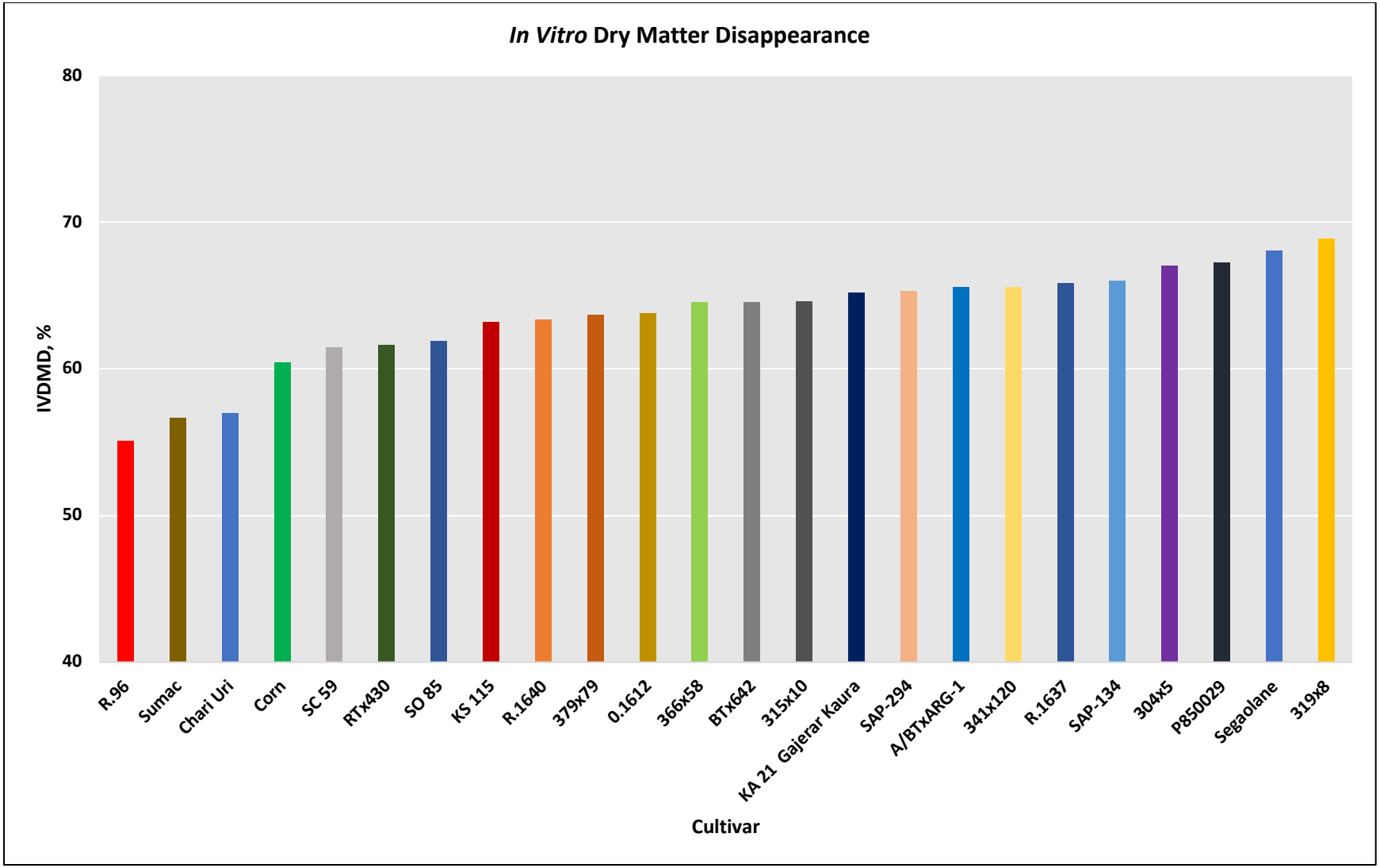

Figure 2.7: Experiment 1 – In vitro dry matter disappearance from cultures taken after incubating grains with mixed ruminal microbes for 24 hours. SEM: 1.0363; $P < 0.01$.

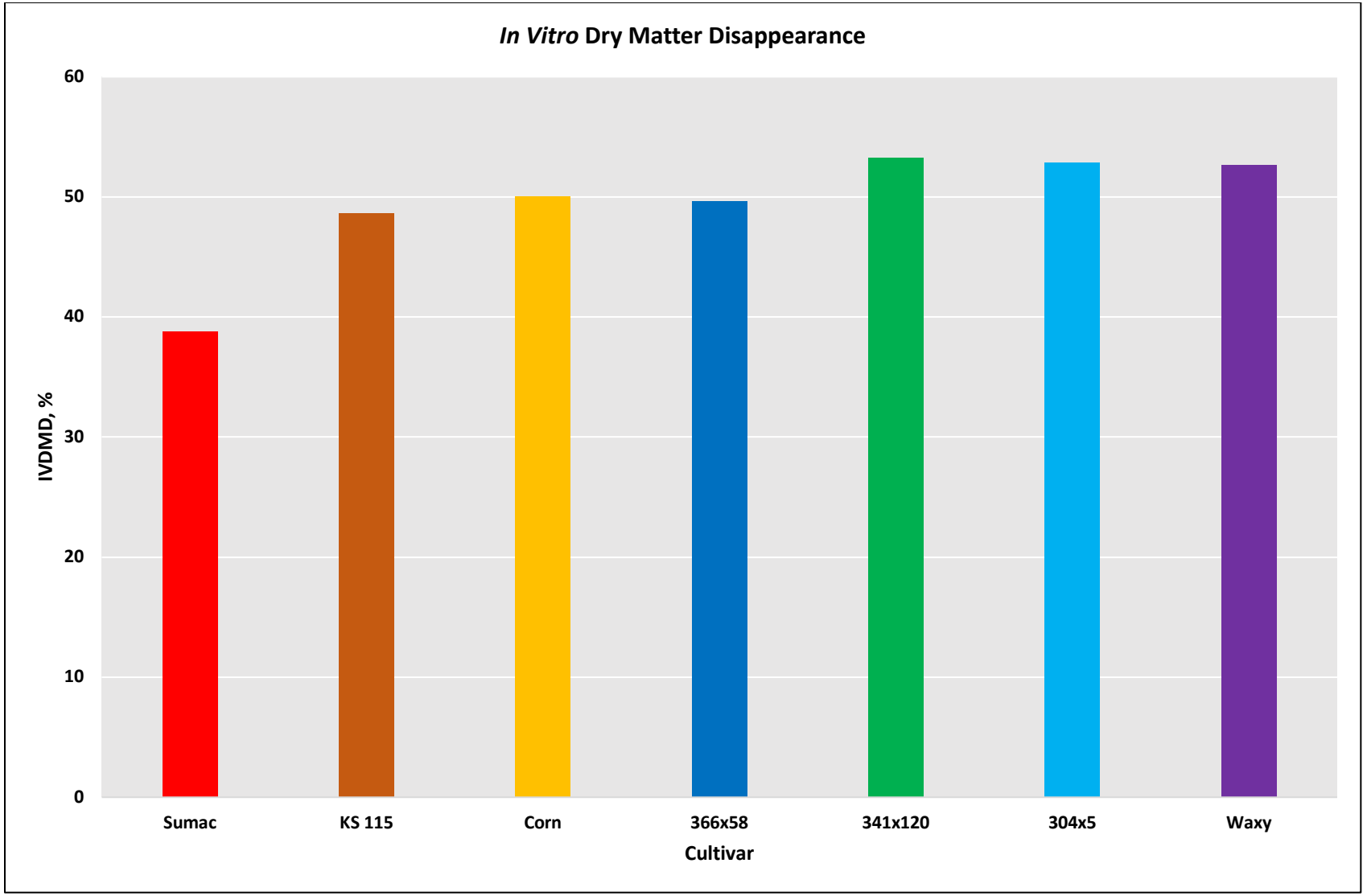

Figure 2.8: Experiment 2 – In vitro dry matter disappearance from cultures taken after incubating grains with mixed ruminal microbes for 30 hours. SEM: 1.0737; $P < 0.0001$.

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Chapter 3 – Backgrounding Cattle Reveal Potential For Sorghum Utilization in Confinement Feeding Systems

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Abstract

Sorghum grains constitute a relatively small proportion of grains utilized in feedlots, primarily as a consequence of increased processing cost and suboptimal animal performance when compared to corn. As a follow up to *in vitro* experiments previously conducted to characterize digestion of sorghum cultivars, a 60-day *in vivo* backgrounding study was conducted to assess performance of crossbred steers (n = 120; initial BW 273.08 kg ± 2.94) fed diets containing 40% (dry matter basis) of dry rolled corn, ground 341x120 sorghum hybrid, or a ground waxy sorghum. Animals were blocked by initial BW and assigned randomly (40 head each) to 1 of 3 pens. Within pens, animals were individually and randomly assigned to a dietary treatment. Pens were equipped with Hokofarm RIC Individual Feeding Bunks (Hokofarm, Netherlands), allowing individual feeding of treatment diets. Average daily gain, dry matter intake, and efficiency of gain were analyzed using the MIXED procedure of SAS (version 9.1; SAS Inst. Inc., Cary, NC), with treatment as the fixed effect, block as the random effect, and animal as the experimental unit. Feeding ground sorghum-based diets to backgrounding steers increased DMI by 0.51 kg/d ± 0.18 ($P < 0.04$) when compared to cattle fed the dry-rolled corn diet. Despite increases in DMI when utilizing sorghum grains, G:F was not influenced by grain source ($P = 0.36$). Average daily gain was greater ($P < 0.005$) for steers fed waxy sorghum compared to corn. When compared to steers fed corn, final body weights were also greater for cattle fed waxy sorghum ($P < 0.003$), with cattle fed 341x120 sorghum being intermediate and not different from other treatments ($P = 0.12$). Sorghum cultivars used in this experiment promoted cattle performance that was equal to or superior to that of corn.

Introduction

From 1950 to 1999 the area of sorghum planted worldwide increased 71%, during that time period, sorghum yields increased 160% (Maunder, 2002). In 2015, 63.5 million metric tons were produced worldwide (Mundia et al., 2019). Approximately 5.7 million acres of sorghum were planted in the Sorghum Belt (Kansas, Texas, Colorado, Nebraska, Oklahoma, and South Dakota) in 2018 (Sorghum checkoff, 2019); however, utilization of the crop still is relatively light in commercial feedlots. In western Kansas, Norwood and Currie (2013) found that sorghum grain yielded 137% more crop than corn when utilizing conventional tillage practices and 85% more than corn with "no-till" practices. Sorghum is among the most efficient crops available in terms of water use and biomass production (Sorghum checkoff, 2019). While sorghum grain has undergone extensive genetic manipulation in recent years, relatively few cattle feeding experiments have been conducted with the resulting varieties. Current dogma pertaining to inferiority of sorghum as a feed grain likely is impacted by historical use of cultivars containing elevated concentrations of tannins. Larraín et al. (2009) found that final BW ($P < 0.01$), ADG ($P < 0.001$), and G:F ($P < 0.01$) were all reduced in feedlot steers when fed high tannin sorghum versus corn. The current study utilized two cultivars of sorghum that were identified from a panel of 24 cultivars through an *in vitro* screening project as having superior digestibility characteristics. Rooney and Pflugfelder (1986) suggested disruption of the endosperm protein matrix is critical to maximizing starch utilization in sorghum grain. Stock (1999) indicated that many commercial hybrids are available for relatively short periods of time, thus making it challenging to identify hybrids suited for use in feeding cattle. With that in mind, it becomes timely to generate studies, such as this, which identify parental lines or specific cultivars which have potential for feeding value in cattle production systems.

Materials and Methods

Animals and Sampling:

Kansas State University Institutional Animal Care and Use committee approved the procedures utilized in this study.

The 60-day backgrounding study took place at the Kansas State University Beef Cattle Research Center, in Manhattan, KS. One hundred and twenty crossbred steers (initial BW = 273.08 kg ± 2.94) were stratified by initial body weight, and then randomly allocated within strata to three dietary treatments. Treatments were based on diets consisting of two different ground sorghum cultivars (341x120 or waxy; mean geometric particle size < 1000 microns) or dry rolled corn (~ 4000 micron). Diet compositions are shown in table 3.1. Steers were equipped with RFID ear tags used with the automated feeding system. Cattle were then randomly assigned to partially covered feeding pens equipped with floors that were one-third concrete and two-thirds dirt. Pens were equipped with automatic water fountains and RIC (Roughage Intake Control) Individual Feeding Bunks (Hokofarm, Netherlands). Steers were fed once daily *ad libitum* (40 animals/treatment). Body weights were measured on days 0, 30, and 60, and feed intakes were monitored daily utilizing the Hokofarm RIC Feeding System. Feed samples were collected weekly and dried at 55°C for 48 h then analyzed for DM, starch, sulfur, CP, NDF, ADF, Ca, P, and K. Portions of these samples were dried using a forced air oven (105°C) for 24 h to determine DM. The remainder of the samples were composited monthly and sent to SDK Laboratories (Hutchinson, KS) for the measurement of starch, CP, NDF, ADF, sulfur, Ca, P, and K.

Average daily gains were calculated by subtracting initial body weight from the final body weight, and then dividing that value by days on feed (DOF). Efficiencies were estimated as ADG divided by the DM intake (DMI).

Statistical Analyses

Animal performance measurements were analyzed using the MIXED procedure of SAS (version 9.1; SAS Inst. Inc., Cary, NC). Animal was used as the experimental unit, with a model including treatment as a fixed effect and block as a random effect. LSMEANS statements were used for calculations of means and standard errors, and the PDIFF function was used to separate means. Means were declared different at $P < 0.05$.

Results and Discussion

One animal from the corn treatment was removed from the study due to reoccurring respiratory disease. The steer started displaying symptoms during the third week of the trial.

Performance data are presented in table 3.2. Initial BW was not different between treatments ($P = 0.24$). Final BWs were 355, 362, and 369 kg for corn, 341x120, and waxy treatments, respectively. Final BW was greater for cattle fed waxy sorghum compared to those fed corn ($P < 0.003$), while cattle fed 341x120 were intermediate and not different from other treatments ($P < 0.10$). Comparatively, Gaebe et al. (1998) observed no differences in final BW for a study which cattle were fed either dry rolled corn or dry rolled grain sorghum, yet BW gain was increased 12.1% for cattle fed corn-based diets ($P < 0.10$). Similarly, Larraín et al. (2009) reported that cattle fed corn-based diets had greater final BW compared to those fed a high-tannin sorghum ($P < 0.01$). Sorghum cultivars used in our experiment are known to contain low concentrations of tannins.

Figure 3.1 illustrates changes in DMI over the entire feeding period. Compared to feeding corn-based diets, feeding ground sorghum-based diets to backgrounding steers increased DMI by 7.85% for the 341x120 cultivar ($P < 0.01$), and by 5.80% for the waxy cultivar ($P = 0.07$). Consistent with our observations, Owens et al. (1997) reported a 10.8% increase in DMI for cattle fed sorghum-based diets compared to those fed corn. Despite the increases in DMI, G:F was not influenced by grain treatment ($P = 0.36$), though ADG increased for cattle fed waxy sorghum or the 341x120 hybrid when compared to those fed corn ($P < 0.001$). We believe a possibility exists for differences in gut fill to attribute to differences in feed intake. By steers consuming greater amount of dry matter when comparing the sorghum-based diets to corn-based diets, they likely experienced greater amounts of gut fill. If the case, final BW values could be reflecting differences in animal fill versus actual gain. Moreover, if true, this would cause G:F values to be different.

Larraín et al. (2009) reported that DMI was not influenced by grain type. Perhaps this was a result of utilizing a high-tannin sorghum cultivar versus the low tannin varieties used in the present study. Conversely, Owens et al. (1997) compared dry rolled corn and sorghum in diets with corn silage and alfalfa as the roughages and observed greater ADG for cattle fed sorghum compared to those fed corn. Schake et al. (1976) also found that DMI was elevated slightly by feeding sorghum. Also in contrast to our results, Gaebe et al. (1998) observed an increase in ADG for cattle fed dry rolled corn over those fed dry rolled sorghum in a study comparing the effects of extruding versus dry rolling corn or grain sorghum ($P < 0.05$). Richard and Hicks (2007) found that total tract starch digestibility of dry rolled grains increased by 8.06% when feeding sorghum compared to corn. This was attributed to the slower passage rate of the sorghum-based diet. These researchers also reported that feed efficiency was greater for dry

rolled corn diets than dry rolled sorghum diets. This may help us understand the ability of steers in this study to generate higher ADG when fed grain sorghum over corn with indifferent feed efficiency. Contradicting our findings, it has been found feeding dry rolled corn improves feed efficiency over dry rolled sorghum by 13.09% (Richards and Hicks, 2007). In a study designed to compared dry-rolled and micronized sorghum grain, Croka and Wagner (1975) found no differences ($P > 0.05$) in feed efficiency, gain, or feed intake.

Some research in the comparison of corn and sorghum comes in the context of steam-flaking the grains. Owens et al. (1997) found that flaking of corn and sorghum increased the metabolizable energy contents by 15 and 21%, respectively. Knowing that extensive processing benefits sorghum grains leads me to question if the steam-flaking process could enhance the feeding capabilities of 341x120 hybrid or waxy sorghum.

Utilizing *in situ* procedures, McAllister et al. (1990) found that once sectioned, sorghum grains were more vulnerable to microbial digestion than corn. After 24 hours of ruminal exposure, sorghum endosperm was colonized by a wider variety of ruminal bacteria than corn (McAllister et al., 1990). This offers some insight as to why sorghum cultivars in our experiment were associated with increased ADG, while sharing equal feed efficiencies with corn. Possible explanations for this would be the conceivable differences in the associative effects of the grain. For instance, if corn simply ferments more rapidly, it is feasible that its impact on fiber digestion is greater than that of the slower digesting sorghum grains. This could help explain the potential that gut fill may have played a role in final BW differences. To ensure our results a finishing trial is needed to verify differences observed in this study, and to confirm the "live-weight" differences were not artifact.

Conclusion

Sorghum cultivars used in this experiment were selected from a group of 24 disparate cultivars that demonstrated a wide range of susceptibility to *in vitro* digestion by mixed ruminal microorganisms. Though sorghum commonly is regarded as being inferior to corn, results of this study provide evidence to suggest some sorghum cultivars can be competitive with corn as energy sources for growing cattle.

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Table 3.1: Diet Composition, DM basis

	%, DM Basis		
Ingredients	**Corn**	**341x120 sorghum**	**Waxy sorghum**
Corn silage	26.00	26.00	26.00
Alfalfa hay	30.00	30.00	30.00
Dry rolled corn	40.00	0.00	0.00
Waxy sorghum, ground	0.00	0.00	40.00
341x120 sorghum, ground	0.00	40.00	0.00
Supplement	4.00	4.00	4.00
Soybean meal, dehulled	1.34	1.34	1.34
Corn, ground	1.65	1.65	1.65
Urea	0.41	0.41	0.41
Limestone	0.29	0.29	0.29
Salt	0.25	0.25	0.25
Trace mineral premix	0.04	0.04	0.04
Vitamin A premix	0.007	0.007	0.007
Rumensin 90	0.02	0.02	0.02

Table 3.2: Impact of diet on animal performance during a backgrounding phase.

	Grain Source				
Item	**Corn**	**341x120**	**Waxy**	***P* <**	**SEM**
No. animals	39	40	40	-	-
Initial BW, kg	272^{a}	271^{a}	276^{a}	0.2399	12.8
Final BW, kg	355^{a}	362^{ab}	369^{b}	0.0141	13.7
DMI, kg/d	8.79^{a}	9.48^{b}	9.30^{ab}	0.0397	0.245
ADG, kg	1.37^{a}	1.52^{b}	1.55^{b}	0.0055	0.041
Gain:Feed	0.1589^{a}	0.1626^{a}	0.1701^{a}	0.3585	0.0059

^{ab}Different superscripts within row represent significant differences $P < 0.05$.

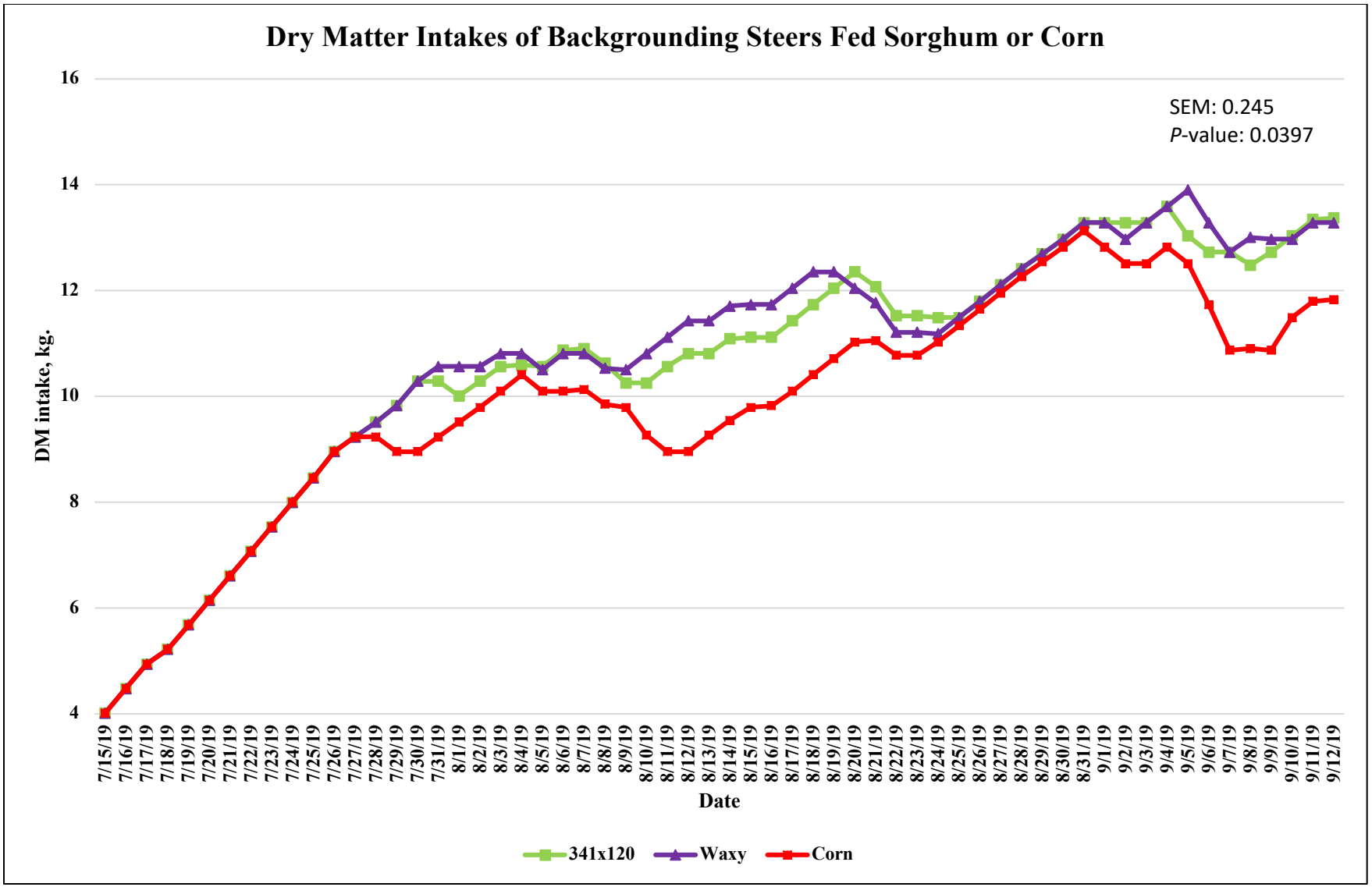

Figure 3.1: Dry matter intakes over the 60-day trial period

Chapter 4 – Literature Cited

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