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Nutritional Quality of Maize Ensiled with Wet Distillers Grains for Sheep

by

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Declaration

I declare that this dissertation for the degree MSc Agric (Animal Nutrition) at the University of Pretoria has not been submitted by me for a degree at any other university.

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Dedication

This dissertation is dedicated to my father Mr. E. Moyo.

List of Abbreviations

ADF	Acid detergent fibre
ADG	Average daily gain
ADI	Average daily intake
ADICP	Acid detergent insoluble crude protein
ADIN	Acid detergent insoluble nitrogen
BMS	Bulk whole maize silage
Ca	Calcium
CDG	Condensed distillers grain
CP	Crude protein
DG	Distillers grain
DGS	Distillers grain with solubles
DDGS	Dried distillers grain with soluble
DIP	Degradable intake protein
DM	Dry matter
DMI	Dry matter intake
DMD	Dry matter degradability
dNDF	Digestible neutral detergent fibre
ED	Effective degradability
EE	Ether extract
FCR	Feed conversion ratio
IVDOM	*In vitro* digestible organic matter
LAB	Lactic acid bacteria
ME	Metabolisable energy
MS DDGS	Maize silage mixed with dried distillers grain with solubles diet
MS SOM	Maize silage mixed with sunflower oilcake meal diet
N	Nitrogen
NDF	Neutral detergent fibre
NDS	Neutral detergent solution
NE	Net energy
NFC	Non-fibre carbohydrates

NH_3-N	Ammonia nitrogen
NPN	Non-protein nitrogen
NSC	Non-structural carbohydrates
OM	Organic matter
P	Phosphorus
RUP	Rumen undegradable protein
SOM	Sunflower oilcake meal
TDN	Total digestible nutrients
TMR	Total mixed ration
UIP	Undegradable intake protein
VFA	Volatile fatty acids
WDG	Wet distillers grain
WDGS	Wet distillers grain with solubles
WDGSMS	Silage blend of wet distillers grain with soluble and whole maize plants
WSC	Water soluble carbohydrates

Table of contents

List of tables

List of figures

Page

Chapter 4

Summary

Nutritional quality of maize ensiled with wet distillers grains for sheep

by

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Four trials were conducted to evaluate the effects of ensiling whole plant maize with wet distillers grains with solubles (WDGS) on its preservation and nutritive value. In the first study, WDGS was blended with whole maize plants at 0% (control), 10%, 20%, 30% and 40%, and ensiled for 120 days in bottle silos in a complete randomized design. Fermentation was monitored by taking samples at day 0, 7, 21, 42 and 120. Results showed a steady decrease ($P<0.05$) in dry matter (DM) concentration with increasing level of WDGS inclusion. Final silage pH was lowest ($P<0.05$) for the 40% WDGS treatment (pH 3.62) and highest for the 10% WDGS treatment (pH 3.79). There was no effect ($P>0.05$) of level of WDGS inclusion on initial buffering capacity (day 0), as well as for day 42 and 120 samples. Lactic acid was higher ($P<0.05$) at day 120 for the control treatment than those blended with WDGS, which did not differ significantly. The concentration of acetic acid was higher value ($P<0.05$) for silage treatments blended with WDGS than the control, with that of 40% WDGS level of inclusion recording the highest ($P<0.05$). The control and 40% WDGS treatments had lower ($P<0.05$) ammonia nitrogen concentration (g/kg N) than the other treatments at day 120.

The second trial involved monitoring ruminal fermentation characteristics of cannulated sheep fed three experimental diets. Formulated diets contained maize silage mixed with 24% dried distillers grains with solubles (MS DDGS treatment), maize silage mixed with 19.5% sunflower oilcake meal (MS SOM treatment), and silage blend of 91% of whole maize plant/9% WDGS (WDGSMS treatment), all on a DM basis, to obtain iso-nutrient diets. The mean value for rumen pH, NH_3N and total VFA concentrations among diets did not differ ($P>0.05$) among treatments. The individual VFA were similar with only butyric acid being lower ($P<0.05$) in sheep fed WDGSMS. There were no differences in the acetic:propionic acid ratio among treatments.

The third trial involved the determination of DM degradability of the three formulated experimental diets using the nylon bag technique. Effective degradability was measured at two outflow rates, 2% and 5%. The diets did not differ ($P>0.05$) for washing losses (a-value), slowly degradable DM fraction (b-value) and rate of degradation of DM (c-value). Treatment MS SOM recorded the highest effective degradability with MS DDGS having the lowest at 2% outflow rate. The effective degradability value at 5% outflow rate for WDGSMS was not significantly different from that of MS SOM and MS DDGS, which differed significantly. In the final study, experimental diets were fed to three groups of eight lambs in a growth performance trial. The groups offered MS DDGS and MS SOM had superior ($P<0.05$) final mass (g/head), average daily intake ($gDM/kg^{0.75}$) and average daily gain (g/head/day), than those offered WDGSMS. The feed conversion ratio did not differ ($P>0.05$) among all treatments.

CHAPTER 1

1.1 Introduction

The ethanol industry is becoming important due to the increasing energy demand on a global level. The increased ethanol production from maize in the United States of America (USA) has been viewed as a way to expand the domestic energy supply and help mitigate growing dependence on imported oil (Collins, 2006). While the growth in ethanol production has been impressive in recent years, ethanol still accounts for only a small portion of USA gasoline use – just 3 percent of total annual consumption (Collins, 2006). In South Africa, plans are being put in place to erect ethanol plants that will utilize the dry milling process to produce ethanol from maize (Köster, 2007). The USA expected to produce nearly 5 million gallons of ethanol for the year 2006 (Collins, 2006). By the end of 2004, the USA produced 7.3 million metric tons of distillers grains, which has become the fastest growing commodity feed for livestock (Kaiser, 2006). However, ethanol's economic importance to agriculture is quite significant.

Distillers grains with solubles can be valuable to agriculture as a feed source for animals. The increased ethanol production from maize has resulted in increased availability of maize distillers grains with solubles (DGS) (Garcia and Kalscheur, 2004), which are ethanol by-products produced after the extraction of ethanol. The process of ethanol production involves the fermentation of maize grain (sorghum and wheat) to produce ethanol and spent grain (whole stillage). According to the review by the Renewable Fuels Association (2009), the spent grain is centrifuged to form distillers grains and thin stillage, which is the liquid fraction of the spent grain. Distillers grains can either be available as a wet (WDGS) or dry (DDGS) product. It must be noted that drying cost significantly increase the commodity price for the distillers by-products. However, it does allow for better handling, transportation, and increased shelf life, resulting in a much wider use of the by-product (Kaiser, 2006).

The growing supply of distillers grains with solubles is likely to lower feed costs without comprising performance, making it more favorable for use as a protein and energy source in the livestock industry (Köster, 2007). Increasing the use of DGS in

growing lamb diets would allow producers to take advantage of these economically priced and readily available feeds (Huls *et al*., 2006). At first glance, DGS appear to be an excellent feed ingredient due to its moderate protein and energy levels. However, it is a by-product that currently has little or no quality control in the production process, which result in a high nutrient variability (Shurson, 2006). The product as well as nutrient composition of DGS may be influenced by several factors, which include raw materials, processing procedures, type of equipment used in the distillation procedure and blending ratios of the various by-products that could originate from the overall process (Köster, 2007).

The DGS is a good source of protein, particularly ruminally undegradable protein (RUP) as a result of cooking and drying. Often assumptions have been made that WDGS had lower concentrations of RUP than the dried form (DDGS), but the differences have been proved to be small (Schroeder, 2003). An approximate three-fold increase in the concentration of protein, fat and fibre was found in distillers grains compared to maize, and unlike maize which is high in starch, distillers grains were practically devoid of starch (Kaiser, 2006). Kaiser (2006) also noted that maize DGS is also an excellent source for energy, attributed to its high concentration of degradable neutral detergent fibre (NDF) and fat.

Many nutritionists have reported high levels of available phosphorus in DGS (Dale and Batal, 2005; Huls *et al.*, 2006). As with other components, the level of total phosphorus was reported to be three times higher in WDGS than in maize (Dale and Batal, 2005). However, the high phosphorous content of DGS raised concerns as to its inclusion rate in growing lamb diets because of environmental impact and potential problems with urinary calculi (Huls *et al*., 2006). When feeding wethers a diet which contained a significant amount of maize distillers by-products, Huls *et al*. (2006) suggested the inclusion of limestone and ammonium chloride to prevent the occurrence of urinary calculi. Huls *et al*. (2006) replaced soybean and a portion of the maize meal with dried distillers grains with solubles (23% of DM) in a lamb diet and there were no negative effects on growth performance, dry matter intake (DMI) and carcass characteristics.

Loy (2006) noted that it was advantageous for distillers to sell WDGS (30-50% dry matter), since this reduced drying costs, increased production capacity and environmental control requirements. Although this significantly reduces WDGS product costs, the costs related to track transportation of water (50-70%) and shorter shelf life must be considered as a major constraint that needs research attention. The effects of transportation, storage costs and losses become more important with wet feeds. Because of the cost of transporting water, wet distillers feeds tend to be utilized locally (Loy, 2006). Opportunities to reduce feed costs and improve profitability of livestock operations are and will continue to be more plentiful by the utilization of these feeds. However, proper ration formulation, economic analysis and feeding management are important in developing the most cost competitive and profitable feeding system. Some of the factors that weighed heavily in decisions relative to by-product feed pricing and inclusion rates included nutrient value of the feeds, nutrient value of competing feeds, consistency of product, reliability of supply, consistency of pricing, transportation and storage losses as outlined by Loy *et al*. (2005). These effects are also greatly influenced by storage methods and handling on the farm. Garcia and Kalscheur (2004) suggested that WDGS can be incorporated in the ensiling of maize to improve its nutritive value and to increase the utilization of the distillers by-products in ruminant diets.

Maize silage is a high energy and low protein-fermented feed which can be suitable for sheep feeding. However, the maize silage protein concentration is usually low to sustain the nutritional requirements for growing lambs (Bell, 1997). Maize silage is composed of the entire maize plant, typically harvested at a whole plant moisture concentration of 65% (McDonald *et al.*, 1991). Up to 50% of the dry matter of maize silage is grain. Maize is generally regarded as an ideal crop for ensiling since it is relatively high in dry matter (DM) content, is of low buffering capacity and contains adequate level of water-soluble carbohydrates (WSC) for satisfactory fermentation to lactic acid (McDonald *et al.*, 1991). When forage maize was ensiled by Harris and Wishart-Smith (1984), changes in composition of both the carbohydrates and protein fraction of the plant occurred. McDonald *et al.* (1991) reported relatively low protein concentration for maize compared to other grasses (8 to 9% protein of DM). However, the protein has a relatively high percentage of sulfur-containing amino

acids, methionine and cystine, but are very low in the essential amino acids lysine and tryptophan (McDonald *et al.*, 1991).

The maize silage protein concentration can be enhanced by blending it with WDGS at ensiling to meet the growth requirements of lambs and this procedure will also adequately preserves the wet distillers co-product (Garcia and Kalscheur, 2004). Garcia and Kalscheur (2004) noted that the advantage of using the WDGS was that it already comes from the processing plant with a pH as low as 3, which resulted in an initial drop in pH of the blend during ensiling. McCullough *et al*. (1963) also reported that distillers grains can be ensiled with forage crops like wheat straw, to enhance preservation and to improve the nutrient concentration of the silage. The possibility of ensiling WDGS with forage crops gives hope for the increased utilization of the ethanol by-product in the feed industry. Ensilage involving WDGS increases production performance in ruminants, and according to McCullough *et al*. (1963), a consistent increase in milk production with Guernsey cows when wet distillers grains were ensiled with wheat straw. However, the information available on the growth performance of lambs or beef steers is very scarce.

Therefore, the overall objective of this study was to prolong the shelf life of WDGS as a component of silage and enhance its contribution to the livestock industry. However, the specific objectives included ensiling of whole maize plants with WDGS to improve the ensiling quality of maize silage with respect to the end products of fermentation. The other specific objective was to evaluate the quality of the WDGS-maize blended silage against the conventional diets in a sheep growth trial.

CHAPTER 2

Literature Review

2.1 Introduction

The fermentation of maize grains to produce ethanol, yields whole spent stillage from which wet distillers grains (WDG) and thin stillage are obtained by screening, pressing and/or centrifugation (White and Johnson, 2003). The thin stillage can be added back to the spent stillage to produce wet distillers grains with solubles (WDGS). Usually WDG is dried to yield dried distillers grains (DDG), or dried distillers grains with solubles (DDGS) if solubles in the thin stillage is added back to the grains at drying (Akayezu *et al*., 1998). The solubles in the thin stillage may also be partially or totally dried to produce condensed distillers solubles (CDS) or dry distillers soluble (DDS) respectively (White and Johnson, 2003). Of these by-products, DDGS is the most commonly used, probably because of ease of handling, storage and shipping. However, the cost of drying has led to the investigation of the possibility of feeding wet distillers by-products to livestock.

Distillers grains with solubles contain moderate levels of protein, and high levels of fat and fiber, which makes them attractive for use in livestock diets, especially for ruminants (Akayezu *et al*., 1998). Garcia and Kalscheur (2004) reported that WDGS should be used fresh, but should be consumed within three to four days in summer or within one week in winter to minimize spoilage. The WDGS should be ensiled if it is not going to be used within the time limits suggested. Ensiling maize plants with WDGS can improve the protein concentration and increase the shelf life of WDGS. Garcia and Kalscheur (2004) also suggested blending distillers grains with forages that have a complementary nutrient profile, such as maize, which is low in protein, energy, fat and phosphorus. According to these authors, combinations of such blends create a dilution effect, making the blend more appropriate to be fed to ruminants, both from a health and environmental perspective.

Silage is the term used for the feed produced by controlled fermentation of high moisture herbage (McDonald *et al*., 1991). When silage is stored under anaerobic conditions (in the absence of oxygen) lactic acid is produced, and at high

concentrations, halts the fermentation process. If silage is undisturbed it may be kept for extended periods. There are many products on the market that are added to the silage at the time of ensiling that increase the quality of the silage by reducing fermentation losses, and some products such as urea (0.5-1%) or anhydrous ammonia actually increase protein concentration (McDonald *et al*., 1991). High quality silage is very palatable, and excellent results can be achieved with growing lambs.

Maize silage contains a moderate to high level of digestible energy, but is low to moderate in digestible protein (McDonald *et al*., 1991). As maize plant matures, fiber content measured in the whole plant maize decreases and the energy concentration increases. This is directly due to the increase in grain content (McDonald *et al*., 1991). Maize silage is low in calcium and trace minerals and contains fair levels of phosphorus. Additional calcium and trace minerals must be supplied. Crude protein of maize silage is 7-9% on a dry matter basis (NRC, 2001).

2.2 Importance of maize distillers grains with solubles in animal feeds

There have been major concerns by animal producers whether they will have access to large quantities of reasonably priced maize in the future (Shurson, 2006). The concerns have been as a result of the rapid growth of the ethanol industry which has turned some maize surplus regions into maize deficit areas in places like the USA. The increased maize demand for ethanol production has pushed maize prices up. Therefore, animal producers who purchase maize must compete with the ethanol industry for supply and price. Shurson (2006) suggested that as the ethanol industry continues to grow, there was going to be an increased supply of the less expensive, multiple types of by-products that will be available for use by the feed and livestock industries. The challenge now is to determine the benefits and limitations of these by-products so that it can be valued and used appropriately.

Optimizing the use of distillers grains is becoming increasingly important as ethanol production increases, with dairy-beef production systems having the potential to use large amounts of DGS (Rinker and Berger, 2003). However, fractionation, changes in enzymes and heat used in the ethanol production process may alter the nutrient composition and digestibility of the ethanol by-products. The challenge for the feed

and livestock production industries is to determine the feeding value and the best applications for this growing portfolio of maize DGS. Ensiling of the wet by-product (WDGS) with forage crops will be one possible way of applying the ethanol by-product into diets suitable for ruminants at high inclusion levels (Garcia and Kalscheur, 2004). It may be cost effective to partially substitute maize with low cost WDGS in livestock feeds. Wet distillers grains solubles can be incorporated in higher inclusion levels than maize, which is becoming an expensive energy source for livestock producers (Garcia and Kalscheur, 2004).

In recognition of the livestock industry concerns, ethanol plants should strive to minimize variability and/or assure consistency of their commodity feeds (Kaiser, 2005). Any by-product feed has challenges and opportunities. Until standards are put in place defining DGS minimum guarantees, it will and/or must be treated as a by-product (Tylutki, 2006). While DGS has high CP and digestible NDF, concerns regarding variability and fatty acid composition will limit widespread use. However, the quantity that may be fed as a percentage of ration dry matter is limited. Much debate exists regarding maximum inclusion rates in rations, with some suggesting as much as 30 percent on a dry matter basis (Schutz *et al.,* 2006). Given the high variability and potential concerns when other high fat and/or maize based diets are fed (i.e. milk fat depression), a safe maximum inclusion rate should be 20 percent (DM basis) (Shurson, 2006). Schingoethe (2004) recommended a maximum inclusion rate of about 20 percent of total ration dry matter as distillers grains in dairy cows. This means 4.5 kg to 6 kg per dairy cow daily of DDGS or 13.5 kg to 18 kg per day of WDGS for most lactating cows. Very little research has been conducted to evaluate the effect of increasing dietary levels of DGS on growth performance of ruminants.

2.3 Process of ethanol production

According to White and Johnson (2003), the process by which DGS is produced is quite easy to understand. Ethanol can be produced from diverse sources, including grains (maize, sorghum, barley and wheat), sugarcane, brewery by-products or lignocellulose biomass such as wheat straw, maize stover and switch grass (Nguessan, 2007). Maize grain is the main feedstock used in the production of ethanol because of its high fermentable starch content compared to other grains (White and Johnson,

2003). Starch is the major carbohydrate storage product in maize kernels, comprising 70-72% of the kernel weight on a DM basis (Bothast and Schlicher, 2005). There are two ethanol production processes: wet and dry milling. According to a review by the Renewable Fuels Association (2009), the main difference between the two production processes is the initial treatment of the grain which determines the initial cost of capital investments. Wet milling is more capital intensive than dry milling because the grain must first be separated into its components, including starch, fiber, gluten and germ. Wet milling typically is used to produce maize oil and maize sweeteners, but the starch can be fermented to produce ethanol (Schingoethe, 2004). With dry milling, the entire maize kernel or other starchy grains are first ground into flour (meal). The maize meal is mixed with water to form a 'mash'. Enzymes are added to the mash which is cooked at a high temperature to convert starch to a simple sugar (dextrose) and to reduce bacteria levels prior to fermentation. Ammonia is added to control pH and as a nutrient for yeast. The mash is cooled and yeast added to promote fermentation of sugar into ethanol and carbon dioxide (Renewable Fuels Association, 2009).

The fermentation process lasts for about 40-50 hours and sulphuric acid is added to halt the fermentation process (Garcia and Kalscheur, 2006). After fermentation, ethanol is removed by distillation and what remains is the spent grain (whole stillage). In most cases, the whole stillage, which is usually 90 percent water, is screened or centrifuged to produce distillers grains and thin stillage. The thin stillage can be added back to the spent grain (WDG) to form the wet distillers grains with solubles (WDGS) or dried to produce distillers dried grains with solubles (DDGS) (White and Johnson, 2003). As the grain is composed of approximately three quarters of starch, which is fermented during ethanol production, the process effectively triples the concentration of oil, fiber, and other minerals. The distillers grains contain primarily unfermented maize residue (protein, fibre, fat). The thin stillage contains yeast cells, soluble nutrients and very small maize particles (Aines *et al*., 1997).

2.4 Nutritional composition of distillers grains with solubles

Distillers grains with solubles (DGS) have important nutritional properties and may offer the animal feed industry a tremendous opportunity to reduce feed costs without

sacrificing performance. Distillers grains by-products are generally characterized by higher protein, fat, NDF (Table 2.1), ash, and lower starch contents compared with the source (maize) grain (Akayezu *et al*., 1998). Factors influencing the composition of distillers grains includes quality of grain used, amount of solubles added and fractionation of particle size (Akayezu *et al*., 1998).

Table 2.1 Chemical composition of distillers grains (Schingoethe *et al.*, 2002)

			% of DM			
DM	CP	Fat	ADF	NDF	Ca	P
30-50	28-35	10-15	15-23	38-44	0.10-0.15	0.43-0.83

Garcia and Kalscheur (2004), suggested that WDGS supplied approximately 10 percent more energy than maize grain and approximately 30 percent protein, 10 percent fat and 1 percent phosphorus, which are highly priced nutrients and thus desirable in a feed. As a rule of thumb for nutrient analysis, DGS is approximately three times the nutrient analysis of maize grain. No significant amount of starch or soluble solids are present in WDGS but the hemicellulose, cellulose and insoluble proteins remain after fermentation of the maize grain (White and Johnson, 2003). Composition of fat essentially remains equivalent to that of maize because maize oil is not recovered during ethanol production. The high fat concentration in WDGS contributes to the energy value of feed and helps negate the energy dilution effects of the high fiber content in DGS (Spiehs *et al*., 2002). The oil is highly polyunsaturated and rich in linoleic acid and therefore, can be a good source of this essential fatty acid and energy.

The amino acid pattern of WDGS generally reflects the maize source (White and Johnson, 2003). The protein in maize has a relatively high percentage of sulfur containing amino acids, methionine and cystine, but are very deficient in the essential amino acids, lysine and tryptophan (White and Johnson, 2003; Spiehs *et al*., 2008). Distillers grains (and all maize products) are naturally low in lysine, the first limiting amino acid in ruminant and monogastric diets. The manufacturing process of WDGS may damage a portion of the protein due to excessive heat during cooking. Heat damage due to drying during DDGS processing may also render protein unavailable

to the animal. In particular, lysine is the first-limiting AA in maize byproducts and is also most susceptible to heat damage because the α-amino group easily binds with reducing sugars in a maillard reaction (Kleinschmit *et al*., 2006). Since lysine is very sensitive to heat damage, it can be expected that increasing acid detergent insoluble nitrogen (ADIN) also decreases lysine levels and lysine digestibility. Variation in ADIN is of particular concern, as this pool is indigestible to mammalian and rumen microbial enzymes (Tylutki, 2006). The WDGS is a good source of ruminally undegradable protein (RUP). Often the assumption is made that wet distillers grains have a lower concentration of RUP than the DDGS, but the differences are slight (Schroeder, 2003). Schroeder (2003) also reported that most of the proteins were degraded by heat during the fermentation process and during drying, so the protein remaining in the DDGS is proportionately higher in RUP than in the original grain.

The nutrient content of the soil can affect the concentration of some nutrients in maize and ultimately distillers grains (DG) (Spiehs *et al*., 2002). However, measuring soil fertility and its correlation with maize nutrient levels is beyond the scope of this study. The ratio of grains and solubles used to manufacture DGS will have more influence on the final nutrient concentration of DGS than soil fertility differences. Most of the variation in the nutrient content of DGS is likely a result of the maize crop used, percentage of solubles added to the distillers grains, and completeness or duration of the fermentation process which affects the degree of starch removal (Spiehs *et al*., 2002).

2.5 Variability of distillers grains with solubles

Due to variation in the methods of production among and within distilling facilities, particularly relative to handling of distillers solubles and dehydration, distillers grains with solubles have been known as a by-product feedstuff with wide variability in both its nutrient concentration and the digestibility of its CP and NDF components (Belyea *et al*., 2004). This has been considered to be detrimental to its economic value, since nutrient consistency among batches, in addition to high nutrient digestibility, increases the economic value of any animal feed. However, many new distillation facilities have made efforts to make production of DGS more consistent among and within facilities by introducing new equipment, standardizing production processes

and treating DGS as a co-product (rather than a by-product) of ethanol production (Belyea *et al*., 2004).

The source of variation in the composition of DGS is not well documented. Belyea *et al*. (2004) suggested that distillers solubles, one of the major parent streams of DGS, has a considerable variation in composition. However, there are probably other sources of variation. An assumption commonly expressed by maize processors, is that the variation in the composition of maize is a major cause of variation in the composition of DGS (White and Johnson, 2003). There are some significant effects of year-to-year variation in maize composition and consequently DGS (Belyea *et al*., 2004). However, there are no significant correlations between composition of maize and components of DGS. Lack of correlation among maize and DGS components is not surprising, considering the transformation in maize components during processing and fermentation (Akayezu *et al*., 1998).

Manufacturing practices may vary among ethanol plants (White and Johnson, 2003). Therefore, the DGS produced in one plant may differ considerably from that produced in another (Spiehs *et al*., 2002). Significant nutrient variation has also occurred within ethanol plants themselves (Belyea *et al*., 2004). As a result, one source of DGS may not sustain the same level of milk production compared to other feed source. Since DGS are formed when two processing streams, wet grains (WG) and distillers solubles (DS), are combined to produce WDGS, Belyea *et al*. (2004) reported that composition of DS can vary significantly from batch to batch. The process of blending of WG and DS prior to drying is not well controlled in the production of DDGS. Therefore, a variation in the proportion of WG and DS may contribute to the variation in protein and other nutrients in DDGS. Because of the substantial differences in nutrient composition between the blended fractions, it is understandable that the proportion of the grains and the solubles blended together will have a significant effect on the final nutrient composition of the distillers grains products (Köster, 2007).

Differences in type of yeast, fermentation, distillation efficiencies and drying processes may also result in nutrient variability (Belyea *et al.,* 2004). The protein in

DGS is derived from two main sources - yeast and maize (Köster, 2007). As yeast grows, it ferments starch and produces cell mass, much of which is yeast protein. Therefore, a proportion of the protein in DGS is of yeast origin. In addition, maize contains moderate amounts of protein NRC (2001). The proportion of yeast protein to maize protein in DGS is not well documented in literature. Belyea *et al*. (2004) suggested that the ratio of DGS amino acid concentration to yeast amino acid concentration varied considerably among amino acids. However, most ratios ranged from 0.45 to 0.70, suggesting that yeast protein may make up approximately half of the protein in DGS. Belyea *et al.* (2004) suggested that for certain amino acids, such as lysine, the high concentration in yeast protein (3.32 g/kg) balanced the typically low concentration in maize (0.23 g/kg) and resulted in relatively high concentrations in the DGS of about 0.77 g/kg.

The variability in lysine and methionine levels among plants is of concern because precise diet formulations require predictability of amino acid levels in DGS (Belyea *et al.*, 2004). Nutritionists need to become familiar with nutrient levels and variability among and within individual plants before selecting a DGS source, because variability does exist between plants (Spiehs *et al*., 2002). Köster (2007) suggested that the difference in lysine digestibility among maize DDGS sources was due to drying time and temperature used to produce DDGS. Since the level and length of heating is highly correlated to lysine digestibility, it is not surprising that a fairly wide range in lysine digestibility exists among DDGS sources. Theoretically, the use of less heat could improve amino acid digestibility of DDGS, but no studies have been reported that specifically determined how these processes will have an impact on the final nutrient composition and digestibility (Köster, 2007).

Variation in fat and protein content affect the market value of DGS. Maize processors typically market DGS with a conservative estimate of nutrient content to ensure that label specifications are met. Belyea *et al*. (2004) reported that marketing DGS by assuming conservative nutrient concentrations short-changed the true potential of DGS as a legitimate feed source. While much attention is given to protein in DGS, fat is also an important nutrient, because it increases available energy concentration (Belyea *et al*., 2004).

2.6 Feeding value of distillers grains with solubles

Distillers by-products have been researched and used in beef cattle for many decades. In general, it improves rumen health, feed palatability and safety, and contributes significantly to the amount of energy, essential minerals such as potassium and phosphorus, and quality metabolisable protein that leaves the rumen (Köster, 2007). Most research has focused on distillers grains as an alternative protein for soybean meal in total mixed rations (Garcia and Kalscheur, 2004). However, in addition to its protein concentration, ethanol by-products are also an excellent source of energy, attributed to high concentration of digestible neutral detergent fiber (NDF) and fat (Schroeder, 2003). Questions have been raised as to the maximum amount of distillers grains to be included in different animal rations. Schroeder (2003) suggested that a maximum of 20 percent distillers grains should be included in the rations (DM basis) of dairy animals. At levels greater than 20 percent of the diet, potential palatability and excessive protein consumption problems may exist. Total dry matter intake may be decreased because the total ration may be too wet when using WDGS above 20 percent (Schroeder, 2003).

Distillers grains are palatable and readily consumed whether wet or dried; however, DM intake may decrease when animals are fed high amounts of DGS, especially wet DGS (Köster, 2007). NRC (2001) reported that gut fill could limit DM intake when diets containing less than 50% DM were fed, especially when fermented feeds (silage or haylage) were included in the total diet.

Feedinfo News Services (2007) mentioned that distillers grains had been proposed to be beneficial for treating animals with acidosis or other liver ailments due to its low starch concentration. It is however not previously proposed that distillers grains can promote cardiac development, thereby treating or ameliorating or preventing arrhythmia (Feedinfo News Services, 2007). It is noted that many cardiovascular pathologies such as arrhythmia are caused by oxidants or by increased calcium influx into the heart (Feedinfo News Services, 2007). As such, wet and/or dry distillers grains may act as an anti-oxidant or may act as a calcium channel antagonist, thereby relieving stress on the cardiovascular system of the animal.

2.6.1 Feeding value in beef cattle

Extensive research with growing-finishing cattle as well as lactating dairy cows gave some insight into when and where distillers grains may fit in for feeding beef cows (Köster, 2007). These situations include feeding as a protein source, particularly for low quality forages (replace soybean meal), as a low starch-high fiber energy source (replace soy hulls), and as a source of supplemental fat (soybean replacement). Beef cows need less supplemental protein than dairy cows, but in many production systems they are fed poor-quality, low-protein forages (Köster, 2007). In these situations distillers grains fit well as a supplemental protein source.

Depending on the animal production goal, DGS can often serve as the sole supplemental source for cattle (Köster, 2007). This could be practiced in lower quality pastures as it is a medium protein feed of which the protein content is approximately 50 percent degradable intake protein (UIP) and 50 percent degradable intake protein (DIP), and also contains other commonly limiting minerals such as phosphorus in relatively high quantities (Huls *et al.,* 2006). Therefore, DGS can be fed successfully as a replacement for other protein sources (such as sunflower oilcake, cotton seed oilcake, urea, etc) in beef cattle supplements on pasture (Köster, 2007). However, it must be emphasized, when feeding DDGS as a sole protein source on low quality pastures, it is important to remember that sufficient quantities must be fed to specifically meet the degradable protein requirements. In such a case, distillers by-products may combine well in a blend with non-protein nitrogen (NPN) source when degradable proteins are the major nutrient of concern (Köster, 2007).

Weaned calves, developing heifers and mature cows on pasture may need extra energy supplements in addition to supplemental protein and phosphorus. It is advantageous if the same commodity can be used for supplemental energy as well as protein. By providing energy as highly digestible fibre, one can avoid further negative associative effects (reduced forage intake and digestibility) associated with feeding starchy feeds as an energy source for grazing cattle. Trenkle (2004) fed 10, 20 and 40% DDGS or 10 and 20% of WDGS without affecting carcass weight, marbling or yield grades in beef cattle on feedlot.

2.6.2 Feeding value in dairy cattle

Distillers by-products provide a unique feed ingredient for the high producing dairy cow as it is an excellent source of UIP, NDF and non fibre carbohydrates (NFC) when compared to, for example, a 60:40 maize and soyabean oilcake blend (Köster, 2007). The unique properties of distillers grains further include high fat concentration of 8 to 12 percent (Kaiser, 2006), which provides a good source of non-carbohydrate energy to compliment that of high starch ingredients. The fat concentration of distillers grains is also high in long-chain unsaturated fatty acids, which may increase processing, healthfulness and marketability of dairy products. Replacing some of the starch with DGS stabilizes rumen pH, which improves rumen health and cow productivity, particularly in the early lactating-higher producing cow (Köster, 2007).

Rations involving maize DGS are low in lysine, while the importance of lysine and methionine to enhance milk production and composition is will documented. Distillers grains with solubles are palatable when fed to dairy cows (Köster, 2007). According to an overview by Köster (2007), DGS combined with the more unpalatable blood meal, improved the overall palatability of the total ration and the high lysine levels in the blood meal complimented the higher methionine levels in maize DGS. This combination is an excellent rumen protected amino acid blend for higher producing dairy cows (Köster, 2007).

Studies with dairy cows showed that dry matter intake was as high as or higher than the intake of control diets (alfalfa hay and maize meal), even with more than 20% DGS in the diet. While DM intake was not affected by inclusion of high amounts of DDGS (Hippen *et al*., 2004; Kalscheur, 2005), DM intake of WDGS diets tended to decrease with more than 20% of the DM as WDGS. Trenkle (2004) reported research results from Iowa State University which revealed that 10, 20 or 40% of the ration dry matter as DDGS could be fed to growing Holstein steers from 196 kg to 317 kg liveweight without affecting feed intake or gain. Köster (2007) reported in his overview that DDGS could be used in lactating dairy diets with yield responses equal to or exceeding that of other protein sources, when the inclusion rate was kept below 20 percent of the dry matter intake (DMI). Other research demonstrated that DDGS can effectively be used at levels up to 30 percent of the total DMI in dairy rations

under normal feeding situations (Köster, 2007). Hippen *et al*. (2003) observed a decreased DM intake with a corresponding decrease in milk production when WDGS supplied more than 20% of the diet DM in rations that contained only 40 to 46% DM. Schingoethe *et al*. (1999) also observed a decreased DM intake when diets contained 31% of DM as WDGS in a 47% DM diet, but milk production was similar to the control diet. Milk production was usually similar to production with control diets, and in many cases higher, when fed any amount of WDGS (Schingoethe *et al.*, 1999). With DDGS, production tended to be highest for diets containing up to 30% DGS, while with WDGS, production was highest when fed up to 20% DGS (Kalscheur, 2005). To illustrate this point, Kleinschmit *et al*. (2006) used a standard, good quality DDGS to evaluate the response to two specially processed DDGS products intended to have even better quality. Milk production was higher for all three DDGS products evaluated than for soybean meal-based control diet with only small additional differences in response due to the improved DDGS quality.

2.7 Ensiling wet distillers grains with solubles

Dried distiller's grains, with or without solubles, are more convenient to store than the wet distillers grains, since it only contains 10-13% moisture (Ganesan *et al*., 2005). Wet distiller's grains (moisture content of 40-70%) will mold and go out of condition in as few as 4 days, although typically, WDGS have about 7 days of shelf life before going out of condition (Loy, 2006). Organic acid may extend shelf life, but the additional cost needs to be considered. Wet distiller's grains have been successfully stored for more than 6 months in silage bags, either bagged alone or in combination with other feeds (Tjardes and Wright, 2002). The conditions required are similar to those needed for any ensiled crop i.e. air exclusion, adequate compaction and low pH. Ensiling in silo bags is probably the method of choice as air exclusion is high, resulting in low spoilage and dry matter losses.

Ensiling distillers by-products with plant forages may provide the solution to the problem of nutrient variability and storage (spoilage) of the ethanol by-product. Wet distillers grains have been successfully preserved alone or in combination with soy hulls, beet pulp or maize silage. One advantage of WDGS is that it sticks well to dry particles of other feeds, increasing the palatability and homogeneity of the diet

(Garcia and Kalscheur, 2004). Garcia and Kalscheur (2004) suggested blending distillers grains with forages that had a complementary nutrient profile such as maize plants. Maize plants have low protein, energy, fat and phosphorus concentrations. Combination of such blends will create a dilution effect, making the blend more appropriate to be fed to ruminants both from a health and environmental perspective (Garcia and Kalscheur, 2004). However, when distillers grains are blended with other feeds that also supply these nutrients (protein, fat and phosphorus), dietary excesses of nitrogen and phosphorus may result in increased nutrient excretion and thus environmental concerns. The fermentation is not a classic ensiling process because wet distillers grain has a very low initial pH (less than 4). However, this acid level, provided by the addition of sulphuric acid to stop the fermentation process during ethanol production (Garcia and Kalscheur, 2006), does aid in preservation.

McCullough *et al*. (1963) ensiled WDGS with forage wheat plants and reported an increase in protein, but with a less fiber concentration. Feeding WDGS-wheat silage to Guernsey cows produced the most consistent influence on milk production. This influence resulted in an increase in milk production. Aines *et al*. (1997) evaluated the ensiling of fescue grass with whole stillage at 0, 15, 30 or 60 percent of the total DM. The fescue-stillage mixture was ensiled for 20 days in small laboratory silos. Lactic acid increased with increasing stillage level. The silages were finally evaluated in a lamb digestibility trial, where digestibilities increased linearly with an increasing level of stillage in the silage. Muntifering *et al*. (1983) concluded that ensiling offered a means of storing WDG, thus saving drying costs, but the feeding value was reduced compared to fresh wet stillage. However, these authors concluded that ensiling reduced the protein-bypass value of WDGS.

Garcia and Kalscheur (2004) reported that blending WDGS with other feeds resulted in a fermentation pattern that shifted from the traditional lactic acid fermentation towards a more acetic acid fermentation. Blends of WDG and maize silage ensiled on an as-fed basis in 50:50 and 75:25 maize to WDG ratios respectively, showed increased aerobic stability upon exposure to air. When compared to pure maize silage, acetic acid had increased by 70 and 286 percent in the 75:25 and 50:50 blends (maize: WDGS) respectively by day 3 post ensiling (Garcia and Kalscheur, 2004). Aerobic

stability of the maize silage and WDGS-maize blends was tested by measuring the number of hours the feed remained stable before rising above ambient temperature. It took a short time (42 hrs) for the pure maize silage to increase its temperature above the ambient temperature, whereas 312 and 648 hours were required for the 75:25 and 50:50 blends (maize: WDGS) respectively (Garcia and Kalscheur, 2004). Increased feed stability after opening the bunker is thus one advantage of ensiling maize silage with distillers grains.

The other feeds that are good alternatives for blends with WDGS include soy hulls and beet pulp (Garcia and Kalscheur, 2004). Both are low in protein, fat and phosphorus, although they provide fermentable energy due to the presence of highly digestible carbohydrates. Soy hulls and beet pulp when blended with WDGS offer the additional advantage of increasing acetate production in the rumen, thus reducing the risk of acidosis (Garcia and Kalscheur, 2006). These authors suggested that blends with WDG were high in energy, making it an ideal substitute for part of the maize grain in diets. The use of blends in high forage diets is also recommended as it provides readily available energy without decreasing rumen pH excessively (Garcia and Kalscheur, 2004), which is known to be detrimental to fiber digestion (McDonald *et al*., 1991). Research at the South Dakota State University, Dairy Science Department, evaluated the anaerobic fermentation characteristics of ensiling WDG alone or mixed with soy hulls. Combining WDG with soy hulls resulted in an immediate acidic condition in the silage mass as a result of the initial low pH of the WDG rather than from fermentation (Garcia and Kalscheur, 2004). It is recommended that feed intakes be restricted to avoid excessive weight gains because of the high energy content and palatability, combined with increased intake potential for rations involving DDGS (Garcia and Kalscheur, 2006).

2.8 Principles of ensilage

McDonald *et al.* (1991) reported that the main objective in the conservation of any crop was to preserve it at the optimum stage of growth for use during those seasons when forage is unavailable. It is essential to achieve anaerobic conditions when preserving crop by natural fermentation. This can be obtained by various methods of which the most efficient way is to store the material in a hermetically sealed container

(McDonald *et al*., 1991). Under these conditions the oxygen trapped in the herbage is rapidly removed by respiratory enzymes in the plant. In the open type silo, the efficiency with which anaerobiosis can be obtained, depends upon the degree of consolidation and the effectiveness of the final sealing (McDonald *et al*., 1991). Sealing prevents re-entry and circulation of air during storage. Where oxygen is in contact with herbage for any period of time, aerobic microbial activity occurs and the material decays to a useless, inedible and frequently toxic product (Church, 1991). Under ideal crop and storage conditions, this phase will last only a few hours. With improper management, it may last for several weeks.

The other objective of ensiling is to discourage the activities of undesirable microorganisms such as Clostridia and Enterobacteria. Clostridia are usually present in the harvested forage in the form of spores, but start to multiply as soon as conditions in the silo become anaerobic (McDonald *et al*., 1991). The growth of these organisms is undesirable, as they produce butyric acid and degrade amino acids to a variety of products, which are of poor nutritive value. The Enterobacteria are non-spore forming, facultative anaerobes (able to grow in both the presence and absence of oxygen), which ferment sugars to acetic acid and other products and also have the ability to degrade amino acids (McDonald *et al*., 1991).

The commonest way of inhibiting the growth of these undesirable microorganisms is to promote lactic acid fermentation (Shaver, 2003). The lactic acid bacteria are also normally present on harvested crops and like the Enterobacteria, are facultative anaerobes (McDonald *et al*., 1991). These organisms ferment the naturally occurring sugars (glucose, sucrose and fructose) in the crop to a mixture of acids, but predominantly lactic acid. The lactic acid product increases the hydrogen ion concentration to a level at which the undesirable bacteria are inhibited (McDonald *et al*., 1991). This inhibition is caused not only by the hydrogen ion concentration but also by the undissociated acids themselves. It is difficult to state an exact pH value of the silage at which this inhibition effect occurs, as the inhibition depends not only on pH but also on the acid concentration and the temperature. The wetter the material, the lower the critical pH value will be. Crops of a DM concentration of about 200 g/kg have an acceptable achievement of a pH value of 4.2, which may preserve the

crop satisfactorily, provided the silo remains airtight and is free from penetration by rain (McDonald *et al*., 1991).

Lactic acid bacteria have a relatively high tolerance to low moisture conditions and are able to dominate fermentation in high moisture (Schroeder, 2004). Clostridia are known to be sensitive to water availability and require very wet conditions for active development. With very wet crops, those with a dry matter (DM) concentration of about 150 g/kg, even the achievement of a pH value of 4.0 may not inhibit Clostridia growth (McDonald *et al*., 1991). The rate of lactic acid production is an important factor inhibiting the growth of undesirable bacteria and in reducing fermentation losses. This depends upon the initial lactic acid bacterial population present on the ensiled crop and upon the substrate availability (Shaver, 2003). This in turn is influenced by the degree of physical damage (laceration, bruising and chopping). Modern silage harvesters are capable of chopping herbage into a particle length of <25mm. With such material, plant sap is readily liberated and the lactic acid bacterial growth is stimulated. Finely chopped silage of this type is more readily consumed by ruminant animals than long or coarsely chopped material (McDonald *et al*., 1991).

2.8.1 Silage types

Gordon (1989) suggested that the objective in silage fermentation is to achieve a stable pH at which biological activity virtually ceases. In this way, preservation is obtained while minimizing nutrient losses and avoiding adverse changes in the chemical composition of the material. This is achieved by discouraging the activities of undesirable micro-organisms and encouraging the development of bacteria which produce lactic acid (Gordon, 1989).

2.8.1.1 Lactic acid silage

Lactic acid should be the primary acid produced in good silage (Gordon, 1989). This acid is stronger than other acids in silage and is primarily responsible for the drop in silage pH (Zimmerman, 2002). Byers *et al.* (1982) recognized that fermentation of maize produced lactic acid which resulted in lower losses in DM and energy concentrations from the crop during storage. Lactic acid should be at least 65 to 75 percent of the total silage acids in good silage (Shaver, 2003). Shaver (2003) reported

that extremely wet silages (<25% DM), prolonged fermentations (due to high buffering capacity), loose packing, or slow silo filling can result in silages with high concentrations of acetic acid (>3 to 4% of DM). In such silages, energy and DM recovery are probably less than ideal (Shaver, 2003).

Lactic acid bacteria, which are the most important species during ensiling, are usually present on maize plants in numbers 1000 times lower than their main competitors, fungi and enterobacteria (McDonald *et al.*, 1991). After ensiling, the micro-organisms capable of anaerobic growth (lactic acid bacteria, enterobacteria, clostridia, some *Bacillus spp*., and yeasts) begin to grow and compete for available nutrients. The changes in the first few days are critical to the success or failure of the subsequent fermentation. If conditions are suitable, the lactic acid bacteria will quickly acidify the environment to such an extent that the competing organisms will not be able to survive and the end result will be a stable, low pH silage (Kung and Shaver, 2001). If the pH is not lowered quickly enough, the undesirable micro-organisms (mainly enterobacteria, clostridia and yeasts) will be able to compete for nutrients and in doing so, reduce the chances even further of obtaining a stable silage, since many of their products do not aid preservation. These organisms are also capable of producing end-products which may reduce the nutritive value of the silage (McDonald *et al*., 1991).

Under anaerobic conditions, the lactic acid bacteria can ferment a wide range of substrates (Shaver, 2003). The fermentation of hexose sugars is used as a basis for the anaerobic bacterial identification. The presence of aldolase also helps to differentiate the bacteria, as does the stereo-isomer of lactic acid produced during fermentation. Unlike higher animals and plants which produce exclusively the L (+) isomer, species of lactic acid bacteria produce either D (-), L (+) or a mixture of both (McDonald *et al*., 1991). Both the D and L isomers are metabolized at equal rates in the rumen. It has been found, that only 10 percent of the rumen lactic acid are absorbed from the rumen, the rest being metabolized mainly to acetate as well as propionate and butyrate. These VFAs are then absorbed from the rumen and used for production (McDonald *et al*., 1991). There is considerable variation in the actual species of bacteria dominating particular silages and both numbers and species present are bound to be affected by the many variables associated with ensiling (McDonald *et al*.,

1991). There is evidence to show that the members of most of the genera of lactic acid bacteria are represented at some stage during ensiling, with *Lactobacillus plantarum* being the most frequently found species. Of all lactic acid bacteria, *L. plantarum* can most successfully colonise freshly ensiled forage, as this species can ferment a wide variety of substrates, is highly competitive and produces large amounts of acid quickly. Other dominant species are *L. brevis* and the *Pediococcus spp*. (McDonald *et al*., 1991).

Lactic acid bacteria are virtually non-proteolytic and therefore, an external supply of amino acids is necessary for their growth (McDonald *et al*., 1991). Their ability to ferment amino acids also appears to be restricted and it is thought that only two, serine and arginine, are extensively attacked by some, but not all, of these organisms. There is some evidence to indicate that a certain lactic acid producing bacteria can decarboxylate amino acids to form amines. In well-preserved silages a small amount of nitrate is reduced to nitrite and most of this is lost from the silo as nitrogen oxide gasses, where as in badly-preserved silages, a larger amount of nitrate is reduced to nitrite which is then further reduced to ammonia (McDonald *et al*., 1991).

2.8.1.2 Acetic acid silage

Enterobacteria represent a minor part of the micro flora on grass but these bacteria are usually present in significantly higher numbers than the lactic acid bacteria, and their numbers increase substantially during the first days of ensiling (McDonald *et al*., 1991). The development of lactic acid bacteria and the subsequent fall in pH normally leads to a rapid decrease in their numbers. According to McDonald *et al*. (1991), under certain ill-defined conditions, acetic acid producing bacteria may dominate the fermentation. Acetic acid silages contain high levels of acetic acid and relatively low levels of lactic acid. Deamination of amino acids is usually extensive, and consequently ammonia levels in these silages are higher than those found in lactic acid silages. Because of the negative correlation of acetic acid concentration and DM intake, it is reasonable to assume that the latter will be low in animals given these silages *ad libitum* (McDonald *et al*., 1991).

The effect of high concentrations of acetic acid in silages fed to animals, was unclear at this time. In the past, some studies can be found where DM intake was depressed

when silage high in acetic acid concentration was fed to ruminants (Shaver, 2003). However, the depression in intake to high acetic acid in the diet has not been consistent. Shaver (2003) speculated that decreased intake may be actually due to unidentified negative factors associated with a poor fermentation, and not to acetic acid itself. For example, in recent studies (Kung and Shaver, 2001; Schroeder, 2004) animals showed no indication of reduced intake when fed silages high in acetic acid due to inoculation with the bacteria *Lactobacillus buchneri* for improved aerobic stability (Shaver, 2003). If a producer has intake problems due to silages with excessively high acetic acid concentrations (0.5 to 6% of DM), the amount of that silage should be reduced in the total mixed ration (Shaver, 2003).

2.8.1.3 Butyric acid silage

McDonald *et al*. (1991) stated that the clostridia bacteria can be divided into two major physiological groups based on its substrates. Saccharolytic clostridia, for example *Clostridia butyricum*, ferment mainly sugars and organic acids and possess little activity against proteins or amino acids. Proteolytic clostridia, such as *C. sporogenes*, ferment mainly amino acids. Some clostridia, for example *C. perfringes*, have both types of activity (McDonald *et al*., 1991). Apart from their presence in silage, clostridia also occur in faeces and in soil. The presence of clostridia in silage is probably the result of soil contamination, since clostridia numbers on green plant material are generally quite low (McDonald *et al*., 1991).

Silages with high concentration of butyric acid have undergone clostridial fermentation (Shaver, 2003). Clostridial growth is stimulated by high storage temperature, low DM concentration (<30%), low WSC concentration, high buffering capacity of the crop and delayed sealing (Jonsson, 1991). It is generally assumed that clostridia multiply either during the initial phase of silage fermentation or at the later stage in secondary-fermented silage. These silages usually have pH values within the range of 5 to 6, and contain low concentration of lactic acid. In such silage material, butyric acid is usually the dominant fermentation product, although acetic acid concentrations are also frequently high (McDonald *et al*., 1991). As butyric acid is a much weaker acid than lactic acid and as only one mole of butyrate is produced from two moles of lactate, it follows that the pH will increase. This makes conditions more

favourable for the growth of proteolytic clostridia, which are inhibited at a higher pH than saccharolytic clostridia (McDonald *et al*., 1991). Because of the extensive breakdown of amino acids caused by clostridia, McDonald *et al*. (1991) concluded that silages of this type will contain high concentrations of ammonia nitrogen (NH_3-N), usually in excess of 200g/kg total N. As a result of these changes, the subsequent utilization by ruminants of the nitrogenous compounds in butyrate silages is likely to be low. The DM intake of ruminants given these silages is low, but whereas there is a close negative correlation between DM intake and the concentration of silage NH_3-N, the exact cause of these reduced intakes by animals is unknown (Shaver, 2003).

Silages high in butyric acid are low in nutritive value and have higher ADF and NDF concentrations because many of the soluble nutrients have been degraded (Shaver, 2003). Such silages may also be high in concentrations of soluble proteins and may contain small protein compounds called amines (due to decarboxylation of amino acids) that have sometimes shown to adversely affect animal performance. High butyric acid has sometimes induced ketosis in lactating cows and because the energy value of silage is low, intake and production can suffer. As with other poor quality silages, total removal or dilution of the poor silage is advised (McDonald *et al*., 1991).

2.8.2 Nutritional composition of maize plants

The maize plant is large and bulk by the time it reaches the end of the vegetative period of growth (ARC, 1978). Consequently it needs to be chopped at the time of harvest in order to facilitate transportation from the field to the silo and consolidation within the silo. The introduction of forage harvesters has improved the problem of reducing particle length which imposes a serious limitation on the speed with which maize could be harvested and ensiled (ARC, 1978). Maize is ensiled to preserve it with minimal loss of nutrients, so that it can be used to maximize the advantage for feeding ruminant livestock during the winter period. When maize is harvested for conservation at its optimal physiological maturity, the crop would have reached maximum yield of DM/ha. Sugars produced by photosynthesis in the leaves, are moved through the stem to the ear, to be stored as starch in the grain. The harvested plants have three main components, the ear shot (consisting of the grain, the central cob to which the grain is attached, and the surrounding husk leaves), large and

elongated stem, which terminates in the male inflorescence or 'tussel', and leaves (ARC, 1978). The three fractions differ not only in its chemical composition but also in the extent to which it contributes to total crop dry weight at harvest. Both these factors can markedly influence the composition and nutritive value of maize silage (ARC, 1978).

The two most important chemical components of the maize plant are carbohydrates and proteins, since both provide the ruminant animal with essential nutrients (ARC, 1978). There are two main types of carbohydrates, those contained within the cells of the plant and those found in the cell walls. Starch is the major non structural carbohydrate (NSC) in maize, whilst cellulose is the major structural carbohydrate. However, the sugars within the plant cell, the water soluble carbohydrates (WSC), are vital to the preservation of the ensiled crop. The protein content of the maize plant is low in relation to that of forage grasses, which are harvested for silage when much less mature, but similar to that of other whole crop cereals (e.g. barley and wheat) harvested at a comparably late stage of development (ARC, 1978).

The structural carbohydrates or cell wall contents of the plant affects both the amount consumed and the proportion digested and absorbed by the animal (ARC, 1978; McDonald *et al.*, 1991). Although the two major cell wall carbohydrates, cellulose and hemicellulose, are theoretically completely digested, in practice, digestibility is reduced by the presence of lignin (ARC, 1978). Lignin gives structural strength to plants by forming bridges between fibers of cellulose and hemicellulose. When plant tissue has both a high cell wall content and a high content of lignin in the cell wall, as is the case in the maize stem, rate of digestion in the rumen is low, extent of digestion is reduced, and as a result the amount which the animal is capable of consuming is also restricted (ARC, 1978). Fortunately, maize has relatively low cell wall content in comparison to other grasses, mainly because of the large contribution of the ear to total crop dry weight. When forage maize is ensiled, changes with the composition of both the carbohydrates and the protein fraction of the plant occur. Maize silage is low in protein and calcium (Umberger, 1996). Nutrition plays a major role in the overall productivity, health and well-being of livestock. It is important that producers consider nutrition management a top priority because feed costs account for

approximately two-thirds of the total cost in sheep production (McDonald *et al.*, 1991). Nutrient requirements of sheep (Table 2.2) vary with differences in age, body weight, and stage of production. However, some guidelines should be followed when incorporating maize silage in ewe and lamb diets.

Table 2.2 Daily nutrient requirements of early weaned lambs (NRC, 1985)

Weight (kg)	DMI (kg)	TDN (kg)	ME (Mcal/kg)	CP (g)	Ca (g)	P (g)
30	1.4	1.1	16.7	216	7.2	3.4
50	1.7	1.29	19.7	240	9.4	4.8

2.8.2.1 Carbohydrates

The major compositional change during ensiling is the fermentation of WSC to short chain organic acids and alcohols. The principal fermentation product is lactic acid which is produced by bacterial activity (ARC, 1978). Glucose and fructose are the principal free single sugars, being found in a 1:1 ratio and 1 to 3 percent of DM in maize plants. Sucrose is the only other sugar found in appreciable amounts. It may often be present at about 4 to 5 percent (ARC, 1978). Other sugars are only found in trace amounts (Church, 1991). According to ARC (1978), the rates of diffusion of soluble carbohydrates from the intact and ruptured cells into the aquas phase, may be more important than absolute amounts in the crop. It is generally considered that most naturally occurring lactic acid bacteria do not have the ability to ferment starch directly (Schroeder, 2004). Two types of *lactobacilli* were identified as playing a major role in the lactic acid fermentation. They are the homofermentative and heterofermentative *lactobacilli*, distinguished by the products of their metabolism (McDonald *et al*., 1991). With forage maize harvested at 25 percent DM, WSC could be almost completely fermented to acids, whilst starch and the cell wall constituents remained relatively unattached by the bacteria. If loss of DM occurred at ensiling, the proportion of starch and cell wall constituents in the DM of the silage could be higher than in the original fresh crop at harvest (ARC, 1978).

McDonald *et al.* (1991) suggested that the production of lactic acid was reflected in the decrease in pH from about 5.5 in the crop at harvest to 4.0 in the silage as fermentation proceeds. Initially, the acids produced by fermentation are buffered by

other components. The buffering constituents of forage maize have not been established, but work on grasses and legumes has shown that buffering was principally brought about by organic acids, such as malic, citric and succinic acids (McDonald *et al.*, 1991). Degradation of proteins may also contribute to the buffering of fermentation acids (ARC, 1978). With the maize crop, relatively less acid is required to reduce the pH to a low level at which bacterial activity ceases, due to its low buffering capacity (ARC, 1978). This property of forage maize is important in the achievement of a predominantly lactic acid fermentation, since with crops of high buffering capacity such as lucerne, other bacteria which are less tolerant to low pH levels can proliferate and convert lactic acid produced early in the fermentation process to butyric acid. This secondary fermentation is associated with substantial loss of both DM and energy, and fortunately, is rarely seen in maize silage (McDonald *et al*., 1991).

2.8.2.2 Protein

Forage maize has a relatively low protein content compared to other grasses and forage crops. Typically, it comprises between 7 to 9 percent CP and there is relatively little change as a result of the ensiling process (ARC, 1978; Wahlberg, 2004). The protein present in forage maize, as distinct from those of seeds, are all enzymatic by nature and are thus concerned with growth and biochemical function of the cell. The amino acid concentration of herbage is affected by stage of growth, although the application of nitrogenous fertilizers can also have a marked effect in increasing the protein concentration (McDonald *et al*., 1991).

McDonald *et al*. (1991) reported that some 10 to 25 percent of the total nitrogen in herbage is made up of non-protein nitrogenous (NPN) components. These include free amino acids and the amides, glutamine and asparagines, peptides of varying chain length, amines, ureides, chlorophyll and nitrates. In forage crops, the free amino acid composition of the protein fraction is relatively stable. The free amino acids are influenced by many factors, such as species, stage of growth and environmental conditions. In addition to the amino acids normally found as components of protein, a number of non-protein amino acids also occur free in grasses and other forage crops.

The amide and nitrate concentration of grasses are influenced by nitrogenous fertilization (McDonald *et al.*, 1991).

ARC (1978) noted that changes in the composition of the protein fraction occurred as a result of the ensiling process. At harvest, about 75 to 90 percent of the total nitrogen in fresh crop is present as true protein (McDonald *et al*., 1991). After ensiling, protein is rapidly degraded, most likely as a result of the activity of plant enzymes (Shaver, 2003). Accumulation of acids in the silage and a reduction of oxygen in the silo eventually inactivates plant enzymes (ARC, 1978). ARC (1978) suggested that the general protein content of maize silage was dependant on the DM concentration of the ensiled crop. On an as-fed basis, typical maize silage that consists of 35-40% DM, approximately has 26% TDN, 3.0-3.2% total protein, 0.11% calcium, and 0.08% phosphorus concentration. Maize silage is low in protein and calcium (ARC, 1978). The minimum dietary nitrogen concentration required in most ruminant diets is approximately 10 g/kg DM, about 62.5 g/kg crude protein (CP); lower concentrations will increasingly impair microbial breakdown of ruminal digesta and retard its onward passage, so that feed intake would be severely reduced (Freer and Dove, 2002).

2.8.3 The influence of stage of maturity on composition and nutritive value of maize silage

As the maize plant develops to physiological maturity, water soluble carbohydrates (WSC) are translocated from the stem and leaf to the ear and deposited as starch in the grain (ARC, 1978). Associated with these changes is an increase in the DM of the whole plant. There is a decrease in stover yield with increasing age of the crop. This is reflected in an increased weight of the ear, which more than balance the loss in yield of stover (ARC, 1978).

Disappearance of sugars from the stover fraction is associated with an increase in the content of starch in the ear. Starch accumulation in the ear is the result of a decrease in cell wall content of the ear fraction whilst the proportion of cell walls in the stover DM increases. The DM content and the content of starch in the ear, increases with advancing maturity, whilst the cell wall contents, total fermentation acids, ash and CP decreases (Forouzmand *et al*., 2005). The WSC are completely fermented in the

material harvested at the medium and late stages, but in the early harvested crop residual WSC remains in the silage. The proportion of insoluble N (protein) in the total N increases in the late harvest silage as does the proportion of N present as ammonia (Forouzmand *et al*., 2005).

Forouzmand *et al*. (2005) suggested that CP content of maize forage and silage tended to decrease as OM content increase with plant maturity. This could be due to the relatively higher leaf content of the plant material at the early maturity stage, and higher ash content of the leaves than the other morphological fraction of the plant. Increasing DM content with advancing maturity is reflected by a reduction in the extent of fermentation in the silo (Forouzmand *et al*., 2005). However, it appears like there is relatively little change in the pH of maize silage with increasing DM content, probably because the content of WSC and the buffering capacity of the crop decreases with advancing maturity. Thus a stable fermentation pattern dominated by the production of lactic acid is almost invariably found in maize silage, regardless of the stage of harvest (Forouzmand *et al*., 2005). A reduced fermentation with increasing DM content of the crop at harvest is also reflected by a reduction in the proportion of the total N, which is degraded to non-protein nitrogen (NPN) during ensiling. Loss of DM during ensiling is related to crop DM content (McDonald *et al*., 1991). Delaying harvest reduces ensiling losses, both as effluent and via the fermentation process. The pH is also affected by kernel maturity of maize silage and increases with advancing maturity (ARC, 1978). Lower pH of less mature maize silage could be related to its higher moisture and WSC contents (McDonald *et al*., 1991). The moisture content of the whole crop maize is inversely related to the stage of maturity at harvest (ARC, 1978).

The voluntary intake of maize silage increases with increasing DM content (ARC, 1978; Forouzmand *et al*., 2005). Harvesting too early (milk stage) can be detrimental to quality of silage produces. This is due to excessive losses of nutrients from silo runoff and because the concentration of energy may be low as a result of the poor starch development in the maize kernel (Table 2.3). In contrast, mature maize silage harvested in the black layer stage of maturity (beyond advanced dough) is low in nutritive value, because of poor starch and fiber digestion (Neylon and Kung, 2003).

Table 2.3 Chemical composition of maize silage at different stages of maturity (NRC, 1985)

Ensiling stage	Nutrient composition (g/kg)									
	DM	CP	EE	CF	NFE	TDN	ME MJ/kg	Ca	P	K
Milk stage	25	90	32	222	571	60.1	9.0	3.8	2.0	12.1
Dough stage	30	70	35	257	578	65.1	9.8	2.3	2.0	13.0
Advanced stage	35	69	36	289	546	66.0	9.9	2.6	2.0	11.4

Reduced degradation of protein to NPN may increase the supply of undegradable plant protein to the abomasum and intestines, and may enhance intake (McDonald, 1981). Digestibility of maize silage organic matter (OM) and cell wall decreases with a delayed stage of harvest. Intake may reveal a relatively small change in increasing DM content, possibly because the proportion of the digested OM of the diet derived from the digestible cell wall, decreases as silage DM content increases (McDonald *et al.*, 1991). Thus, animals will obtain a greater proportion of digested nutrients from the non-cell wall fraction as the DM content of silage increases. This increase may result in an improvement in net energy (NE) value. According to Neylon and Kung (2003), the greatest change in the non-cell wall fraction with a delayed harvest is in the starch concentration of the crop. Harvesting at late maturity stage causes a decline in the concentration of CP, but an increase in the concentration of starch. The concentration of ADF and NDF are not different due to maturity at harvest (Neylon and Kung, 2003). Concentrations of lactic and acetic acids are reduced in more mature maize silage and is most likely due to a combination of lower WSC and a reduction in growth of lactic acid bacteria, because of a lower water activity in the drier and more mature crop (ARC, 1978).

The site of digestion of non-cell wall fractions may also change from the rumen to the intestines, as the crop becomes more mature (Neylon and Kung, 2003). The estimated NE value increases as DM content and the proportion of the ear in the crop increases. This increase is attributed to the accumulation of starch in the ear and an increase in crude fibre content, as stage of harvest and crop maturity advances (ARC, 1978). The optimum stage of harvest for forage maize should therefore be at a stage when yield

per hectare is maximal, since the period of ear development and grain filling is associated with a very large increase in yield (ARC, 1978).

2.9 Conclusion

Availability of distillers grains as a feed for ruminants has increased as the ethanol industry expands due to higher demand for greener fuels. Distillers grains contain high concentrations of protein, fiber and fat as a result of starch fermentation during ethanol production. Distillers grains can be fed to animals as a protein source when fed at less than 15% DM or as an energy source when included at levels greater than 15% DM in finishing diets. However, the drying process for DDGS appears to reduce the energy value of the distillers by-product. Wet distillers grains with solubles contain high levels of moisture (low DM concentration) which significantly reduces its shelf life and ease of handling. The possibility of blending WDGS with forage crops as an ensilage is encouraging as this can preserve WDGS for extended periods, improve nutritive value of forage crops and allow for better handling.

2.10 Hypothesis

The hypotheses tested in the present study were as follows:

- It was hypothesised that ensiling whole maize plants with varying levels of wet distillers grains with soluble (WDGS) inclusion will not affect the ensiling quality of the silage, in terms of the end products of fermentation. The WDGS was included at 0% WDGS (control), 10% WDGS, 20% WDGS, 30% WDGS and 40% WDGS.
- The animal evaluation trial hypothesised that the utilisation of distillers grains with solubles (dry or wet) in maize silage diets will not affect the rumen fermentation parameters, dry matter degradability and growth performance of lambs.

CHAPTER 3

Materials and Methods

3.1 Location

This study was conducted at University of Pretoria's Experimental Farm during the period January 2007 until December 2007 after the approval of the proposal by the Ethics Committee (No. EC070412-016). The area is at an altitude of 1372 m and receives an average rainfall of 674 mm per annum.

3.2 Maize cultivation

The maize plants were planted at the Plant Production Section of the farm. The Phb 3442 (Pioneer) maize plant hybrid was planted on the 11th of January 2007. This hybrid was selected as it was the conventional maize plant commonly grown at the farm for silage production and is a medium season hybrid (time to maturity), appropriate to grow in this region. It provides high yield benefits in environments with low to medium yield potential. This hybrid had an above average drought tolerance and resistance to local diseases. The seeds were sown into a cultivated soil bed using a maize planter machine. Pre-emergence herbicides (Atrazine & Lasso) were applied at planting to help control weeds before maize germination. Post-emergence herbicides were added a week after germination. Fertiliser application was as per recommendations by the Experimental farm chief Agronomist, after conducting soil nutrient analysis. The plants were irrigated during the period of March 2007 to time of harvesting. Harvesting was conducted at the hard dough stage of maturity on the 22nd of May 2007 using a silage harvester.

3.3 Ensiling in laboratory silos

3.3.1 Procedure

The whole maize plants were harvested by a silage harvester at the hard dough stage and samples were taken for chemical analysis for plant nutrient composition before ensiling. Wet distillers grains with solubles (WDGS) were purchased from a commercial ethanol plant in Ventersdorp (about 160 km from Pretoria). Representative samples of WDGS were taken at the point of packaging into plastic

bags for chemical analysis. On arrival at the Experimental farm, the WDGS consignment was immediately placed in cold storage (5-7°C) until time of ensiling.
Five treatments were ensiled using whole maize plants cut at a height of 10 cm above ground level, partly to avoid soil contamination of the plant material. The maize plants were chopped to a length of between 10 mm and 20 mm using a maize silage harvester. The different treatments were ensiled in 3ℓ canned fruit bottles (mini-silos). The treatments included the ensiling of whole maize plants (37% DM) with 0, 10, 20, 30 and 40% of WDGS (25% DM). These five treatments involved mixing WDGS and maize plants on an as is basis (weight ratio).

Ensiling was performed by filling and compacting as much treatment material (WDGS-maize blend) into the bottle as possible to exclude air and create anaerobic conditions. Each bottle was tightly sealed with a lid after ensiling. There were 12 bottles ensiled per treatment (60 bottles in total). The bottles were then stored in a dark room with a temperature ranging between 20-25°C. Three samples per treatment that represented zero fermentation (day 0) were placed in sealable plastic bags and immediately frozen (-20°C) until time of chemical analysis.

3.3.2 Monitoring of the fermentation process

The ensiling of different treatments was for a period of 120 days. For each treatment, three bottles were opened at day 7, 21, 42 and 120. Bottles representing each of the corresponding sampling times were opened and all the material emptied into plastic bags, sealed and stored in a freezer at -20ºC.

During the time of analysis, three samples at a time were thawed overnight and one fraction (80g) of the sample was used for obtaining a silage extract (liquid supernatant). This extract was used for the determination of pH, ammonia N concentration (NH_3-N), water soluble carbohydrates (WSC), lactic acid concentration and volatile fatty acid (VFA) concentration. The second fraction of each of the three samples was oven-dried at 55°C for 48 hours and analysed in triplicate for nitrogen (N) concentration. The third fraction of each of the three samples was stored in a freezer (-20°C) until analysis for buffering capacity (Playne and McDonald, 1966). The same procedure was followed for all treatment samples.

3.3.3 Analytical methods

The samples which were oven-dried at 55°C were used to determine N concentration. The liquid supernatant obtained from the one fraction of the silage sample was used to determine pH value, NH_3-N, lactic acid and VFA concentration of the silage. The frozen silage fraction was then later used to determine the buffering capacity of the silage (Bechaz, 2000). The remainder of the unused fraction was kept in the freezer in case there was need to repeat any of the analyses.

The dry matter and nitrogen concentration (Kjedahl method) of silage samples were determined according to the procedures of AOAC (1990). The NDF and ADF concentrations were determined according to the procedure outlined by Robertson and Van Soest (1981).

3.3.3.1 Silage extraction process

The silage extract (supernatant) from each sample was obtained for the determination of fermentation characteristics. About 80 g of each silage sample was weighed into 500 ml sealable bottle containers to which 320 ml of distilled water was added. The bottles were sealed and shaken for 6 hours at 180 rpm using a horizontal shaker (Bechaz, 2000). The extracted silage was then filtered through four layers of cheese cloth to remove the plant material. The silage extract recovered from each sample was transferred into four different 25 ml plastic bottle containers and stored in a freezer (-15ºC) until time of analysis for fermentation characteristics (VFA, NH_3-N, WSC and lactic acid) (Bechaz, 2000).

3.3.3.2 Silage pH

The remainder of the silage extract obtained after filling up the 25 ml was immediately measured for silage pH. The pH was measured by means of an electrode pH meter (Mettler Toledo) after it had been calibrated by means of pH 4 and pH 7 buffer solutions. Readings were noted to the nearest 0.001 pH unit (Bechaz, 2000).

3.3.3.3 Buffering capacity

Buffering capacity is expressed as the number of milli-equivalents alkali required to change the pH of silage (100g DM) from pH 4 to pH 6 (Playne and McDonald, 1966).

About 5 g of silage sample was placed in a plastic honey jar, 100 ml of distilled water was then added and the sample macerated in a blender for 2 minutes, switching on and off every 20 seconds. The material was then filtered through a Whatman No.1 filter paper and an aliquot of the filtrate (50 ml) was used to determine the pH.

The filtrate was titrated to pH 3 with 0.1 M hydrochloric acid in order to release bicarbonate as carbon dioxide. The same filtrate was then titrated to pH 4 with 0.2 M sodium hydroxide and the burette reading recorded (R1). The titration with 0.1 M sodium hydroxide to pH 6 was conducted and the burette reading recorded (R2). Buffering capacity is calculated as:

Buffering capacity (meq/100g DM) = (390/(R2-R1)ml) x DM% sample.

A correction was made for the titration value of 50ml water blank (Playne and McDonald, 1966).

3.3.3.3 Ammonia nitrogen concentration

The silage supernatant for the analysis of NH_3-N was left to defrost to room temperature. About 50 µl of standard, sample and 0.1 N HCl (blank) were measured into test tubes in duplicates using pipettes. About 2.5 ml of phenol reagent and 2 ml hypochlorite reagent were added and mixed. The tubes were then placed in a water-bath at 95ºC for 5 minutes. The tubes were removed and cooled to between 25 and 30ºC and then read on a spectrophotometer at 630 nm. The NH_3-N concentrations were reported as g/kg DM (Broderick and Kang, 1980).

3.3.3.5 Volatile fatty acids concentration

The frozen silage extracts for VFA were removed from the freezer and allowed to reach room temperature. The extracts were centrifuge at 4500 rpm for 20 minutes in a cooler chamber (less than 10ºC). The samples were then filtered through a Cameo 30 (0.45 µm) filter. About 1µl of sample was injected into a capillary column of a Gas chromatograph (Bechaz, 2000).

Approximately 50 ml of cooled distilled water and 2 ml of ortho-phosphoric acid were added to a clean 100 ml volumetric flask. The flask was then placed on an accurate scale and fatty acids to be determined were added using a pipette. The fatty

acids were added as follows; 120 mg acetic acid, 30 mg propionic acid, 30 mg n-butyric acid, 40 mg iso-butyric acid and 20 mg n-valeric acid. Distilled water was added to volume. A standard was injected repeatedly until consecutive results were comparable (Bechaz, 2000).

The results were calculated as follows;

- Peak area of sample/ Peak area of standard X standard concentration X dilution factor = mg/100 ml acid in the sample
- Divide by molecular mass of acid to obtain the concentration in mmol/100ml sample (Webb, 1994).

3.3.3.6 Lactic acid concentration

The silage supernatant for the analysis of lactic acid concentration was left to defrost to room temperature. About 7.9 ml of precipitating reagent was transferred into a centrifuge tube and 0.10 ml of sample fluid was added. This mixture was centrifuged for 5 minutes at 2000 r.p.m. About 1 ml of supernatant was transfer to a test tube (about 15 x 2.5cm). Sulphuric acid (6 ml) was rapidly added using a fast flowing pipette. It was then allowed to stand for about 2 minutes and mixed. The tube was then placed in running tap water to cool for 2 to 3 minutes (below 20ºC) (Pryce, 1969). About 0.1 ml of p-hydroxydiphenyl solution was added to the tube, mixed well and allowed to stand for 10 minutes. The tube was placed in a boiling water bath for 90 seconds. The tube was cooled again in tap water and then optical density was measured in 1 cm cuvetts at 564 nm wave length (Pryce, 1969). The method was based on the conversion of lactic acid to acetaldehyde by heating with sulphuric acid. The acetaldehyde was reacted with p-hydroxydiphenyl to form a coloured complex which is read through a spectrophotometer. By dissolving the p-hydroxydiphenyl in dimethylformamide instead of an alkali, a reagent is obtained which is indefinitely stable (Pryce, 1969).

3.3.3.7 Water soluble carbohydrates

Water soluble carbohydrates (WSC) concentration of the silage samples was determined according to Harris (1970). The silage supernatant was left to defrost at room temperature. About 1ml of silage was diluted with 9ml of distilled water

(solution 1) in a test tube and thoroughly mixed. About 1ml of solution 1 was also diluted with 9ml of water and mixed (solution 2). About 1ml of solution 2 was added into a test tube and mixed with 0.15ml of Phenol (80%), 5.0ml H_2SO_4 (95-98%), and read on a spectrophotometer after thirty minutes (Harris, 1970).

3.3.3.8 In vitro digestible organic matter (IVDOM)

The procedure used in this study was according to Tilley and Terry (1963), as modified by Engels and Van der Merwe (1967) which involved two digestion phases. During the first digestion phase, dried plant materials at each sampling time (0, 7, 21, 42 &120) were incubated in triplicate under anaerobic conditions with rumen liquor for 48 hours at 39°C. This was followed by a 24 hour acid pepsin digestion phase at 39°C, under anaerobic conditions. Following the 72 hour incubation, the residual plant materials were collected and oven dried at 105°C for 12 hours. Ash contents were determined by combustion (550°C for 2 hours) (Engels and Van der Merwe, 1967).

Calculation

% IVDOM = (1 – wd – wb/ws) x 100

Where: wd=weight of dry plant residue
wb=weight of dry residues from blank
ws=weight of original plant sample (Engels and Van der Merwe, 1967)

3.3.4 Statistical analysis

The GLM (General Linear Models) procedure of SAS (1990) for repeated measure analysis was used to test for statistical differences between treatments (0, 10, 20, 30 and 40% WDGS) and between sampling periods (Day 0, 7, 21, 42 and 120).

The three bottles per sampling period per treatment were used as replicates. The Benferroni's mean separation test (Samuels, 1989) was used to determine differences of parameters measured.

3.4 Rumen fermentation

3.4.1 Procedure

Three treatments were used to obtain certain rumen fermentation parameters. The diets for the sheep used were formulated on an iso-nutrient basis. The maize plants were chopped between 10 mm and 20 mm in length using a maize silage cutter. Chopped whole maize plants were either ensiled alone (BMS) or blended with 27% wet distillers grains with solubles (WDGS-maize silage) on an as is basis. Therefore, on a DM basis, it means that 6.75% DM of WDGS was mixed with 37% DM of maize plants for the production of WDGS-maize silage (WDGSMS). The remainder of the WDGS after ensiling was oven dried at 55ºC for 48 hours to produce DDGS with a moisture concentration of between 10 and 15% DM.

This range of DDGS moisture concentration is recommended for the ideal preservation of distillers grains with solubles (Schroeder, 2003). The two silage treatments (BMS and WDGSMS) were ensiled in bulk using plastic bags (0.8 m x 1.05 m) for a period of 120 days. The amount of BMS silage was double that of WDGSMS blend since two ingredients (sunflower oilcake meal and DDGS) were going to be mixed with BMS at feeding, to create two separate diets.

Part of whole plant maize silage (BMS) was blended with sunflower oilcake meal (SOM) at the point of feeding. This was the positive control diet (MS SOM). The other part of BMS was blended with dried distillers grains with solubles (DDGS) at the point of feeding to create a maize silage-DDGS diet. The WDGS-maize silage was further mixed with sunflower oil cake, mineral mixture and maize meal at the point of feeding to adjust the feed to iso-nutrient levels required for growing lambs. This adjustment was repeated in the other two treatments. The iso-nutrient basis formulation was designed to meet the requirements for growing lambs and was based on CP, ME, NDF, Ca and P concentrations.

Table 3.1 represents the mixture of feed ingredients for the three different treatments during diet formulations conducted by using the Format International Software Inc.

Table 3.1 Ingredient and nutritional composition of the experimental diets during feed formulation

Item	Diet			
	MS DDGS	MS SOM	WDGSMS	% DM
Ingredients (%DM)				
Maize silage	67.7	75.4	-	33.5
Maize meal	7.6	4.1	2.5	92.8
WDGSMS	-	-	91.1	30.1
DDGS	23.8	-	-	96.9
SOM	-	19.5	5.5	92.1
Limestone (36%)	0.4	0.5	0.4	-
Monocalcium PO_4	0.5	-	0.5	-
Chemical composition				
ME (MJ/kg DM)	10.5	10.5	10.4	
CP (g/kg DM)	146.7	147.1	147.2	
NDF (g/kg DM)	436.7	428.1	431.5	
Ca (g/kg DM)	7.4	7.6	7.7	
P (g/kg DM)	3.5	3.8	3.6	

MS DDGS – dried distillers grains with solubles/whole maize silage diet

MS SOM – sunflower oilcake meal/whole maize silage diet

WDGSMS – wet distillers grains with solubles blended with whole maize at ensiling diet

Three rumen cannulated sheep were individually housed in metabolism crates. Each individual sheep was randomly allocated to one of the three experimental diets, (MS SOM (control), MS DDGS and WDGSMS). The sheep were dosed against internal parasites on arrival at the experimental site. The experiment was conducted over three periods and was of a 3 x 3 Latin square changeover design. The duration of this trial was for a total of thirty nine days, which was divided into three periods. Each period consisted of thirteen days. The first ten days were for adaptation, followed by three days of data collection, which involved collection of rumen fluid samples for pH, volatile fatty acids (VFA) and ammonia nitrogen concentration (NH_3-N) analyses. Diets were offered *ad libitum* once daily at 0600 hours. Water was available all times. Each diet was sampled every day and pooled by period for each animal. Orts were removed daily, weighed, sampled, and pooled by period for each animal.

Sampling was conducted from day eleven until the end of each sampling period. The difference between the quantity of DM offered and the quantity refused from day eight to nine was used to calculate the *ad libitum* DMI for each sheep.

3.4.2 Rumen sampling

The sampling period lasted for three days to reduce rumen environment disturbances which were expected to occur when opening the cannulae frequently (Bechaz, 2000). Ruminal fluid samples were collected at 2 hour intervals after feeding over an equivalent of a 24 hour period. On the first day, sampling occurred at 06:00, 12:00, 18:00 and 00:00; on the second day at 08:00, 14:00, 20:00 and 02:00; and on the third day at 10:00, 16:00, 22:00 and 04:00. The rumen fluid was extracted via the rumen cannulae using a suction pump (vacuum fluid extractor). Representative samples of the rumen fluid were collected at different locations within the rumen using a suction pump with a 0.02 mm sieve and collected in a 500ml plastic container and pH was determined. The filtrate was subdivided for the appropriate analysis (NH_3-N and VFA) and preserved as follows: 30 ml of rumen fluid preserved with 5 ml of 50% sulphiric acid (H_2SO_4) and frozen (-20°C) for further analysis for rumen ammonia nitrogen concentration; and 20ml of rumen fluid was preserved with 4ml of 25% H_3PO_4 and frozen at -20°C for further analysis for rumen VFA concentration (Kennelly *et al.*, 1999). The remainder of the rumen fluid was discarded.

3.4.3 Analytical methods

Samples of rumen fluid were stored in a freezer at -20°C prior to the analysis of NH_3-N and VFA. Dry matter intake was calculated as the difference between the quantity of DM offered and the quantity refused by each animal. Feed and orts samples were collected and analysed for DM feed intake.

3.4.3.1 Rumen pH

After the rumen fluid extraction, the fluid was poured into a 100ml container and the rumen pH was immediately measured using a battery powered Mettler Toledo portable pH meter.

3.4.3.2 Rumen ammonia nitrogen

The NH_3-N concentration of the rumen fluid samples was determined as described by Broderick and Kang (1980). See section 3.3.4.4.

3.4.3.3 Rumen volatile fatty acids

The analysis of rumen VFA was similar to that of silage (section 3.3.4.5), with the only difference being the dilution factor for the different fatty acids. The standard fatty acids were added as follows: 450 mg acetic acid, 200 mg propionic acid, 70 mg n-butyric acid, 25 mg iso-butyric acid and 25 mg n-valeric acid (Bechaz, 2000).

3.4.4 Statistical analysis

Data was analyzed statistically by using the General Linear Models procedure of SAS (1990) for the Latin square design. The Benferroni's mean separation test (Samuels, 1989) was used to determine differences of parameters measured.

3.5 Dry matter degradability trial

3.5.1 Procedure

Three rumen cannulated sheep were used as in the rumen fermentation trial, where sheep were fed MS SOM, MS DDGS and WDGSMS diets in a 3 x 3 Latin square changeover design. The duration of this trial was for a total of thirty six days, which was subdivided into three periods. Each period consisted of twelve days, which included ten days of adaptation, and two days of data collection.

There were three test feeds which were separately incubated in the rumen of the cannulated sheep, that is, MS SOM, MS DDGS and WDGSMS. The test feed samples were dried at 60°C for 48 hours and then ground to pass through a 2mm screen. The dry matter of the grounded samples was determined as in section 3.3.4.1. The nylon bags (14 x 6.5 cm) were oven dried at 60°C for 30 minutes and their empty weights measured after allowing cooling to room temperature in a desiccator. About 5g DM of sample was placed in each nylon bag (each sample prepared in duplicate at each time of incubation) and tightly tied using nylon strings (Osuji *et al.*, 1993). The nylon bags were then tied to a round stainless steel disc (135g, 4cm diameter and 5mm thick)

with ten evenly spaced small holes drilled through the periphery of the disc. The disc was tied to a 30 cm nylon string which was secured at the rumen cannulae

3.5.2 Incubation and analytic methods

Each test material (MS SOM, MS DDGS and WDGSMS) was incubated separately in the rumen of cannulated sheep fed lucerne hay. Samples were incubated at different times (0, 2, 4, 6, 8, 12, 24, and 48 hours), and withdrawn at the same time (sequential addition) (Osuji *et al.*, 1993). At each incubation time, 2 bags of the test material were placed into the rumen of a corresponding sheep, which translated to 14 bags per sheep after all bags were incubated (excluding the 0 hours incubation). Two discs with seven bags each were incubated and separated by placing the supporting string through a small plastic tube (25cm, 6 mm diameter) to avoid tangling of the separate strings. Bags of the zero hour interval as well as the incubated ones were washed with cold water using a pressure washing machine (Sputnik 3) immediately after removing the incubated bags. The washed bags were dried in an oven at 60 °C for about 48 hours and then cooled in a desiccator before weighing to determine DM of the residue samples (Ørskov and McDonald, 1979). The disappearance of DM was calculated according to Osuji *et al.* (1993) as follows:

$$\%DMD = \frac{(SWa - BW) \times DMa - (SWb - BW) \times DMb}{(SWa - BW) \times DMa} \times 100$$

Where: SWa = weight of the original sample + nylon bag

BW = weight of empty nylon bag

SWb = weight of the sample + nylon bag after incubation

DMa = dry matter of feed sample

DMb = dry matter of residue sample

The DMD values at different time intervals were used to calculate degradation constants of DM using the nonlinear model: $y = a + b\,(1-e^{-ct})$ suggested by Ørskov and McDonald (1979) where; y = disappearance of DM at time t

a = rapidly soluble (washing loss) fraction

b = the slowly degradable fraction

c = the rate (%/h) of degradation of fraction b.

These degradation constants were used to estimate effective degradability (ED) following the model of Ørskov and McDonald (1979): ED = a + [bc/(k + c)], where k is the passage rate from the rumen, estimated to be 4%/h.

3.5.3 Statistical analysis

Analysis of variance was performed using the General Linear Model (SAS, 1990) for a 3 x 3 Latin square design to establish the effect of treatment on DMD. Means were separated using Bonferroni's test (Samuels, 1989) at $P < 0.05$ confidence limit.

3.6 Growth performance trial

3.6.1 Procedure

A total of twenty four ram lambs (Merino-type) acquired from the University Experimental Farm, were used in this growth performance trial to evaluate three treatments in a completely randomized design. Prior to the start of the trial, lambs were dosed against internal parasites, and vaccinated against pulpy kidney and pasteurella. The lambs were stratified by weight (29.7±6.3) and assigned randomly to the three different experimental diets (MS SOM, MS DDGS and WDGSMS). Each treatment was assigned to eight animals that were individually housed in metabolic crates. Diets were offered *ad libitum* three times a day at 0600, 1300, and 2100hrs, that is, same time every day. Fresh water was provided every day.

Lambs were fed the experimental diets for the adaptation period for ten days, to accustomise the animals to the diet, adjusted their feed intake and cleared the gastro-intestinal tract of the residues of the previous feed. This was followed by the data collection period which lasted for 45 days. Animal weights were recorded on two consecutive days at the start of the trial and averaged to establish their average initial weights. The lambs were weighed every seven days (weekly) during the data collection period (45 days). Each diet offered to an individual animal was weighed and recorded prior to feeding. Representative samples for each diet were collected each time before feeding and pooled over the entire trial period. Orts were removed, weighed, recorded and representative samples collected everyday. A composite sample of orts for each experimental unit (animal) were pooled over the trial period and stored in a freezer until the time of analysis (Drouillard *et al.*, 1991).

3.6.2 Analytical methods

Representative samples of the raw materials and the three experimental diets were taken prior to and during the trial for the determination of DM, CP, ME, Ca and P concentration.

3.6.2.1 Determination of dry matter and nitrogen concentration

The concentrations of DM and N were conducted on both raw material and diet samples. These parameters (DM and N) were determined according to the methods described in section 3.3.4.1 and 3.3.4.2 respectively.

3.6.2.2 Fat concentration

Sample for fat concentration were determined according to the Soxtec procedure as described by Harris (1970).

3.6.2.3 Metabolisable energy concentration

Samples from the raw materials were determined for ME concentration. A computer program spreadsheet to predict the ME value of feedstuff by Robinson (2003) was used to determine ME concentration of the samples. The spreadsheet can be downloaded from the author's website. The program requires the determination of sample parameters that include DM, organic matter (OM), fat, N, acid detergent insoluble crude protein (ADICP), NDF and 30 hour *in vitro* NDF digestibility (dNDF) to calculate the ME value (Robinson, 2003). The values of the above mentioned analyses were entered into the simple spreadsheet to estimate the energy value of the feeds.

3.6.2.3.1 Dry matter and nitrogen concentration

The dry matter and nitrogen concentration were determined according to the methods described in section 3.3.3.

3.6.2.3.2 Ash concentration and Acid detergent insoluble crude protein

The ash and acid detergent insoluble crude protein (ADICP) concentration were determined according to the procedure outlined by AOAC (1990). Ash concentration

was required to calculate the OM concentration for the subsequent calculation of ME concentration.

3.6.2.3.3 Organic matter concentration

The OM concentration was calculated by utilizing the DM and ash concentration as shown below;

% OM (DM) = 100% - % Ash (DM)

3.6.2.3.4 Neutral detergent fibre concentration

The NDF and ADICP concentration of the samples was determined as described by Robertson and Van Soest (1981).

3.6.2.3.5 30 hour in vitro NDF digestibility

The *in vitro* NDF assay that was used in this study was the 30h *in vitro* NDF, which simply means that the sample of feed was incubated in a 20ml test tube with rumen fluid for 30 hours. The procedure was outlined in detail by Robinson (2003). The 30h period was selected since it best correlated to digestion of feeds in the rumen at maintenance.

3.6.2.4 Calcium concentration

The calcium concentration of the samples was determined using the procedure described by Giron (1973).

3.6.2.5 Phosphorus concentration

Phosphorus concentration was determined as outlined by AOAC (2000).

3.6.3 Statistical analysis

The GLM (General Linear Models) procedure of SAS (1990), was used in a complete randomized design to test for statistical differences between the three experimental diets (MS DDGS, MS SOM and WDGSMS), with animals as the experimental units. Significance of difference between least square means was determined by Bonferroni's test (Samuels, 1989) at $P < 0.05$ confidence limit.

CHAPTER 4

Results and Discussion

4.1 Nutrient composition of raw materials

It is important to describe the quality of raw materials and other ingredients used in this study. The chemical composition of feedstuffs (Table 4.1.1) used during ensiling generally determines the pattern of silage fermentation and ultimately the quality of the silage post ensiling.

Table 4.1.1 Chemical composition of feed ingredients in g/kg DM

Item	Maize	SOM	WDGS	DDGS	BMS	WDGSMS
DM	370	921	251	969	350	314
CP	67.5	418.5	334.1	348.4	84.9	137.5
NDF	454.4	346.8	519.0	557.8	532.9	491.9
Fat	64.1	14.4	155.1	93.0	80.9	49.9
Ca	2.9	5.4	2.9	3.6	4.1	3.7
P	1.8	11.3	8.5	6.8	2.6	2.4

The dry matter (DM) concentration of wet distillers grains (WDGS) was lower while that of SOM and DDGS was higher (251, 921 and 969 g/kg as is, respectively). After ensiling the bulk whole maize plants (BMS), DM concentration remained unchanged. However, when WDGS was blended with maize plants, DM concentration was higher (314 g/kg) than that of WDGS alone. The low DM concentration in WDGS was complemented by the higher DM concentration in whole plant maize, resulting in reduced moisture concentration in WDGS-whole plant maize mixture (Garcia and Kalscheur, 2004). The WDGS already has a fixed DM concentration; therefore the mixture resulted in a lower DM concentration (that is WDGSMS). This complementary effect was essential for creating an ideal environment necessary for a desirable fermentation process. McDonald *et al*. (1991) reported that a high moisture concentration (<30% DM) at ensiling resulted in an undesirable fermentation process due to a slow decline in pH. Very wet silages can result in high concentrations of acetic acid (> 3 to 4% of DM) and may lead to less than ideal recovery of energy and DM (Kung and Shaver, 2001).

One notable thing was that distillers grains (wet and dry) supplied more nutrients (NDF, fat and P) than other feedstuffs, except for SOM which was higher in CP and Ca concentration. When compared to whole maize plants, CP concentration in DDGS and WDGS was about five times higher. However, sunflower oilcake meal had the highest CP concentration (418.5 g/kg DM) compared to whole maize plants, WDGS and DDGS (67.5, 334.1 and 348.4 g/kg DM respectively). However, when WDGS and whole maize plants were ensiled, the two feedstuffs exhibited a complementary nutrient profile for CP, fat and P, as presented in Table 4.1.1. Garcia and Kalscheur (2006) recommended the ensiling of WDGS with forage crops that have a complementary nutrient profile such as low protein, fat, energy and phosphorus.

The neutral detergent fibre (NDF) concentration for SOM was lower than in other feedstuffs. The NDF concentrations for whole maize plants and BMS was consistent with the range (300-580 g/kg DM) suggested by Schroeder (2004) for maize plants ensiled at hard dough stage of maturity. This NDF fraction was not expected to change during the ensiling period (Shaver, 2003) for distillers grains and maize plants, as the main substrate of fermentation was expected to be the water soluble carbohydrate fraction of the ensiled material, mainly derived from whole maize plants (McDonald *et al*, 1991).

4.2 Ensiling maize plants with WDGS in laboratory silos

4.2.1 Dry matter concentration

The DM concentrations of five different inclusion levels of WDGS are presented in Table 4.2.1. Dry matter concentration decreased with increasing level of WDGS inclusion prior to ensiling (day 0). There was a significant decrease in DM concentration for all treatments between day 0 and 7, which was in agreement with McDonald *et al.* (1991) who reported decreases in DM concentration during the first week of ensiling. The immediate DM losses could probably be due to rapid fermentation of carbohydrates, mainly water soluble carbohydrates (WSC), which is the primary substrate for lactic acid fermentation (Muck, 1988). Dry matter losses in wetter silages (30% and 40% WDGS) were probably due to packing pressure during ensiling which squeezes out soluble sugars, proteins and minerals along with water,

resulting in a silage effluent (evident in this study) representing losses of nutrients which are part of DM (Charmley, 2004).

Table 4.2.1 Mean values (±s.e) of DM (g/kg) concentration for maize ensiled at five inclusion levels of wet distillers grains with solubles

		Time (days)					
Parameter	**Treatment**	**0**	**7**	**21**	**42**	**120**	**SEM***
DM (g/kg)	0% WDGS	$366^{a}{}_{1}$	$352^{bc}{}_{1}$	$350^{c}{}_{1}$	$361^{ab}{}_{1}$	$360^{ab}{}_{1}$	±0.252
	10% WDGS	$356^{a}{}_{1}$	$321^{b}{}_{2}$	$319^{b}{}_{2}$	$322^{b}{}_{2}$	$309^{b}{}_{2}$	±0.252
	20% WDGS	$334^{a}{}_{2}$	$311^{b}{}_{2}$	$309^{b}{}_{2}$	$298^{c}{}_{3}$	$292^{c}{}_{3}$	±0.252
	30% WDGS	$328^{a}{}_{2}$	$298^{b}{}_{3}$	$293^{b}{}_{3}$	$286^{bc}{}_{4}$	$275^{c}{}_{4}$	±0.252
	40% WDGS	$311^{a}{}_{3}$	$288^{b}{}_{3}$	$272^{c}{}_{4}$	$270^{c}{}_{5}$	$266^{c}{}_{4}$	±0.252
SEM*		±0.113	±0.113	±0.113	±0.113	±0.113	

^{a,b,c,d,e} For each row means with common superscripts do not differ (P>0.05)

$_{1,2,3,4,5}$ For each column means with common subscripts do not differ (P>0.05)

* Standard error of the mean

The DM concentration for 0% WDGS further decreased at day 21, but increased thereafter until day 120 post ensiling. At 10% WDGS inclusion, the DM concentration remained constant over the entire ensiling period from day 7 onwards. A significant decrease in DM concentration occurred after day 21 for 20% WDGS inclusion, but remained constant until day 120. The DM concentration of silage ensiled at 30% WDGS remained constant after day 7 until day 42, but decreased at day 120. At 40% WDGS inclusion, there was a significant decrease in DM concentration at day 7 and 21, but DM concentration did not change until day 120.

The DM losses at day 120 post ensiling for the control and 10% WDGS were lower than for other three treatments. The control treatment lost 0.6% of DM compared to treatments blended with WDGS which recorded losses of more than 4% of DM. Wet silages usually lead to less than ideal recovery of DM (Muck, 1988), which was consistent with the results of the present study where DM losses were high with increasing moisture concentration, especially for 30% and 40% WDGS treatments. In contrast, Kalscheur *et al*. (2003) ensiled chopped maize plants with wet distillers grains (WDG) at 0%, 25%, 50% and 100% level of inclusion and reported increased DM concentrations with increasing level of WDG inclusion. This was probably due to higher moisture concentration in chopped maize plants than in WDG, which did not contain solubles. In the present study, the lower DM concentration with increased

level of WDGS could have been as a result of high moisture concentration present in WDGS (Table 4.1.1).

Very limited data is available on the preservation of WDGS using the bottle ensiling method. However, in the study of Muck (1987) the DM concentration affected the rate of fermentation and thus pH pattern as well as proteolysis. Muck (1987) reported that DM concentration influences the growth of lactic acid bacteria (LAB) and thus the pH time course. An interaction between initial numbers of LAB and DM content on pH time course may cause a large variation with the amount of proteolysis (Muck, 1987).

4.2.2 Silage pH and buffering capacity

The changes in pH for five different inclusion levels of WDGS are presented in Table 4.2.2. Variations in pH across treatments at day 0 were noted, with lower pH values as WDGS inclusion level increased. The originally low pH of WDGS (3.2), as a result of sulphuric acid addition during ethanol production (Garcia and Kalscheur, 2006), may have resulted in lower pH values of WDGS blended silages prior to ensiling.

Table 4.2.2 Mean values (±s.e) of pH and buffering capacity (meq/100g DM) for maize ensiled at five levels of WDGS inclusion

		Time (days)					
Parameter	**Treatment**	**0**	**7**	**21**	**42**	**120**	**SEM***
pH	0% WDGS	$5.89^{a}{}_{1}$	$3.92^{b}{}_{3}$	$3.81^{c}{}_{3}$	$3.78^{c}{}_{2}$	$3.77^{c}{}_{1,2}$	±0.014
	10% WDGS	$5.39^{a}{}_{2}$	$4.14^{b}{}_{2}$	$3.89^{c}{}_{2}$	$3.80^{d}{}_{1}$	$3.79^{d}{}_{1}$	±0.014
	20% WDGS	$5.20^{a}{}_{3}$	$4.24^{b}{}_{1}$	$3.87^{c}{}_{2}$	$3.84^{c}{}_{1}$	$3.73^{d}{}_{2}$	±0.014
	30% WDGS	$5.22^{a}{}_{3}$	$4.28^{b}{}_{1}$	$3.89^{c}{}_{2}$	$3.78^{d}{}_{2}$	$3.71^{e}{}_{2}$	±0.014
	40% WDGS	$4.89^{a}{}_{4}$	$4.24^{b}{}_{1}$	$3.98^{c}{}_{1}$	$3.65^{d}{}_{3}$	$3.62^{d}{}_{3}$	±0.014
SEM*		±0.006	±0.006	±0.006	±0.006	±0.006	
Buffering capacity (meq/100g DM)	0% WDGS	141.2^{a}	$53.4^{b}{}_{4}$	$53.20^{b}{}_{1,2}$	49.74^{b}	$41.24^{c}{}_{1}$	±1.571
	10% WDGS	142.3^{a}	$80.9^{b}{}_{2}$	$59.53^{c}{}_{1}$	48.04^{d}	$34.18^{d}{}_{2}$	±1.571
	20% WDGS	146.0^{a}	$70.8^{b}{}_{3}$	$58.01^{c}{}_{1}$	44.52^{d}	$31.06^{e}{}_{2,3}$	±1.571
	30% WDGS	145.5^{a}	$81.0^{b}{}_{2}$	$51.84^{c}{}_{2}$	42.89^{d}	$27.89^{e}{}_{3}$	±1.571
	40% WDGS	147.5^{a}	$94.9^{b}{}_{1}$	$50.52^{c}{}_{2}$	46.76^{c}	$34.18^{d}{}_{2}$	±1.571
SEM*		±0.702	±0.702	±0.702	±0.702	±0.702	

^{a,b,c,d,e} For each row means with common superscripts do not differ (P>0.05)

$_{1,2,3,4,5}$ For each column means with common subscripts do not differ (P>0.05)

*Standard error of the mean

There was a significant decrease in pH values for all treatments at day 7 with the 0% WDGS (control) having the highest decrease compared to maize blended with WDGS silage. The rate at which pH dropped at day 7 decreased with an increasing inclusion level of WDGS. Garcia and Kalscheur (2006) recorded pH levels below 4 at day 3 when ensiling a blend of chopped maize and wet distillers grains (WDG). Their findings did not agree with the results of this study where pH values for maize blended with WDGS were above 4 after day 7. However, the 0% WDGS treatment recorded pH values below 4 at day 7, suggesting a rapid fermentation in the early stages of ensiling which assists in the prevention of clostridial activity (Zimmerman, 2002). The reduced rate of a pH decline in silage blended with WDGS at day 7, as compared to the control, could have been caused by reduced WSC supply (Table 4.2.3) as well as an increased moisture concentration which reduced the activity of lactic acid bacteria (Kung and Stokes, 2005).

The silage pH values for the control continued to decrease ($P<0.05$) at day 21, with no further decrease ($P>0.05$) until day 120. It was interesting to note that the pH values for all treatments blended with WDGS continued to decrease until day 120, contrary to the study of Garcia and Kalscheur (2006) who reported no further pH decline after day 3. The mean pH value for the 40% WDGS level of inclusion was the lowest at day 120 post ensiling (3.62) with that of 10% WDGS level of inclusion being the highest and comparable ($P>0.05$) to that of the control treatment. The low pH for 40% WDGS could have been partly as a result of a high concentration of sulphuric acid present in WDGS (Garcia and Kalscheur, 2006) and partly due to an increased lactic acid production (Table 4.2.3)

Kung and Shaver (2001) reported that the typical pH range for well fermented maize silage should be between 3.7 to 4.2, which was consistent with results of the control treatment (whole maize plant) at day 120 post ensiling. The pH values for silage blended with 10% to 30% WDGS fell within the pH range of 3.7 to 4.2 at day 120 post ensiling despite having increasing levels of moisture. At 40% WDGS level of inclusion, the final pH values was below the pH range of 3.7 to 4.2 as suggested by Kung and Shaver (2001). Contrary to other reports (Zimmerman, 2002), pH was not affected by high moisture concentration. The low DM concentration exhibited in the

30% and 40% WDGS treatments did not affect the final pH of the silage. The low pH values recorded by maize silage blended with WDGS could also be influenced by the presence of sulphuric acid in WDGS (Garcia and Kalscheur, 2006) and the continued fermentation of the silage.

In this study, changes in the buffering capacity of five different inclusion levels of WDGS are presented in Table 4.2.2. The initial buffering capacity value for all treatments were similar (P>0.05). Therefore, the different inclusion levels of WDGS did not seem to have an effect on the initial buffering capacity. The initial buffering capacity is an important measure as it determines the degree to which the ensiled material resists changes in pH (McDonald *et al.*, 1991). In the present study, the initial buffering capacity ranged between 141.2 to 147.5 meq/100g DM and this was within the range (<200 meq/100g DM) reported by Kung and Stokes (2005) for whole maize plant silage. Buffering capacity for 0% WDGS drastically decreased between day 0 and 7 when compared to other treatments. The slow drop in buffering capacity for maize silage blended with WDGS at day 7 could have been due to the presence of a high protein content (Horvey, 2003) which extends the start of the fermentation process, thus longer time required to lower buffering capacity. This may explain the continued slow decrease in buffering capacity for whole maize silage blended with WDGS treatments throughout the ensiling period. The resistance to change pH for maize silage blended with WDGS increased with the inclusion level of WDGS. This increase in buffering capacity may be related to a high CP concentration in WDGS as well as lower fermentable carbohydrates (Horvey, 2003). Buffering capacity is generally associated with pH (McDonald *et al*., 1991); and this association was evident in this study, as proportionate decrease in pH led to a subsequent decrease in buffering capacity for all treatments.

4.2.3 Water soluble carbohydrates

The changes in the water soluble carbohydrate (WSC) concentration of five different inclusion levels of WDGS are presented in Table 4.2.3. The water soluble carbohydrate concentration decreased with increasing levels of WDGS prior to ensiling, with the control treatment having the highest concentration. This is because the sugars and starch in WDGS are expected to be completely fermented during

ethanol production (Kaiser, 2006). Therefore, the decrease in WSC was expected with the proportionate increase in WDGS inclusion to the chopped maize plants. At day 0, WSC concentrations of all treatments were lower (Table 4.2.3) compared to WSC values reported for initial maize silage by Wilson and Webb (1937). Horvey (2003) suggested that a minimum of between 60 and 120 g/kg DM of WSC is required for desirable silage fermentation. In this study, the initial WSC concentration for all treatments were within the ranged (60 to 120 g/kg DM) reported by Horvey (2003).

Table 4.2.3 Mean values (±s.e) of WSC (g/kg DM) for maize ensiled at five levels of WDGS inclusion

		Time (days					
Parameter	**Treatment**	**0**	**7**	**21**	**42**	**120**	**SEM***
WSC (g/kg DM)	0% WDGS	$105.15^{a}{}_{1}$	$5.14^{c}{}_{2}$	10.51^{b}	$9.47^{b}{}_{2}$	$10.66^{b}{}_{2}$	±0.913
	10% WDGS	$75.49^{a}{}_{2}$	$6.66_{1,2}{}^{c}$	7.31^{bc}	$7.82_{2}{}^{bc}$	$10.48^{b}{}_{2}$	±0.913
	20% WDGS	$72.24^{a}{}_{2}$	$6.54_{1.2}{}^{d}$	9.59^{cd}	$11.59_{2}{}^{c}$	$18.72^{b}{}_{1}$	±0.913
	30% WDGS	$67.64^{a}{}_{2,3}$	$6.75_{1,2}{}^{d}$	8.49^{d}	$14.50_{1}{}^{c}$	$20.90^{b}{}_{1}$	±0.913
	40% WDGS	$64.11^{a}{}_{3}$	$10.01_{1}{}^{d}$	9.68^{d}	$15.48_{1}{}^{c}$	$22.11^{b}{}_{1}$	±0.913
SEM*		±0.408	±0.408	±0.408	±0.408	±0.408	

^{a,b,c,d,e} For each row means with common superscripts do not differ (P>0.05)

$_{1,2,3,4,5}$ For each column means with common subscripts do not differ (P>0.05)

* Standard error of the mean

There was a drastic decrease in WSC concentrations for all treatments at day 7 compared to any other day during the ensiling period, a finding which was in agreement with other studies on maize silages (Wilson and Webb, 1937; McDonald *et al*., 1991; Kim and Adesogan, 2006). Water soluble carbohydrates are utilized by homo- and hetero-fermentative microorganisms as an energy source for growth during fermentation. The WSC include sugars present in plants like fructose, glucose, sucrose and fructosans (Kung, 2000). Soluble carbohydrates from sugar and starch are also needed by lactic acid bacteria to produce lactic acid during the fermentation process (Kung, 2000; Downing *et al*., 2008). Based on results presented in Table 4.2.6, more lactic acid was expected to be produced in the control treatment compared to other treatments. There was a small increase in WSC concentration at day 120 for all treatments. The reason for this increase in WSC towards the end of the ensiling

period could not be explained by the researcher at the time when this study was conducted.

4.2.4 Lactic acid

The changes in the lactic acid concentration of the five different inclusion levels of WDGS are presented in Table 4.2.4. The lactic acid concentration did not differ (P>0.05) among treatments prior to ensiling (day 0), but drastically increased at different rates at day 7. Lactic acid was the highest for the control treatment at day 7, which could have been as a result of sudden proliferation of *Lactobacillus* spp., resulting in lactic acid production (Kung and Shaver, 2001). It is possible that the low initial pH of WDGS (pH 3.2) inhibited the proliferation of homofermentative bacteria which are responsible for lactic acid production (Seglar, 2003) in maize silage blended with WDGS during the early stages of fermentation.

Table 4.2.4 Mean values (±s.e) of lactic acid concentration (g/kg DM) for maize ensiled at five levels of WDGS inclusion

		Time (days)					
Parameter	**Treatment**	**0**	**7**	**21**	**42**	**120**	**SEM***
Lactic acid (g/kg DM)	0% WDGS	0.03^{d}	$18.52^{c}{}_{1}$	$22.34^{b}{}_{1}$	$23.11^{b}{}_{1}$	$24.90^{a}{}_{1}$	±0.415
	10% WDGS	0.29^{d}	$12.75^{c}{}_{2}$	$21.32^{b}{}_{1}$	$23.42^{a}{}_{1}$	$22.29^{ab}{}_{2}$	±0.415
	20% WDGS	0.43^{d}	$10.40^{c}{}_{3}$	$21.47^{a}{}_{1}$	$19.56^{b}{}_{2}$	$21.67^{a}{}_{2}$	±0.415
	30% WDGS	0.24^{d}	$8.74^{c}{}_{3,4}$	$21.50^{a}{}_{1}$	$19.73^{b}{}_{2}$	$21.90^{a}{}_{2}$	±0.415
	40% WDGS	0.90^{d}	$7.77^{c}{}_{4}$	$18.54^{b}{}_{2}$	$22.67^{a}{}_{1}$	$21.32^{a}{}_{2}$	±0.415
SEM*		±0.186	±0.186	±0.186	±0.186	±0.186	

^{a,b,c,d,e} For each row means with common superscripts do not differ (P>0.05)

$_{1,2,3,4,5}$ For each column means with common subscripts do not differ (P>0.05)

*Standard error of the mean

Significant increases in lactic acid occurred between day 7 and 21 for all treatments, but no further increases were recorded for 20% and 30% WDGS level of inclusion until day 120. In most silage trials (Seglar, 2003; Garcia and Kalscheur, 2006), the peak lactic acid concentrations were reached between day 7 and 21, which was consistent with results for 20% and 30% WDGS level of inclusion. An early peak in lactic acid is beneficial as it result in a pH drop and assist to preserve the silage quicker with less nutrient losses (Selgar, 2003). In this study, more lactic acid was

produced in the control treatment compared to maize silage blended with varying levels of WDGS at day 120 post ensiling. The reduced proportion of chopped maize plants with an increasing level of WDGS inclusion may also have reduced lactic acid production due to the absence of a fermentative substrate (water soluble carbohydrates) contributed by maize, which is used in the fermentation process. The WDGS is devoid of WSC and starch (Kaiser, 2006). Lactic acid is the strongest and most abundant acid produced during an ideal fermentation (Kung and Stokes, 2005), and responsible for most of the drop in silage pH. The blending of WDGS with whole maize plants normally resulted in fermentation patterns that follow an acetic acid production (Garcia and Kalscheur, 2004), leading to a lower lactic acid production.

4.2.5 Volatile fatty acids

4.2.5.1 Acetic acid

The changes in the acetic acid concentration of the five different inclusion levels of WDGS are presented in Table 4.2.5.

Table 4.2.5 Mean values (±s.e) of acetic and propionic acid concentrations (g/kg DM) for maize ensiled at five levels of WDGS inclusion

		Time (days)					
Parameter	**Treatment**	**0**	**7**	**21**	**42**	**120**	**SEM***
Acetic acid (g/kg DM)	0% WDGS	0.65^{c}	$8.01^{b}{}_{1}$	$10.81^{ab}{}_{1}$	$11.68^{ab}{}_{1}$	$12.48^{a}{}_{2}$	±0.704
	10% WDGS	1.16^{d}	$5.87^{c}{}_{2}$	$7.89^{bc}{}_{2}$	$9.07^{b}{}_{2}$	$17.10^{a}{}_{1,2}$	±0.704
	20% WDGS	1.02^{c}	$5.05^{b}{}_{2}$	$7.28^{b}{}_{2}$	$7.81^{b}{}_{2}$	$20.03^{a}{}_{1}$	±0.704
	30% WDGS	1.05^{d}	$5.08^{c}{}_{2}$	$6.03^{c}{}_{2}$	$9.57^{b}{}_{2}$	$19.51^{a}{}_{1}$	±0.704
	40% WDGS	0.79^{d}	$3.72^{c}{}_{2}$	$7.15^{b}{}_{2}$	$9.83^{b}{}_{2}$	$24.53^{a}{}_{1}$	±0.704
SEM*		±0.315	±0.315	±0.315	±0.315	±0.315	
Propionic acid (g/kg DM)	0% WDGS	0.06^{c}	$1.39^{a}{}_{1}$	$1.33^{a}{}_{1}$	$0.94^{b}{}_{1}$	$1.07^{ab}{}_{2}$	±0.579
	10% WDGS	0.13^{b}	$0.43^{b}{}_{2,3}$	$0.84^{a}{}_{2}$	$0.84^{a}{}_{1}$	$1.02^{a}{}_{2}$	±0.579
	20% WDGS	0.11^{d}	$0.64^{c}{}_{2}$	$1.12^{b}{}_{1,2}$	$0.98^{bc}{}_{1}$	$1.76^{a}{}_{1}$	±0.579
	30% WDGS	0.12^{b}	$0.34^{b}{}_{2,3}$	$0.84^{a}{}_{2}$	$1.03^{a}{}_{1}$	$1.18^{a}{}_{2}$	±0.579
	40% WDGS	0.13	0.13_{3}	0.19_{3}	0.18_{2}	0.33_{3}	±0.579
SEM*		±0.259	±0.259	±0.259	±0.259	±0.259	

^{a,b,c,d,e} For each row means with common superscripts do not differ (P>0.05)

^{1,2,3,4,5} For each column means with common subscripts do not differ (P>0.05)

* Standard error of the mean

The concentration of acetic acid did not differ (P>0.05) among treatments at day 0 and slowly increased at different rates with time of ensiling. However, there was a significant increase (P<0.05) in the acetic acid concentration for all silage treatments at day 7, with the control treatment having the highest concentration.

Selgar (2003) reported that small amounts of acetic acid are produced by anaerobic heterofermentative bacteria during early ensiling. The proportions of acetic acid produced, however, depend on crop maturity, moisture and epiphytic bacteria populations of the harvested crop (Seglar, 2003). There was a slow but significant increase in acetic acid concentration between day 7 and 42 for 10% to 40% WDGS treatments. This slow increase can be explained by the low initial pH in WDGS (3.2) (Garcia and Kalscheur, 2006), which inhibited the proliferation of heterofermentative bacteria responsible for acetic acid production, during the early ensiling phases. Acetic acid concentration between day 7 and 42 was kept low for maize silage blended with WDGS treatments compared to the control. Acetic acid concentration was the highest for maize silage blended with WDGS treatments (with lower initial pH compared to the control) at day 120 post ensiling, which was contrary to reports by Garcia and Kalscheur (2006) who suggested that low pH at time of ensiling inhibited homofermentative bacteria and allowed for heterofermentative bacterial proliferation responsible for acetic acid production during the early phases of ensiling.

The acetic acid concentration value for the control treatment at day 120 post ensiling was (12.5 g/kg DM) higher than values reported by Garcia and Kalscheur (2006), who recorded a final acetic acid concentration of 2.3 g/kg DM for maize silage. When Garcia and Kalscheur (2006) ensiled blends of 25% DM of WDG (without solubles) and 75% DM of chopped maize, 50% DM of WDG and 50% DM of chopped maize, and 100% DM of WDGS and 0% chopped maize, they recorded 43.2, 56.7 and 38.9 g/kg DM respectively. These values were higher compared to those of the present study for maize silage treatments blended with WDGS. Blending WDGS with other feeds result in fermentation patterns that differ from the traditional lactic acid fermentation towards more production of acetic acid (Garcia and Kalscheur, 2004). This suggestion was in agreement with the results of this study where acetic acid concentration was higher in maize silage treatments blended with WDGS compared to

the control treatment at day 120. In the present study, the high concentration of crude protein due to WDGS inclusion could also have resulted in higher concentrations of acetic acid as suggested by Kung and Shaver (2001).

4.2.5.2 Propionic acid

The changes in the propionic acid concentration of the five different inclusion levels of WDGS are presented in Table 4.2.5. Prior to ensiling (day 0), propionic acid did not differ ($P>0.05$) among treatments. Propionic acid concentration significantly increased ($P<0.05$) at day 7 for most treatments, excluding 40% WDGS, and peak concentrations were recorded at day 120. The 40% WDGS treatment remained unchanged throughout the ensiling period and recorded the lowest propionic concentration (0.33% g/kg DM) post ensiling. In well preserved silages, propionic acid is usually produced at lower concentrations during fermentation and is responsible for maintaining aerobic stability (Kung and Stokes, 2005). It has a characteristic sharp sweet smell and taste. Kung and Stokes (2005) suggested that well fermented silages should contain very low propionic acid concentrations (<0.2 to 0.3% of DM), which was within the range (0.033% - 0.176%) reported in the present study. Very wet silages (<30% DM) usually lead to high propionic acid concentrations (Kung and Stokes, 2005). In the present study, all treatments had DM concentrations higher than 300 g/kg DM, with the 40% WDGS having the lowest concentration (310 g/kg DM). Therefore, high concentrations of propionic acid were expected to be recorded in 40% WDGS compared to other treatments but the lowest propionic concentration was recorded in this treatment. This was probably due to early preservation of the silage as a result of low initial pH of WDGS.

4.2.5.3 Butyric acid

Butyric acid was detected prior to ensiling but significantly decreased ($P<0.05$) for all treatments, to undetectable levels at day 7 until day 120 post ensiling (Table 4.2.6). This was in agreement to Dermarquilly (1988), McDonald *et al*. (1991), and Kung and Shaver (2001) who reported that the concentration of butyric acid in well preserved silage was undetectable. The butyric acid detected prior to ensiling was due to clostridial fermentation, which usually occurs during the initial ensiling phase (Jonsson, 1991).

Table 4.2.6 Mean values (±s.e) of butyric acid concentrations (g/kg DM) for maize ensiled at five levels of WDGS inclusion

		Time					
Parameter	**Treatment**	**0**	**7**	**21**	**42**	**120**	**SEM***
Butyric acid (mmol/100mL)	0% WDGS	$0.083^{a}{}_{3}$	0.000^{b}	0.000^{b}	0.000^{b}	0.000^{b}	±0.071
	10% WDGS	$0.134^{a}{}_{2}$	0.000^{b}	0.000^{b}	0.000^{b}	0.012^{b}	±0.071
	20% WDGS	$0.086^{a}{}_{3}$	0.000^{b}	0.011^{b}	0.000^{b}	0.014^{b}	±0.071
	30% WDGS	$0.184^{a}{}_{1}$	0.000^{b}	0.000^{b}	0.015^{b}	0.000^{b}	±0.071
	40% WDGS	$0.095^{a}{}_{3}$	0.000^{b}	0.000^{b}	0.000^{b}	0.013^{b}	±0.071
SEM*		±0.032	±0.032	±0.03	±0.03	±0.03	

^{a,b,c,d,e} For each row means with common superscripts do not differ (P>0.05)

$_{1,2,3,4,5}$ For each column means with common subscripts do not differ (P>0.05)

* Standard error of the mean

This clostridial fermentation process could have been due to soil contamination of the plant material at ensiling (McDonald *et al*., 1991) or delayed sealing of silos (Jonsson, 1991). Elevated levels of butyric acid are undesirable in silages. This indicates silage deterioration from secondary fermentation, which in the presence of unpalatable nitrogenous end products such as amines and amides, may lead to a significant reduction in DM intake and energy level of the forage (Kung and Shaver, 2001).

4.2.6 Nitrogen and ammonia nitrogen

4.2.6.1 Nitrogen

Changes over time for nitrogen concentration (N) are presented in Table 4.2.7. Nitrogen concentration at day 0 (prior to ensiling) increased with an increasing level of WDGS inclusion across treatments, as was the case in the study of Anderson *et al*. (2009). This could have been due to the high concentration of N present in WDGS (53.4 g/kg DM) after ethanol extraction (Kaiser, 2006). The content of N at 0% WDGS inclusion remained unchanged until day 21. It decreased at day 42 and was constant until day 120. This was in agreement with reports presented by Kung and Shaver (2001) and McDonald *et al*. (1991) who recorded a decrease in N concentration for well preserved maize silages. The N concentration for maize silage blended with WDGS remained unchanged throughout the ensiling period. The results of treatments associated with blending of WDGS contradicts with other studies of silage were the N content actually decreased (Castro *et al*., 2006), probably due to the high content of N in WDGS compared to the control treatment. During ensiling of the

whole maize plants, protein was expected to be broken down to non-protein nitrogen (NPN) (Bergen *et al*., 1994). Results from our control treatment confirmed this observation, but showed that major proteolysis occurred later during ensiling and reached a steady state after 42 days. Silage proteolytic activity evidently declined in maize silage blended with WDGS.

Table 4.2.7 Mean values (±s.e) of nitrogen (g/kg DM) and NH_3-N concentrations (g/kg N) for maize ensiled at five levels of WDGS inclusion

		Time (days)					
Parameter	**Treatment**	**0**	**7**	**21**	**42**	**120**	**SEM***
N (g/kg DM)	0% WDGS	$16.16^{a}{}_{5}$	$15.04^{ab}{}_{4}$	$15.04^{ab}{}_{5}$	$14.72^{b}{}_{5}$	$13.92^{b}{}_{5}$	±0.371
	10% WDGS	$23.62^{ab}{}_{4}$	$22.08^{b}{}_{3}$	$20.64^{b}{}_{4}$	$21.12^{b}{}_{4}$	$25.44^{a}{}_{4}$	±0.371
	20% WDGS	$26.88^{ab}{}_{3}$	$25.28^{b}{}_{3}$	$25.76^{b}{}_{3}$	$27.04^{b}{}_{3}$	$30.40^{a}{}_{3}$	±0.371
	30% WDGS	$32.40^{ab}{}_{2}$	$30.88^{b}{}_{2}$	$30.40^{b}{}_{2}$	$30.88^{b}{}_{2}$	$34.48^{a}{}_{2}$	±0.371
	40% WDGS	$39.60^{ab}{}_{1}$	$36.48^{c}{}_{1}$	$38.32^{abc}{}_{1}$	$39.20^{ab}{}_{1}$	$40.40^{a}{}_{1}$	±0.371
SEM*		±0.166	±0.166	±0.166	±0.166	±0.166	
NH_3-N (g/kg N)	0% WDGS	$1.62^{d}{}_{1}$	$22.05^{c}{}_{1}$	$30.14^{b}{}_{1}$	$30.36^{b}{}_{2}$	$35.36^{a}{}_{2}$	±0.055
	10% WDGS	$1.70^{d}{}_{1}$	$24.72^{c}{}_{1}$	$33.57^{b}{}_{1}$	$33.63^{b}{}_{1,2}$	$44.77^{a}{}_{1}$	±0.055
	20% WDGS	$1.63^{d}{}_{1}$	$21.98^{c}{}_{1}$	$33.20^{b}{}_{1}$	$36.35^{b}{}_{1}$	$47.95^{a}{}_{1}$	±0.055
	30% WDGS	$1.89^{d}{}_{1}$	$21.77^{c}{}_{1}$	$33.40^{b}{}_{1}$	$37.59^{b}{}_{1}$	$48.56^{a}{}_{1}$	±0.055
	40% WDGS	$1.28^{e}{}_{2}$	$14.72^{d}{}_{2}$	$23.00^{c}{}_{2}$	$29.54^{b}{}_{2}$	$36.21^{a}{}_{2}$	±0.055
SEM*		±0.025	±0.025	±0.025	±0.025	±0.025	

^{a,b,c,d,e} For each row means with common superscripts do not differ (P>0.05)

$_{1,2,3,4,5}$ For each column means with common subscripts do not differ (P>0.05)

* Standard error of the mean

Bergen *et al*. (1994) suggested that plant enzymes are usually related to early proteolysis, but in this study, they may not have been responsible for this proteolysis since it was low.

4.2.6.2 Ammonia nitrogen

The changes in the silage ammonia nitrogen concentration (NH_3-N) of the five different inclusion levels of WDGS are presented in Table 4.2.7. Ammonia nitrogen was the lowest for 40% WDGS inclusion level (P<0.05) at day 0, compared to other treatments which were higher. Prior to ensiling, no proteolytic activity was expected to have occurred (Seglar, 2003), and values at day 0 were the lowest.

The NH_3-N concentration for 0% WDGS significantly increased between day 0 and 21 ($P<0.05$), and did not change between day 21 and 42. However, an increase in NH_3-N was also recorded between day 42 and 120 post ensiling. The tendency of a NH_3-N increase for 10%, 20% and 30% WDGS did not differ ($P>0.05$) from that of the control. There was a continual increase in the NH_3-N value for 40% WDGS between day 0 and 120, but at different rates. The NH_3-N concentration of 40% WDGS treatment was the lowest at day 7 and this can be related to the presence of a high sulphuric acid concentration, which was expected to be at a higher concentration at 40% WDGS level of inclusion compared to other treatments. It is well known that sulphuric acid inhibits the activities and proliferation of fermentative bacteria (desirable or undesirable) responsible for proteolysis (Garcia and Kalscheur, 2006). Proteolysis is usually due to plant degrading enzymes and/or undesirable clostridial microorganisms (Seglar, 2003).

The 0% and 40% WDGS treatments did not differ ($P>0.05$) in concentration of NH_3-N on day 120, and were lower compared to NH_3-N concentrations of 10%, 20% and 30% WDGS treatments which did not differ ($P>0.05$). The final NH_3-N concentrations of all treatments (ranged between 35.4 to 48.6 g/kg N) in this study were lower than those indicated by Seglar (2003), and Kung and Shaver (2001) for maize silage. Therefore, this suggested that the fermentation process was ideal, with less proteolysis associated with clostridial activity. This was indicated by a low reduction in N concentration during the ensiling period for all the treatments.

According to McDonald *et al*. (1991), NH_3-N provides some indication of protein degradation during silage preservation, with extensive proteolysis occurring in silages which are stored too wet (< 30 to 35% DM) (Dermarquilly, 1988). This was not consistent with the results of this study for the 20%, 30% and 40% WDGS treatments with high moisture concentrations. However, the increase in NH_3-N concentration was not due to the moisture content of the silage, contrary to suggestions by Dermarquilly (1988). This clearly shows that silages ensiled with a blend of WDGS might have a different pattern of fermentation from that expected from silages with a low DM content. The recommended level of NH_3-N concentration in well preserved silages is less than 10% of N (Kung and Shaver, 2001), which was in agreement with

results of this study. Theoretically, high amounts of NH_3-N in silage should not have a negative effect on animal performance if the total dietary N fractions are in balance (Kung and Shaver, 2001) as it does not alter palatability and therefore, intake. However, high levels of NH_3-N in silages usually correlate with high butyric acid and amine concentrations, which are undesirable to ruminants due to the negative effect on palatability and intake of the silage (Seglar, 2003).

4.2.7 Neutral detergent fibre and acid detergent fibre

4.2.7.1 Neutral detergent fibre

The concentration of NDF in all treatments was determined at the start and the end of the ensiling period, and the results are presented in Table 4.2.8. There were no significant increases in the NDF concentration at day 0 for all treatments, which was in agreement with a study by Anderson *et al*. (2009).

Table 4.2.8 Mean values (±s.e) of NDF and ADF concentrations (g/kg DM for maize ensiled at five levels of WDGS inclusion

		Time (days)		
Parameter	**Treatment**	**0**	**120**	**SEM***
NDF (g/kg DM)	0% WDGS	507.6	481.6_{2}	±0.643
	10% WDGS	519.4	493.5_{2}	±0.643
	20% WDGS	519.2^{a}	$473.1^{b}{}_{2}$	±0.643
	30% WDGS	526.4	512.2_{1}	±0.643
	40% WDGS	521.6	522.3_{1}	±0.643
SEM*		±0.288	±0.288	
ADF (g/kg DM)	0% WDGS	238.6	250.7_{2}	±0.491
	10% WDGS	236.7	255.5_{2}	±0.491
	20% WDGS	224.9^{b}	$265.7^{a}{}_{1,2}$	±0.491
	30% WDGS	226.4^{b}	$278.9^{a}{}_{1}$	±0.491
	40% WDGS	223.4^{b}	$279.1^{a}{}_{1}$	±0.491
SEM*		±0.220	±0.220	

^{a,b,c,d,e} For each row means with common superscripts do not differ (P>0.05)

$_{1,2,3,4,5}$ For each column means with common subscripts do not differ (P>0.05)

* Standard error of the mean

Surprisingly, NDF concentration was decreased (P<0.05) in the 20% WDGS treatment at day 120, contrary to reports by Seglar (2003) who reported that NDF

concentrations will slightly increase after ensiling due to a reduction of WSC concentration at ensiling. The NDF fraction of WDGS is readily available for degradation by microorganisms as an energy source (Schingoethe, 2004). Therefore, some of the reduction in NDF concentration at the 20% WDGS level of inclusion could have been utilised during the fermentation process.

At day 120, significant differences among treatments were recorded, with 30% and 40% WDGS treatments ending with higher NDF values ($P<0.05$) compared to the other three treatments. As expected, the NDF concentration was higher for treatments with higher inclusion levels of WDGS after day 120 post ensiling. Whole maize plants had an NDF concentration of 45.4% and the WDGS contained an average of 51.9% (DM basis) NDF concentration (Table 4.1.1). This indicated that the NDF concentration increased with increased level of WDGS inclusion when the two ingredients were blended in different proportions. Kung and Shaver (2001) suggested that high concentrations of NDF in silage usually indicates that many of the soluble nutrients have been degraded which reduces the nutrient value of the silage, as was the case with the 30% and 40% WDGS treatments.

The energy content of maize silage can vary greatly depending on the level of the fibre ratio of grain to stover in silage, as well as the digestibility of the fibre (Garcia *et al*., 2003). Tjardes and Wright (2002), recorded that the concentrations of NDF in whole plant maize silage will vary greatly (46.0 ± 6.5% of DM). In the present study, the NDF concentration at day 120 for all treatments was within the range reported by Tjardes and Wright (2002), with no significant differences occurring between the start and end of the ensiling period.

4.2.7.2 Acid detergent fibre

The changes in ADF concentration for all treatments are presented in Table 4.2.8. There were no differences in ADF concentration at day 0 for all treatments. This proved that most of the ADF concentration was embedded in the chopped maize plants (McDonald *et al*., 1991). The ADF concentration increased at day 120 for treatments blended with 20%, 30% and 40% WDGS. The increase in ADF concentration in 20%, 30% and 40% WDGS treatments could have been due to

utilization of readily digestible NDF of WDGS by fermentative bacteria (Schingoethe, 2004), since WSC in those treatments was lower. In a study by Kalscheur and Garcia (2003), ADF and NDF concentrations increased with the increasing level of WDGS ensiled with whole maize plants. This was in agreement with results of ADF concentration in the present study. Kalscheur *et al*. (2003) ensiled a blend of WDGS and chopped maize plants at ratios of 25:75 and 50:50 (WDGS: whole plant maize), for a period of 129 days and recorded a final ADF concentration of 240 and 200 g/kg of DM respectively. The range for the final ADF presented in this study for WDGS treated silage was more than that recorded by Kalscheur *et al.* (2003), probably due to a high variability of WDGS from both within and across production plants (Kononoff and Christiansen, 2007). The higher the ADF, the less digestible the feed and the less energy it will contain (McDonald *et al*., 1991). This ADF fraction is closely related to indigestibility and is a major factor in calculating energy concentration of feeds (Garcia *et al*., 2003)

4.2.8 In vitro digestible organic matter

The *in vitro* digestible organic matter (IVDOM) was determined at day 0 and 120, as presented in Table 4.2.9. There were no significant differences observed at day 0 across treatments.

Table 4.2.9 Mean values (±s.e) of IVDOM concentration (g/kg DM) for maize ensiled at five levels of WDGS inclusion

		Time (days)		
Parameter	**Treatment**	**0**	**120**	**SEM***
IVDOM (g/kg DM)	0% WDGS	782.3	766.5	±0.835
	10% WDGS	786.8	766.7	±0.835
	20% WDGS	790.7	767.5	±0.835
	30% WDGS	795.6	776.7	±0.835
	40% WDGS	813.9	777.5	±0.835
SEM*		±0.373	±0.373	

^{a,b,c,d,e} For each row means with common superscripts do not differ (P>0.05)

^{1,2,3,4,5} For each column means with common subscripts do not differ (P>0.05)

***** Standard error of the mean

The range of IVDOM values (78.2% to 79.6% of DM) recorded prior to ensiling of 0%, 10% 20% and 30% WDGS treatments were in agreement with values for whole

maize plants of some hybrids recorded by Johnson and Gates (1999) and by Meeske *et al*. (2000) for chopped maize silage. The IVDOM values for the 40% WDGS treatment prior to ensiling were similar to values for highly digestible maize grains (Ferreira and Mertens, 2005), probably due to a high proportion of WDGS which is highly degradable (Schingoethe, 2004).

There were no changes in the IVDOM concentration recorded between day 0 and 120 for all treatments. The IVDOM concentration among treatments did not differ ($P>0.05$) at day 120. The IVDOM values at day 120 for all treatments were higher than those of Ferreira and Mertens (2005) for maize ensiled at different chopping lengths. Colenbrander *et al*. (1971) determined the IVDOM of the whole maize plant silage and recorded a digestibility of 60.5% DM which was lower compared to the IVDOM of the control in the present study. The high values of IVDOM probably indicated that all treatments were highly digestible, and that the inclusion of WDGS had no affect on the digestibility of the silage. This was probably due to lower concentrations of structural material like lignin, cellulose and acid detergent insoluble crude protein in WDGS (Schingoethe *et al*., 2002). In general, ensiling produced little or no improvements in IVDOM, and according to Christiansen and Wagner (1974), there was no reduction in digestibility due to an increase in the moisture concentration of the higher levels of WDGS inclusion. The higher values of IVDOM concentration in the control treatment was due to the high concentration of maize grains (Kamalak *et al*., 2004), that improved digestibility. The IVDOM values of the non-grain proportion (fodder) of maize plants are lower but much more variable compared to the grain proportion (Johnson and Gates, 1999). Keep in mind that the two-stage Tilley and Terry (1963) IVDOM method used in this study may produce accurate results for fresh grasses, but giving probably less accurate predictions of digestibility in silage samples (Givens *et al*., 1995).

4.3 Animal evaluation trials

4.3.1 Nutritional composition of experimental diets

Three treatment diets were formulated and used in the rumen fermentation, dry matter degradability (DMD), and growth performance trials. The chemical composition of the experimental diets is presented in Table 4.3.1. The dry matter (DM) content of the

treatment diets were different, with the MS DDGS diet having the highest DM concentration of 429.7 g/kg DM and WDGSMS recording the lowest (336.5 g/kg DM), with that of MS SOM being intermediate at 381.4 g/kg DM. The DM concentration of the experimental diets during feeding was important as it may affect voluntary feed intake and the general performance of animals (McDonald *et al*., 1990).

Table 4.3.1 Chemical composition of diet treatments in g/kg DM

Treatment	NDF	ADF	CP	Fat	ME (MJ/kg)	Ca	P
MS DDGS	467.51	264.36	144.24	38.72	11.095	4.95	3.16
MS SOM	438.87	250.59	153.62	38.47	9.226	5.76	4.33
WDGSMS	466.04	268.97	150.25	41.97	10.248	5.82	3.77

Charmley (2004) mentioned that maximum DM intake of maize silage occurred at a DM concentration of about 500 g/kg, and that DM concentration below 250 g/kg DM resulted in lower silage DM intakes. The DM concentration in the present study (381.4 to 429.7 g/kg DM) was within the range reported by Charmley (2004), which should meet the DM requirements for sheep.

The NDF concentrations of MS DDGS, MS SOM and WDGSMS were 467.5, 438.9 and 466.0 g/kg of DM respectively, which was higher than the optimal requirement for growing lambs (420 to 430 g/kg DM) (NRC, 2001). The high NDF concentration in the MS DDGS and WDGSMS diets was probably due to the high concentration of readily digestible NDF present in the distillers grain (350 g/kg DM) fraction blended in those diets (Kaiser, 2006). It is generally assumed that ruminants will eat a maximum total NDF of close to 12% of their body weight (McDonald *et al*., 1990). The minimal dietary N concentration in ruminant feed is approximately 10 g/kg N (62.5 g/kg CP) (Freer and Dove, 2002). In the present study, CP concentrations in the three formulated diets were within the range (140 to 160 g/kg DM) reported by the NRC (2001) for growing lambs (between 20 to 35 kg liveweight). However, Black *et al*. (1973) noted that differences in feedstuffs used for the various determinations, and particularly differences in the extent of degradation of dietary protein within the rumen and subsequent amino acid absorption, are the variations that exists in

estimating protein requirements of growing lambs. The CP requirements for ruminants however, have little meaning unless energy requirements have been satisfied (Black *et al*., 1973).

The metabolisable energy requirements were met for the MS DDGS and WDGSMS diets, with that of MS SOM being slightly lower (Table 4.3.1). The optimal ME requirements for growing lambs was suggested to be 10.5 g/kg DM by the NRC (2001). This high ME concentration in the MS DDGS and WDGSMS diets was probably due to the high levels of digestible NDF and fat in distillers grains. The high ME concentration in diets involving DGS were as a result of a higher energy concentration present in ethanol by-products, which is estimated to be about 13.3 MJ/kg DM (NRC, 2001). The NRC (2001) also suggested that the ME requirements increases with the increase in body weight of the lambs. Dietary fat concentration (Table 4.3.1) for all diets in the present study, were considered as adequate and not expected to interfere with the digestion of other nutrients in the rumen (NRC, 2001). According to the NRC (2001), the recommend dietary fat level of inclusion is 30 g/kg DM. However, dietary fats have been used to manipulate digestion and absorption of different nutrients, for instance, limiting ruminal acidosis resulting from high carbohydrates and low fiber diets (Chillard, 1993).

In this study, the calcium (Ca) and phosphorus (P) concentration for the three experimental diets were within the requirements for growing lambs (5.1 and 2.4 g/kg for Ca and P, respectively), as recommended by NRC (2001). However, the P concentration was slightly higher than the recommended concentration. Huls *et al*. (2006) reported that high P content present in DDGS raises concerns as to its inclusion rate in finishing lamb diets because of environmental impact and potential problems with urinary calculi. This may also off-set the recommended Ca:P ratio of 2:1. Calcium: Phosphorus ratios lower than unity (1:1) result in reduced growth and poor nutrient conversion (Wise *et al*., 1963). However, Wise *et al*. (1963) reported that optimal Ca:P ratio for ruminants is higher than that of non-ruminant animals and that the ruminant will tolerate wide ratios of Ca:P, but ratios lower than unity are deleterious. Distillers grains and sunflower oil cake used in the formulation of the three diets had high concentrations of P with that of whole plant maize being low, as

presented in Table 4.3.1. However, the dilution factor was evidently inadequate to reduce the concentration of P to the required ratio in the final diets. The Ca:P ratios for the diets used in this study were 1.6:1, 1.3:1 and 1.5:1 for MS DDGS, MS SOM and WDGSMS respectively.

4.3.2 Rumen fermentation parameters

The average mean values of rumen fermentation parameters of cannulated sheep fed MS DDGS, MS SOM and WDGSMS diets are presented in Table 4.3.2.

Table 4.3.2 Mean values (±s.e) for rumen fermentation characteristics of sheep fed three experimental diets

Parameter	Treatment			SEM*
	MS DDGS	MS SOM	WDGS MS	
pH	6.3	6.1	6.0	±0.102
NH_3-N (mg/100mL)	4.10	6.24	4.72	±2.631
Total VFA (mmol/L)	71.71	81.93	71.25	±1.125
Acetic (mmol/ L)	47.37	57.05	51.46	±1.028
Propionic (mmol/L)	14.59	17.24	13.62	±0.015
Butyric (mmol/ L)	8.03[a]	7.33[a]	5.83[b]	±0.129
Valeric (mmol/ L)	0.69	0.73	0.73	±0.180
Isobutyric (mmol/ L)	0.51	0.65	0.47	±0.283
[#]A/P	3.2	3.3	3.8	±0.247

[#]A/P = acetic and propionic acid ratio

[a, b,c] For each parameter, row means with common superscripts do not differ (P>0.05)

* Standard error of the mean

4.3.2.1 Rumen pH

The average rumen pH values varied between 6.0 and 6.3 (Table 4.3.2). However, the rumen pH of the sheep fed the three experimental diets did not differ (P>0.05). According to Erfle *et al*. (1982), the ruminal pH varied from higher than 7 to less than 5, depending on the type of diet fed. In this study, the ruminal pH values obtained in sheep fed the three diets did not indicate any possibility of acidosis and were within the range of optimal pH suggested by Erfle *et al*. (1982) for a complete ration involving maize silage, soybean meal and a mineral premix. The optimal pH for fiber digestion (6.2 and 6.8), as suggested by Hoover (1986), was obtained in sheep fed MS DDGS diet, with those fed MS SOM and WDGSMS diets being slightly lower. The pH levels less than 6 may have an adverse effect on the rumen microbial growth

(Hoover, 1986), and can be associated with reduction in both cellulose digestion and voluntary intake of roughages (Ørskov and Fraser, 1975). Corrigan *et al*. (2008), indicated that cattle fed 40% WDGS (DM basis) in diets which consisted of dry rolled maize with alfalfa hay, had a lower ruminal pH than the control diet fed without WDGS. This suggestion was not consistent with the results obtained in the present study, as sheep fed a diet involving the inclusion of WDGS recorded similar values of pH to those fed MS DDGS and MS SOM.

Rumen pH changes after feeding were compared among treatments (Figure 4.3.1) over a 24 hour sampling period. The rumen pH did not differ among the sheep fed the three diets except only at 18 hour post feeding.

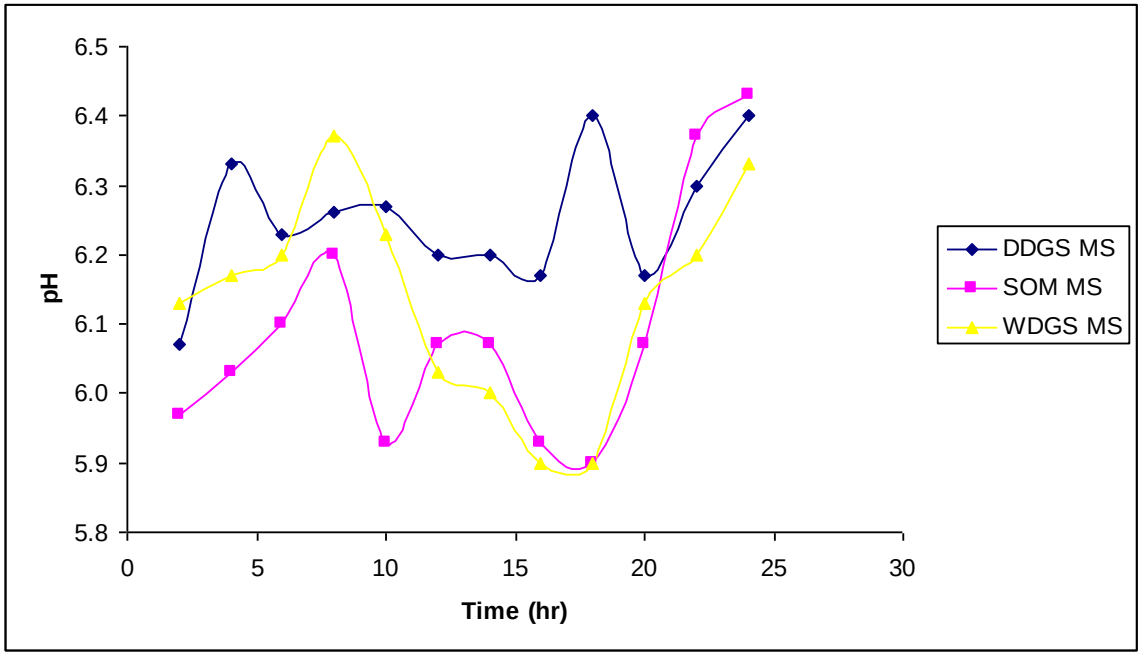

Figure 4.3.1 Post feeding changes in rumen pH for MS SOM, MS DDGS and WDGSMS at two hour intervals

At 18 hours post feeding, the pH of MS DDGS was higher ($P<0.05$) than both MS SOM and WDGSMS diets, which did not differ ($P>0.05$). May *et al*. (2007) reported that steers fed 25% DDGS steam flaked maize diets with maize silage as the roughage source, had lower ruminal pH after 18 hours of feeding compared to other diets fed without DDGS. However, in the present study sheep fed the diet involving DDGS recorded a higher rumen pH compare to the other two diets at 18 hour post feeding. Feeding DDGS probably increased ruminal pH due to lower starch and higher NDF

concentrations (Köster, 2007) in the diet. The rise in pH after 20 hours post feeding in all diets indicated the end of VFA production due to minimal presence of fermentable organic matter (Erfle *et al.*, 1982).

4.3.2.2 Ruminal Ammonia Nitrogen

The average ruminal ammonia concentration (NH_3-N) did not differ (P>0.05) among the three diets, ranging between 4.10 and 6.24 mg/100mL (Table 4.3.2). Ruminal NH_3-N concentration changes over a 24-hour sampling period post feeding of the three experimental diets are presented in Figure 4.3.2.

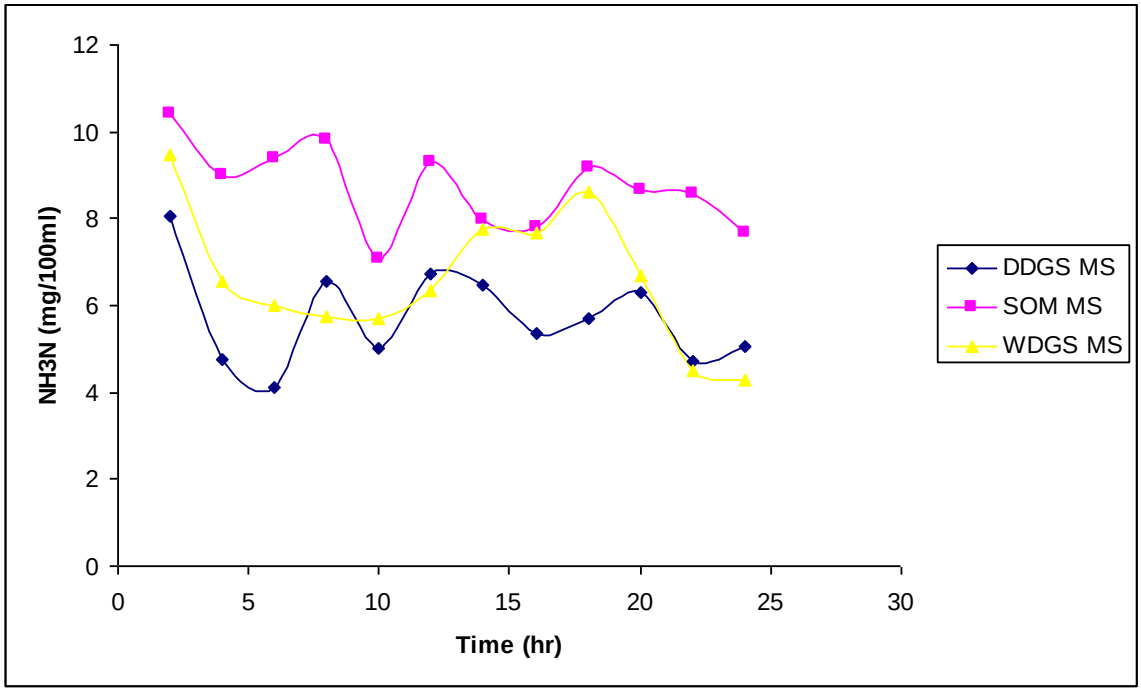

Figure 4.3.2 Changes in rumen NH_3-N for MS SOM, MS DDGS and WDGSMS at two hour interval

There was a steady decline in the ruminal NH_3-N concentration for all the diets with distinct significant differences at 18 and 22 hours post feeding. At 2 hours post feeding, all diets had a peak concentration of NH_3-N, but declined rapidly thereafter, a finding similar to a report by Schingoethe *et al.* (2001). All treatments had a rumen NH_3-N peak at 2 hours. At 18 hours post feeding, the sheep fed the MS DDGS diet recorded the lowest concentration of NH_3-N compared to those offered MS SOM and WDGSMS diets which were higher and did not differ (P>0.05). Sheep fed MS SOM had higher (P<0.05) ruminal NH_3-N concentrations compared to those offered MS DDGS and WDGSMS which were lower and similar at 22 hours post feeding. The

optimum NH_3-N concentration is defined as the minimum concentration of NH_3-N necessary to support maximum synthesis of microbial production (Satter and Slyter, 1974) and maximum rumen degradability of DM. The reduction in NH_3-N concentration throughout the sampling period were probably due to a high level of proliferation of rumen microbes which increase the demand of NH_3-N, as well as a reduced protein substrate which can be degraded to NH_3-N in the rumen (Roffler and Satter, 1975). However, other factors like time after feeding, location of ruminal sampling, protein concentration in the diet, protein degradability and ruminal volume may had an effect on ruminal NH_3-N concentration in sheep (Roffler and Satter, 1975).

Ruminal NH_3-N production resulted from protein degradation in the rumen, which could become a source of non-protein N necessary for ruminal microbial protein synthesis (Bach *et al.,* 2005). Ruminal microbial protein represents 50 to 80% of total absorbable protein in ruminants (Storm and Ørskov, 1983). Norton (1994) pointed out that 70 mg/L of NH_3-N concentration is the minimum level required by the microbial population in the rumen to support optimum microbial proliferation. In the present study, the average NH_3-N concentration of experimental diets (MS DDGS, MS SOM and WDGSMS was 41.0, 62.4 and 59.7 mg/L respectively).

The values obtained from the present study were lower than those suggested by Norton (1994), which indicated that microbial growth was below optimal in the rumen for all diets. This suggests that NH_3-N level in the rumen less that 70 mg/L is associated with hindrance in microbial activity and is indicative of low proteolysis in the rumen, due to low supply of dietary non-protein nitrogen (Norton, 1994). Norton (1994) also concluded that feeds containing 1.35% N are considered to be N deficient since they cannot provide the minimum NH_3-N levels required. Rate and extent of ruminal proteolysis not only affect microbial protein synthesis but also the quantity and quality of undegraded dietary protein that reach the small intestines (Stern *et al.*, 2006). Although microbial protein alone may be adequate for low producing ruminants, it can be inadequate for supporting higher levels of growth (Stern *et al.*, 2006).

4.3.2.3 Ruminal VFA

The average total VFA concentration of experimental diets fed to cannulated sheep did not differ (P>0.05), as presented in Table 4.3.2. The results of this study were consistent with those of Ham *et al*. (1994), where average total VFA concentrations did not differ among treatments involving 40% DDGS and 40% WDGS (DM basis) in dry rolled maize with maize silage as a roughage source. However, in the study by May (2007), cattle fed DDGS with maize silage as the roughage source, had lower average total VFA compared to the control involving maize silage mixed with steam-flaked maize. Ruminal pH is related to VFA production (McDonald *et al.*, 1990). In the present study, pH did not differ among the experimental diets, hence the similar production of ruminal VFA. The total VFA concentration indicates that there was no effect in feeding DDGS or WDGS to sheep when compared to the control, as reported by Schingoethe *et al*. (1999).

The changes over time in total volatile fatty acid (VFA) concentrations were measured at two hour intervals (Figure 4.3.3).

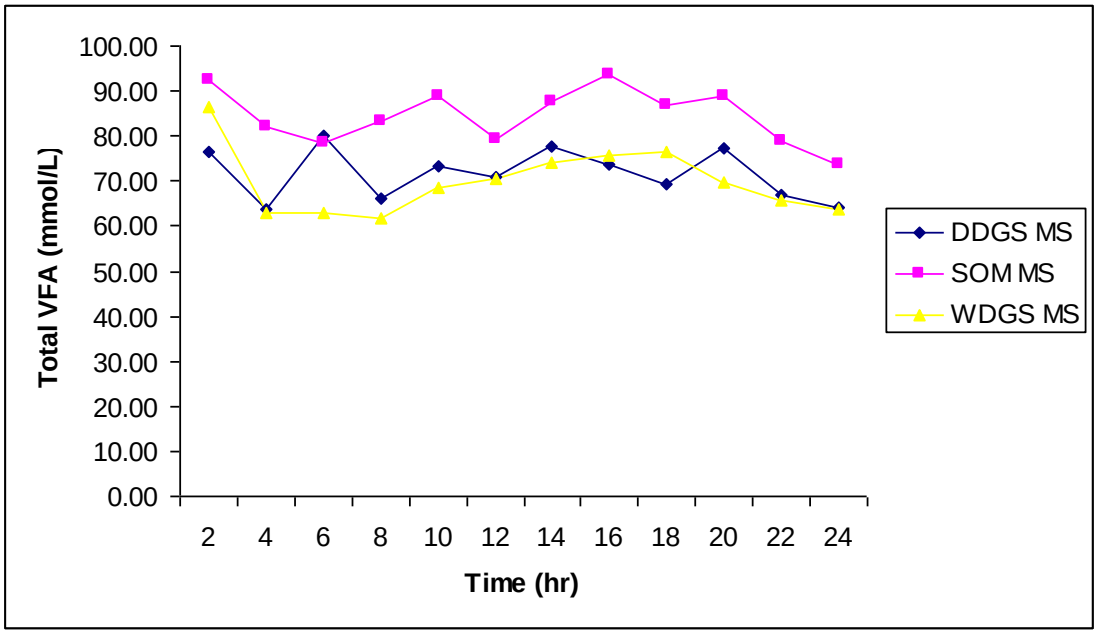

Figure 4.3.3 Rumen total VFA concentrations for cannulated sheep fed MS DDGS, MS SOM and WDGSMS diets measured at two hour intervals

At 20 hours post feeding, sheep fed MS SOM had the highest (P<0.05) total VFA concentration than that of MS DDGS and WDGSMS which did not differ. The lower

total VFA concentration observed in sheep fed MS DDGS and WDGSMS (Figure 4.3.3) were likely to be intake driven (Dijkstra, 1994) because sheep fed these diets had lower intake compared to sheep fed MS SOM diet. Generally, the ruminal VFA concentration dropped during the second half of the sampling period for MS DDGS, with that of MS SOM and WDGSMS dropping in the last quarter. This indicated a decrease of fermentable organic matter in the rumen (May, 2007). Owens *et al*. (1998) suggested that total VFA accumulation dictates whether the rumen is acidotic, as ruminal acidosis represents varying degrees of acidity in the rumen. The average acetic, propionic, valeric and isobutyric acid concentrations of sheep fed MS DDGS, MS SOM (control) and WDGSMS diets did not differ ($P>0.05$). However, average butyric acid concentration was significantly different ($P<0.05$) among diet treatments as animals fed WDGSMS diet had lower average rumen butyric acid concentrations compared to those offered MS DDGS and MS SOM diets (Table 4.3.2). The main end products of anaerobic fermentation within the rumen are acetic, propionic and butyric acids, which are themselves the main form in which energy from plant material is absorbed (McDonald *et al.*, 1990). The results of this study was consistent with those of Peter *et al*. (2000) who reported different levels of butyric acid when Simmental x Angus heifers were fed cracked maize hay supplemented with three maize by-products; dry maize gluten, maize dried distillers grains and modified maize fiber.

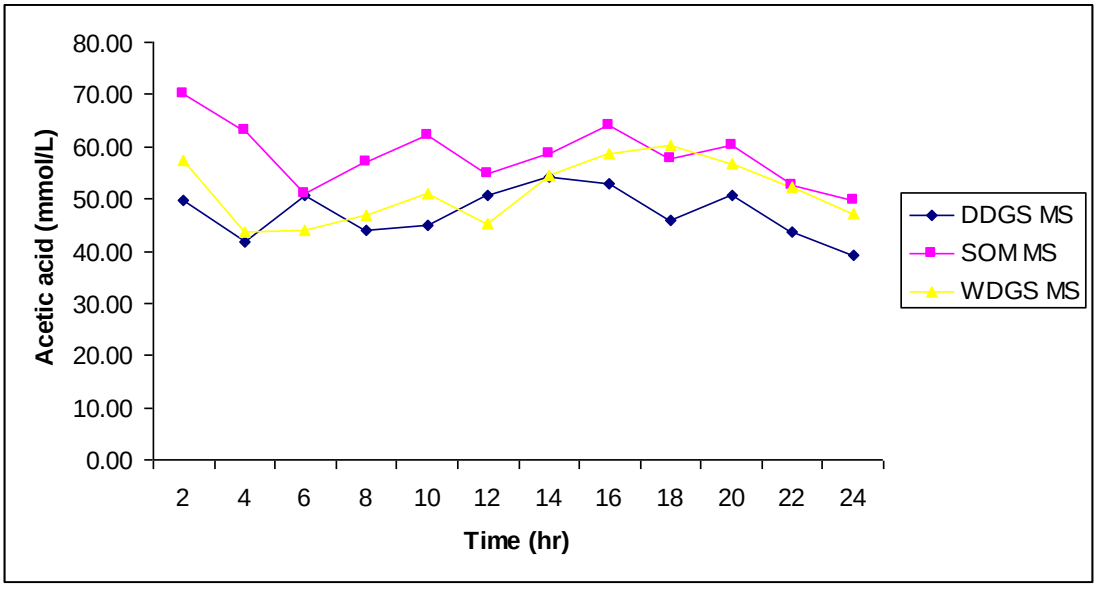

Figure 4.3.4 Rumen acetic acid concentrations for cannulated sheep fed MS DDGS, MS SOM and WDGSMS diets measured at two hour intervals

On average, the acetic acid concentrations in the rumen were 47.6, 57.1 and 51.5 mmol/L for sheep fed MS DDGS, MS SOM and WDGSMS respectively (Table 4.3.2). Post feeding sampling time had an effect on acetic acid concentration in the rumen ($P<0.05$) at the 4 and 20 hour sampling periods (Figure 4.3.4). At 4 hours post feeding, the sheep offered MS SOM diet had higher ($P<0.05$) acetic acid concentrations compared to those offered MS DDGS and WDGSMS diets which had lower acetic acid concentrations but the latter two did not differ ($P>0.05$). At 20 hours post feeding, MS DDGS diet resulted in lower ($P<0.05$) acetic acid concentrations compared to MS SOM and WDGSMS diets which were similar. Peak acetic acid concentration for all diets was reached after 2 hours post feeding. The average propionic acid concentrations were 14.6, 17.2 and 13.6 for MS DDGS, MS SOM and WDGSMS respectively, and did not differ ($P>0.05$) significantly. These values were lower than propionic acid levels recorded by May (2007) when steers were fed a steam-flaked-maize based diet and 25% DDGS (DM).

Changes in propionic acid concentration post feeding were recorded for 24 hour sampling period (Figure 4.3.5). There were no significant differences in the propionic acid concentration among the diets at each of the sampling times.

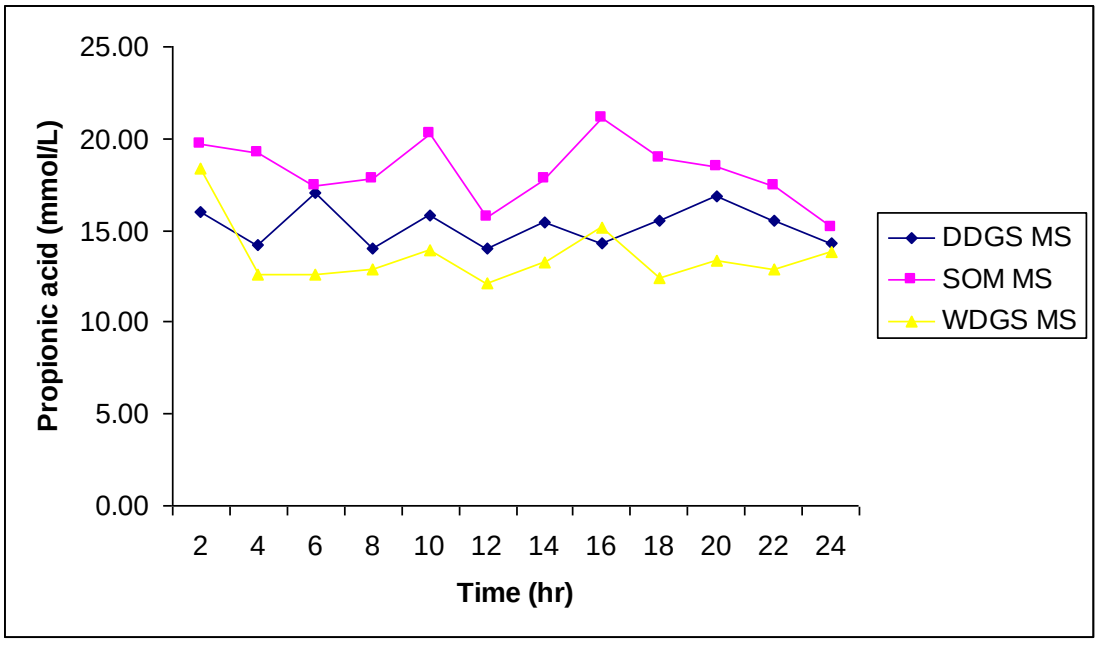

Figure 4.3.5 Rumen propionic acid concentrations for cannulated sheep fed MS DDGS, MS SOM and WDGSMS diets measured at two hour intervals

Lower values of propionic acid were recorded for all treatments at day 120 in the present study, which could have been due to low concentrations of starch as a result of feeding distillers grains which were deficient in starch. Linington *et al.* (1998) recorded low concentrations of propionic acid in Merino sheep fed high fibre compared to low fibre diets, which was in agreement with results obtained in the present study. The experimental diets offered to sheep in the present study were also high in fibre with maize silage as the main structural fibre source; therefore, low propionic acid concentrations were expected.

The acetic/propionic acid ratio did not differ ($P>0.05$) among treatments (Table 4.3.2). The lower the acetic/propionic ratio, the more propionic acid would be produced compared to acetic acid. In the present study, the acetic/propionic acid ratio was high for all diets due to low production of propionic acid. High fibre concentration favors the production of acetic acid in the rumen (McDonald *et al.*, 1990). As the proportion of propionic acid decreased, it may have a negative effect on the efficiency of ME utilization (Ham *et al.*, 1994). May (2007) reported that feeding 25% DDGS with maize silage in a steam flaked maize based diets decreased ruminal concentrations of total VFA, acetic and propionic acids when compared to the steam-flaked maize mixed with maize silage (control).

The average butyric acid concentrations for WDGSMS was lower ($P<0.05$) compared to MS DDGS and MS SOM diets which did not differ ($P>0.05$). On average, the butyric acid concentrations in the rumen were 8.0, 7.3 and 5.8 mmol/L for MS DDGS, MS SOM and WDGSMS respectively. The low butyric acid concentration on sheep fed WDGSMS was likely to be intake driven because these sheep had lower intake compared to other two groups.

The changes over time post feeding of the ruminal butyric acid concentration were presented in Figure 4.3.6. Differences among treatments were recorded at 6 hours and 14 hours post feeding. In both sampling times, sheep fed WDGSMS diet had lower rumen butyric acid concentration compared to those fed MS DDGS and MS SOM diets.

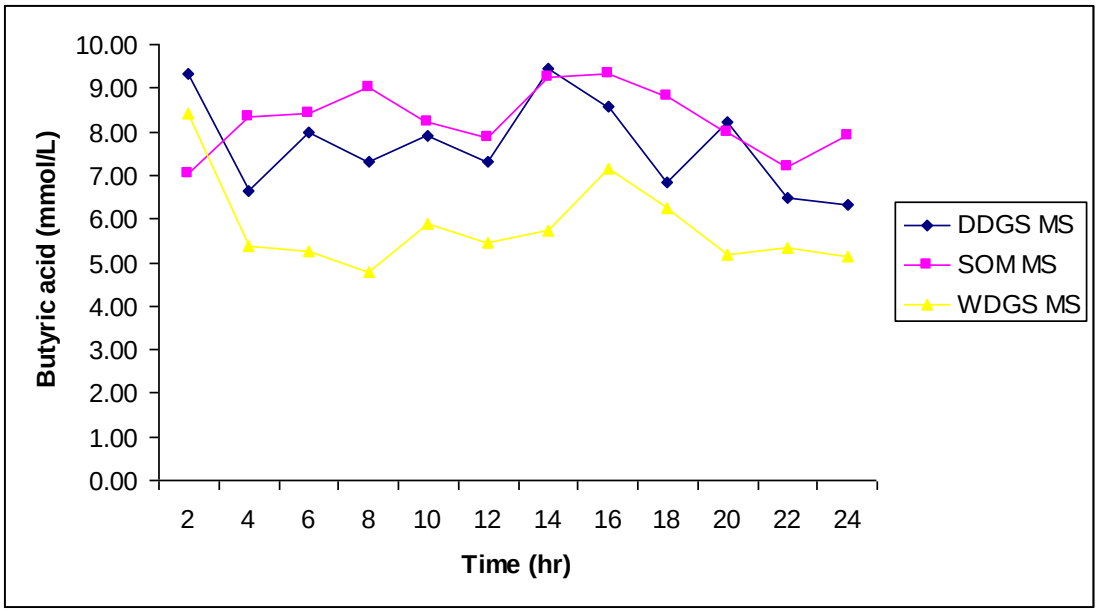

Figure 4.3.6 Rumen butyric acid concentrations for cannulated sheep fed MS DDGS, MS SOM and WDGSMS diets measured at two hour intervals

Unlike in the study by Schingoethe *et al*. (1999) who fed WDGS to dairy cattle, the results of the present study indicated that there was an effect in feeding WDGS to sheep on the concentration of butyric acid when compared to the control diet (MS SOM).

Changes over time post feeding for rumen valeric acid concentration are presented in Figure 4.3.7. The sheep fed MS DDGS, MS SOM and WDGSMS recorded similar ($P>0.05$) valeric acid concentrations at each sampling times.

Therefore, the inclusion of DDGS and WDGS did not have an effect on the valeric acid concentration when compared to the control (MS SOM) diet. This was in agreement with the results of Schingoethe *et al*. (1999) who found no differences in the valeric acid concentrations when distillers grains with solubles (DGS) containing diets were fed together with maize silage to dairy cattle compared to maize silage as a roughage source in a concentrate diet.

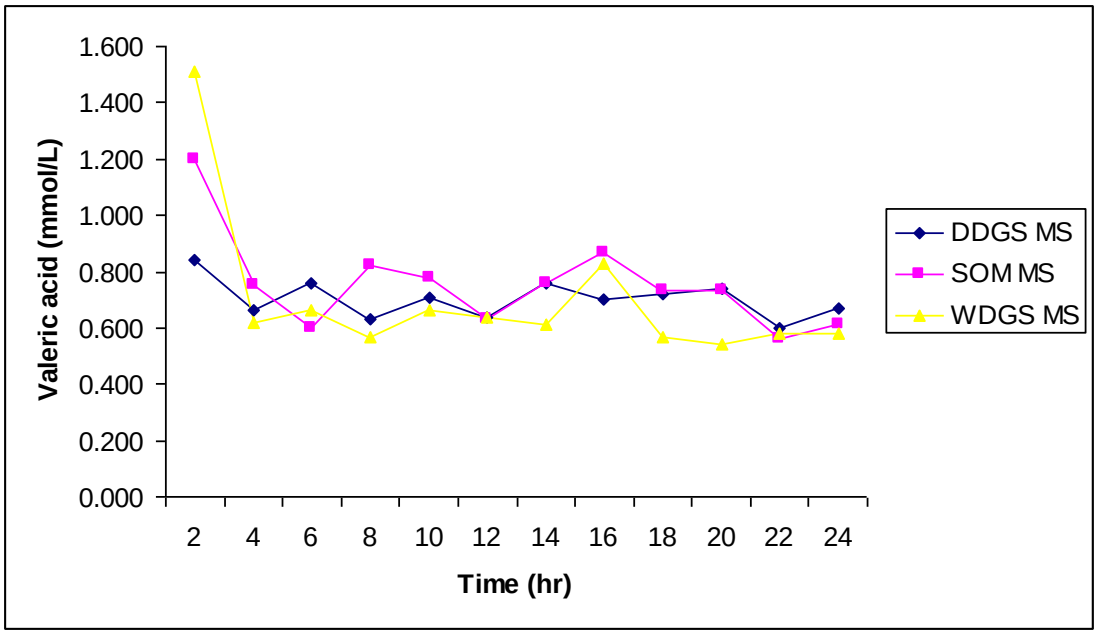

Figure 4.3.7 Rumen valeric acid concentrations for cannulated sheep fed MS DDGS, MS SOM and WDGSMS diets measured at two hour intervals for 24 hours

Significant differences were recorded with isobutyric acid among diet treatments as shown in Figure 4.3.8.

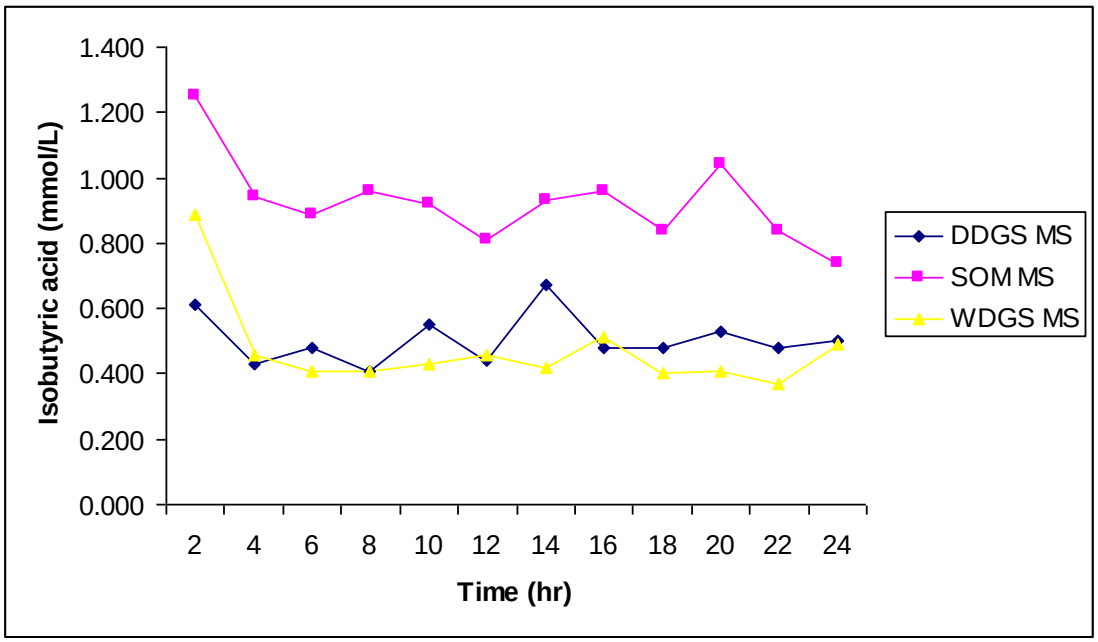

Figure 4.3.8 Rumen isobutyric acid concentrations for cannulated sheep fed MS DDGS, MS SOM and WDGSMS diets measured at two hour intervals for 24 hours

The rumen isobutyric acid concentration of sheep fed WDGSMS and MS DDGS were lower than those of sheep fed MS SOM from 4 hours until 12 hours post feeding and also at 20 hours post feeding. However, at 22 hours, the isobutyric acid concentration of MS DDGS did not differ from both MS SOM and WDGSMS, although MS SOM was higher ($P<0.05$) than WDGMS. The isobutyric acid concentration of sheep fed diets involving DDGS and WDGS was lower ($P<0.05$) compared to the control. This was in agreement with data recorded by Schingoethe *et al*. (1999), who also suggested that the lower branched-chain fatty acid possibly reflected the relatively low content of branched-chain amino acids in the diets high in maize by-products.

Branched-chain volatile fatty acids (valeric and isobutyric acids) are products of degradation of branched amino acids, but they are also growth factors primarily metabolized in the rumen by fiber digesting bacteria in the rumen (McDonald *et al*., 1990). The level of ruminal concentration may thus indicate either the level of protein degradation or fiber digestion in the rumen (May, 2007). Previous research indicated that feeding 25% DDGS (DM basis) in steam-flaked maize diets (May, 2007), or feeding 40% DDGS (DM basis) in dry-rolled maize based diets (Ham *et al.*, 1994), did not affect the concentration of isobutyric and valeric acids. In the present study, DGS inclusion (WDGSMS and MS DDGS) had an effect in the isobutyric acid concentration in the rumen compared to the control diet (MS SOM) between day 4 and 12, and at day 16 to 120 (Figure 4.3.8).

There was no effect of diet offered on valeric acid concentration, which was partly in agreement with the study by May (2007). However, contrary to the present study, Ham *et al.* (1994) fed 40% WDGS (DM basis) in dry-rolled maize diets which increased concentration of valeric acid but had no effect on the isobutyric acid concentration.

4.3.3 Dry matter degradability trial

The dry matter degradability (DMD) characteristics of MS DDGS, MS SOM and WDGSMS are presented in Table 4.3.3. The zero time intercept (a), slowly degradable DM fraction (b) and the rate of DM degradation of fraction b (c) mean values did not differ among the three experimental diets. The rate of ruminal

degradation ranged between 0.043% and 0.057% per hour but the values did not differ (P>0.05) among diets. This result was consistent with the study of Tuah *et al*. (1996) who incubated whole maize plants in cannulated sheep for 96 hours and found degradation rates ranging between 0.04% and 0.06% per hour. The rate of disappearance of each feed diet in the present study increased progressively as incubation period increased (Figure 4.3.9).

Table 4.3.3 Mean values (± s.e.) of ruminal dry matter degradation parameters for the three experimental diets

Parameter	Treatment			SEM*
	MS DDGS	MS SOM	WDGS MS	
a (g/kg DM)	278.2	287.1	294.9	±0.762
b (g/kg DM)	485.7	474.7	442.8	±0.712
c (rate constant/h)	0.043	0.055	0.057	±0.002
$ED_{(1)}$ (g/kg DM)	608.2^{c}	634.8^{a}	622.5^{b}	±0.078
$ED_{(2)}$ (g/kg DM)	501.4^{b}	535.4^{a}	530.5^{ab}	±0.219

a, b,c For each degradability parameter row means with common superscripts do not differ (P>0.05)

a, b and c are described by the equation $y=a+b\ (1-e^{-ct})$, where y is the percentage degraded DM at time t (h); a the zero time intercept (washing loss); b the slowly degradable DM fraction , and c the rate of degradation (fractional rate constant/h) of fraction b. ED is the effective degradability calculated using the equation ED= a+ (bc/(c+k)), where k is outflow rate from the rumen.

(1) where outflow rate =0.02

(2) where outflow rate =0.05

* Standard error of the mean

The effective degradability (ED) was measured at two outflow rates, that is, at 0.02 ($ED_{(1)}$) and 0.05 ($ED_{(2)}$). The ED is a function of *a*, *b*, *c* and the rate of passage of *b* fraction through the rumen (Ørskov and McDonald, 1979). The $ED_{(1)}$ ranged between 608.2 and 634.8 g DM/kg, with incubations from MS DDGS feed material having the lowest effective DM degradability and MS SOM with the highest. The $ED_{(2)}$ values for the three diets ranged between 501.4 and 535.4g DM/kg. The value for the MS SOM diet was higher (P<0.05) than that of MS DDGS diet. The WDGSMS diet value did not differ from that of the other two diets. Wilkens *et al*. (2009) recorded DMD values similar to those for MS DDGS and WDGSMS diets (at 0.05 outflow rate),

when they incubated diets involving DDGS and modified wet distillers grains plus solubles in steers. The ED for MS DDGS diet was lower than other feeds because almost 50% of DM contained in DDGS was CP (Promkot *et al*., 2007). Low degradability of MS DDGS diet could be due to the effect of heat treatment during ethanol production and drying of DDGS which can possibly cause a Maillard reaction and thus render DDGS less degradable (Dale and Batal, 2005). The lower rumen NH_3-N might be another reason for the reduced degradability. Mehrez *et al*. (1977) studied rate of rumen fermentation in relation to NH_3-N concentration by varying the amount of urea added to a whole barley diet (from 0 to 10 g/kg diet) and then incubating bags in the rumen of animals given these diets. These authors found that the disappearance of dry matter from the bag was positively correlated with increasing level of rumen NH_3-N from below 10 to 24 mg% at which point degradability was at a plateau. However, rumen NH_3-N levels in the present study, were in range of NH_3-N reported by Satter and Slyter (1974) in mixture culture batch that ammonia-N lower than 2.0 mg% of rumen fluid can be limiting for microbial growth.

The proportions of DM disappearance from MS DDGS, MS SOM and WDGSMS diets at various periods of incubations in the rumen were presented in Figure 4.3.9.

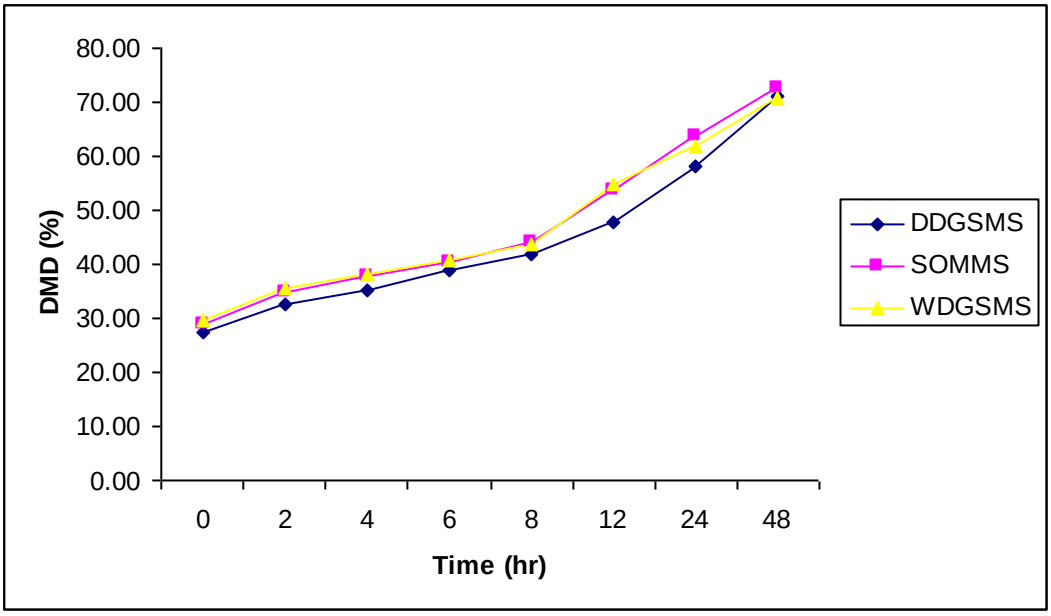

Figure 4.3.9 Proportions of DM disappearance for MS DDGS, MS SOM and WDGSMS diets incubated in the rumen of cannulated sheep

As was expected, 0 hour incubation time (washing losses) had less DM disappearance than any other sampling time for all diets. The average amount of soluble fractions at zero hour of incubation when samples were washed was lowest in the MS DDGS diet (27.3%), followed by the MS SOM diet (28.9%) and the WDGSMS diet (29.6%) having the highest DMD.

In the present study, the DM disappearance was more profound with increasing time of incubation in all diets. The rate of DM disappearance for each diet increased progressively as the incubation period increased, with that of the MS DDGS diet being lower at some points during the incubation period. This progressive increase with time of incubation was consistent with observations by Aina *et al*. (2004) for agricultural by-products like rice bran and wheat offals incubated in sheep. In addition, the MS DDGS diet had a lower ($P<0.05$) DM disappearance than that of the other two diets at 12 and 24 hours post incubation. Contrary to the study by Akbar *et al*. (2001) who incubated maize silage harvested at different maturity stages in sheep, DM disappearances in the present study were similar at all incubation times (except for 12 and 24 hour incubation). The incubation of all treatments was for 48 hours and this duration seemed inadequate as the plateau in the Figure 4.3.9 was not reached. The 48 hour incubation is good for high quality maize silage (NRC, 2001). Therefore, 72 hour incubation could have been appropriate for the diets used in the present study.

The MS DDGS diet had different degradability coefficients at 12 and 24 hours incubation time given the same incubation periods and that the rate of release of nutrients in the rumen also varied with the other two diets. The DMD allowed the description of the quantity of nutrients effectively degraded in the rumen of livestock and the outflow rate of food from the rumen (Susmel *et al*., 1990). The variations in degradability characteristics observed for the three diets could have been partly due to differences in fibre content (Smith *et al*., 1989). The MS SOM diet had a lower fibre concentration and could have affected microbial activity in the rumen.

Disappearance of DM material from the rumen is the sum of the material degraded by microbial fermentation and material of suitable particle size washed from the rumen (Kempton, 1980). The effective percentage of degradability of dry matter was

dependent on the course of degradation of the DM particles in the rumen and time distribution of the DM stay in the rumen. The DM degradation decreased when there was an increase in the rate of passage of the particles (McDonald *et al.*, 1990). In consequence, the rate of passage could have exerted a direct effect on the digestion process, absorption of nutrients and feed intake.

4.3.4 Growth performance trial

The inclusion of WDGS in a silage blend negatively affected dry matter intake of lambs when compared to MS DDGS and MS SOM diets. The DMI of the lambs fed WDGSMS diet was lower ($P<0.05$) than the other two diets throughout the experimental period (Table 4.3.4).

Table 4.3.4 Mean values (±s.e) of dry matter intake (g/head/day) changes for Merino lambs given three treatment diets

		Treatment			
Parameter	**Days**	**MS DDGS**	**MS SOM**	**WDGS MS**	**SEM***
DMI (g/head/day)	7	730[a]	810[a]	600[b]	±0.034
	14	1010[a]	1110[a]	820[b]	±0.045
	21	1120[a]	1170[a]	900[b]	±0.040
	28	1180[a]	1190[a]	930[b]	±0.034
	35	1180[a]	1190[a]	870[b]	±0.043
	42	1250[a]	1280[a]	820[b]	±0.039
	49	1280[a]	1290[a]	810[b]	±0.045

[a, b,c] For each row means with common superscripts do not differ ($P>0.05$)

* Standard error of the mean

Lambs offered MS DDGS and MS SOM did not differ ($P>0.05$) in DM intake. This observation was consistent with that of Anderson *et al.* (2006), who found that Holstein heifers offered silage of maize plants blended with 20% WDGS, recorded a lower DM intake compared to control (maize silage) and maize silage mixed with 20% DDGS at feeding. Decreased intake may be expected when there is a high inclusion level of WDGS because of the high moisture concentration (Table 4.2.1) and possibly sulphuric acid in the diet (Anderson *et al.*, 2006).

When diet DM concentration decreased below 50%, gut fill might have limited DMI (Lahr *et al.*, 1983). This is typically common when high moisture feed product is

combined with fermented feeds, as was the case in this study. Although all diets in the present study had a DM concentration below 50%, DMI was significantly lower in WDGSMS diet compared to other diets. Hippen *et al*. (2003) observed decreased DMI when cows were fed 30% or less of the ration DM as WDGS; such diets contained less than 50% DM. Larson *et al*. (1993) obtained a linear decrease in DMI in growing lamb when they increased the inclusion level of WDGS from 0 to 40% of the diet DM. In the present study, the intake by lambs on WDGSMS diet was decreased compared to the other diets with higher DM which suggested that intake decreased with decreasing DM content of the diet. May *et al*. (2007) fed steers 25% DDGS (DM basis) with steam-flaked maize and the DMI was not affected compared to the control (steam-flaked maize), as was the case in this study.

The average changes in weight gain, average daily intake (ADI) and feed conversion ratio (FCR) for lambs fed MS DDGS, MS SOM and WDGS diets are represented in Table 4.3.5.

Table 4.3.5 Mean values (±s.e) for liveweight changes, feed intake and feed conversion ratios of Merino lambs given three treatment diets

	Treatment			
Parameter	**MS DDGS**	**MS SOM**	**WDGS MS**	**SEM***
ADI (g DM/kg $W^{0.75}$)	95.71[a]	98.96[a]	73.11[b]	±1.316
Initial mass (kg)	31.75	32.38	31.00	±1.316
Final mass (kg)	42.21[a]	41.81[a]	39.98[b]	±1.316
ADG (g/day)	220[a]	200[a]	170[b]	±1.316
FCR	5.35	5.77	5.10	±1.316

[a, b,c] For each row means with common superscripts do not differ (P>0.05)

* Standard error of the mean

The average daily intake per unit metabolic body weight (g DM/kg $W^{0.75}$) for lambs fed WDGSMS diet was lower compared to those fed MS DDGS and MS SOM diets. This finding confirms previous research by Spiehs *et al.* (2008) who fed finishing steers increasing levels of WDGS in the diet, and recorded decreased body weight gain, primarily due to low DMI. In the present study, the ADI (g DM/kg $W^{0.75}$) for WDGSMS diet was reduced partly due to the high moisture content of the feed which reduced intake (McDonald *et al*., 1990).

The lambs on all diets gained weight but at different proportions. The average final body weight was 42.21, 41.81 and 39.98 kg for lambs fed the MS DDGS, MS SOM and WDGSMS diets respectively. The body weight of lambs fed the WDGSMS diet were lower ($P<0.05$) than those of the other two diets. This was confirmed in the study of Vander Pol *et al*. (2005) who fed increasing levels of WDGS in high moisture maize, dry-rolled maize and alfalfa hay basal diet and reported lower weight gains compared to the control which was without WDGS. There was a tendency for a linear decrease in final body weight as level of DM concentration decreased from 43.0% for the MS DDGS diet to 33.6% for the WDGSMS diet.

Changes over time for body weight gained of lambs fed the three experimental diets are presented Figure 4.3.10. In all the diets, there were no significant differences in weekly weight gains of sheep among the treatments. Based on DMI, this scenario indicated that sheep on separate diets consumed different amounts of DM but had similar or a higher feed utilisation, as live weights did not differ throughout the experimental period.

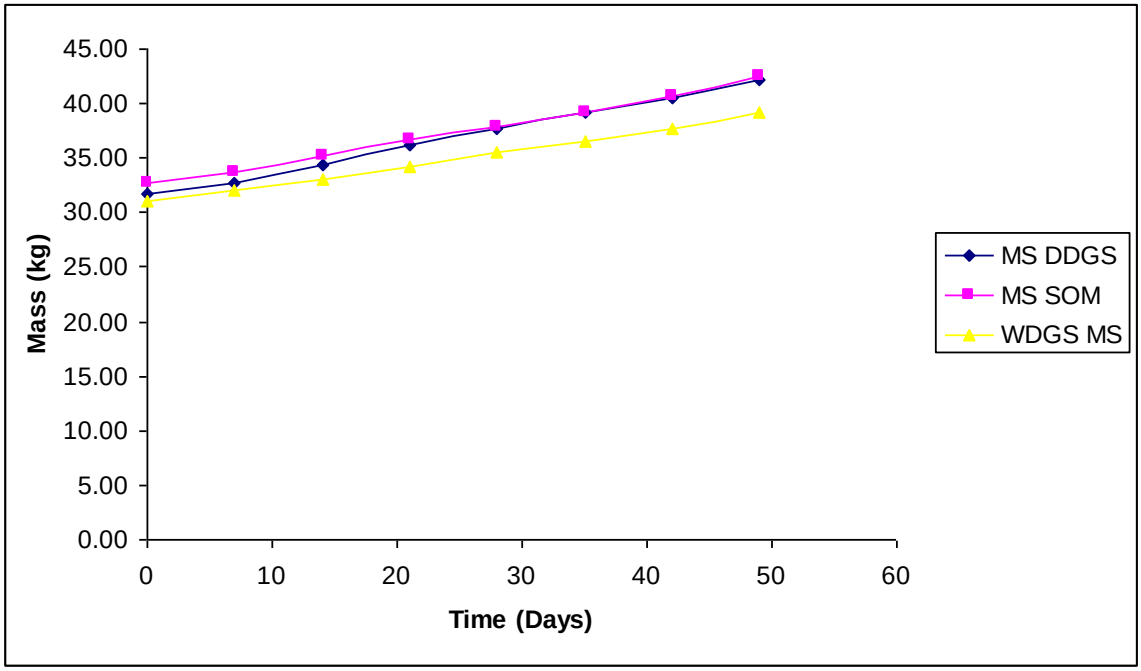

Figure 4.3.10 Mean values for weekly live weight changes of Merino lambs fed MS DDGS, MS SOM and WDGSMS diets

As with ADI, lambs offered the MS DDGS and the control diets had higher daily gains (220 and 200g/d) compared to those animals fed the WDGSMS diet (Table

4.3.6). The average daily gain over the entire trial period of lambs fed the three experimental diets is presented in Table 4.5.3.

The weight gain (ADG) on week 1 for lambs fed the MS SOM diet was higher (P<0.05) compared to lambs fed the MS DDGS and WDGSMS diets. The lambs offered WDGSMS diet had the lowest gain in the second week whilst those fed the MS DDGS and MS SOM diets had the highest daily gains. Average daily gains recorded for the MS DDGS and WDGSMS diets were significantly different but did not differ from that of the animals offered the MS SOM diet during the third week.

The tendency of weight gains among the three treatment diets on week five were similar to those of week two, with higher gains recorded for MS DDGS and MS SOM diets, with lambs offered WDGSMS having the lowest gain. In the final two weeks of the trial, there were no significant differences in the weight gains between the three diets.

Table 4.3.6 Mean values (±s.e) of weekly average daily gain for Merino lambs fed three experimental diets

		Treatment			
Parameter	**Days**	**MS DDGS**	**MS SOM**	**WDGS MS**	**SEM***
	7	140[b]	200[a]	140[b]	±0.015
	14	230[a]	200[a]	140[b]	±0.021
ADG (g)	21	250[a]	200[ab]	160[b]	±0.023
	28	210	180	200	±0.026
	35	210[a]	200[a]	140[b]	±0.022
	42	200	200	180	±0.024
	49	250	270	230	±0.023

[a, b,c] For each row means with common superscripts do not differ (P>0.05)

* Standard error of the mean

During the entire study, ADG was superior for lambs fed the control (MS SOM) and MS DDGS diets compared with those fed WDGSMS diet. As noted with DMI and final body weight, there was a linear decrease in ADG as the level of DM decreased. Larson *et al*. (1993) reported that ADG increased as level of WDGS increased in dry-rolled maize (DRC) based diets. This was contrary to the results of the present study where lambs fed diets involving WDGS (WDGSMS) had lower ADG compared to the control diet (MS SOM). Ham *et al*. (1994) reported significantly higher ADG for

cattle fed DRC diets supplemented with either wet or dry distillers grains. Meanwhile Al-Suwaiegh *et al*. (2002) demonstrated higher ADG when they fed up to 40% of DDGS to steers. This was in agreement with results recorded in the present study as lambs fed MS DDGS diet had higher ADG compared to lambs offered the WDGSMS diet. May *et al*. (2007) observed no differences when 25% of DDGS was fed compared to the diets offered without DDGS inclusion.

There were no significant differences recorded for the average FCR among the experimental diets (Table 4.3.5). Larson *et al*. (1993) noted that conversion efficiency increased as level of wet distillers grains increased. As was in this study, Lodge *et al*. (1997) noted lower ADG, but reported improved FCR for cattle fed WDGS over those fed a basal diet involving dry-rolled maize. In spite of low DMI for the diet containing WDGS, feed conversion of lambs fed WDGSMS diet were similar to those of lambs fed MS DDGS and MS SOM. This may be attributed to higher dietary fat concentration (May *et al.,* 2007) for lambs fed WDGSMS compared to lambs fed MS SOM and MS DDGS.

Despite the differences in degradability of DM (Table 4.3.3), the average FCR of lambs for the three diets were similar (Table 4.3.5). Based on the rumen fermentation results of this study, DM degradation was low for all diets, which could have translated to similar FCR performances across treatments. This was due to higher amounts of rumen undegradable protein present in distillers grains and sunflower oilcake meal as these are pre-heated feeds that could reduce degradability of protein in the rumen (McDonald *et al*., 1990).

The week-on-week FCR demonstrated that the first five weeks of the experimental period, the FCR was similar ($P>0.05$) for all diets (Table 4.3.7). However, feed conversion ratio decreased ($P<0.05$) for lambs fed WDGSMS diet compared to lambs fed MS SOM and MS DDGS diets at week 6 and 7. This decrease in FCR for lambs fed WDGSMS was due to the lower ADG and DMI (Bremer *et al.,* 2008) during the corresponding weeks of the sampling period.

Table 4.3.7 Mean values (±s.e) of weekly feed conversion ratios for Merino lambs given three treatment diets.

		Treatment			
Parameter	**Days**	**MS DDGS**	**MS SOM**	**WDGS MS**	**SEM***
	7	5.12	4.48	4.27	±0.306
	14	4.75	6.42	5.71	±0.573
	21	4.89	6.59	5.76	±0.594
FCR	28	6.19	7.14	5.29	±0.633
	35	6.14	6.72	6.07	±0.649
	42	6.95^{ab}	7.18^{a}	5.32^{b}	±0.619
	49	6.19^{a}	5.74^{ab}	4.62^{b}	±0.497

$^{a, b,c}$ For each row means with common superscripts do not differ (P>0.05)

* Standard error of the mean

CHAPTER 5

Conclusion and Recommendations

The amount of data that was collected in the initial part of this study revealed that ensiling wet distillers grains with solubles (WDGS) as a preservation method can be viable. However, there were concerns over the high proportion of acetic acid with increasing level of WDGS inclusion which is suspected to reduce feed intake (DMI), but is not yet clear in the literature at this point. The best quality silage is one that has a low pH, low NH_3-N and high lactic acid concentration compared to acetic acid concentration (Kung and Shaver, 2001). Low dry matter concentration of silage (283.0 g/kg DM) decreases DMI (Muck, 1988). Treatments containing 30% and 40% WDGS had a lower DM concentration than the range mentioned by Muck (1988) for final silage DM concentration. The DM concentration of such silages can be improved by ensiling WDGS with wilted whole maize plants which can probably increase the DM content of the silage.

The final silage pH for 0, 10, 20 and 30% WDGS treatments was within the pH range of 3.7 to 4.2 suggested by Kung and Shaver (2001) for well preserved whole maize silage. The 40% WDGS treatment recorded lower final pH values compared to other treatments. This low pH for 40% WDGS was due to the presence of a higher sulphuric acid concentration that contributed to the low silage pH, especially with treatments involving higher levels of WDGS inclusion. The low silage pH of WDGS blended silage suggested that preservation could be enhanced by combining WDGS and whole maize plants, as pH decreased with increasing level of WDGS inclusion. Therefore, based on the silage pH, all the silage treatments were well preserved.

Lactic acid concentration decreased with increasing level of WDGS inclusion. Some researchers (Garcia and Kalscheur, 2006) noted that the main acid contained in WDGS is acetic acid. In the present study, acetic acid concentration increased with increasing level of WDGS inclusion. Lactic acid is the important acid in the preservation of silage and therefore should dominate in any well preserved silage (Kung and Stokes, 2005). Our results revealed that the lactic acid concentration was higher for the control treatment. It may be important to inoculate lactic acid producing

bacteria in silages blended with WDGS. This may promote increased lactic acid production, and possibly decrease the fears of reduced DMI with silages containing a high proportion of acetic acid.

The silage NH_3-N concentration was less than 10% of the total nitrogen concentration for all treatments, indicating that there was minimal breakdown of CP during the ensiling process. This suggested that silage for all treatments were well preserved. There were no changes in the initial NDF concentration for all treatments, except at the 20% WDGS inclusion were NDF actually decreased. The level of NDF concentration after 120 days post ensiling was increased with increasing level of WDGS inclusion, indicating a relatively lower NDF degradation in the WDGS by-product. This also revealed that there was enough substrate for microbial utilization during the ensiling period, although the WSC concentration seemed to decrease with increasing level of WDGS inclusion. The ADF concentration increased for treatments with greater than 10% level of WDGS inclusion, but did not change significantly for control and 10% WDGS treatments. It could have been interesting to analyze for both NDF and ADF for the entire ensiling period as was with other measured parameters. Unfortunately this was not possible due to the limited financial assistance available for this study.

In the animal evaluation trial, the inclusion of WDGS as a blend with whole maize plants at ensiling (WDGSMS) and mixing DDGS with whole maize silage at feeding (MS DDGS) did not have a significant effect on average ruminal fermentation parameters when compared to the control diet (MS SOM). Data obtained from the rumen fermentation trial indicated how experimental diets (MS SOM, MS DDGS and WDGSMS) influenced the rumen environment based on the measures of rumen parameters (pH, NH_3-N and VFA). The average value observed for pH, NH_3-N, total VFA and individual VFA concentrations showed no major statistical differences for each of the experimental diets. However, lower concentrations of butyric acid were observed in sheep offered WDGSMS diets. This indicated that diets with WDGS produced less butyric acid in the rumen compared to the other two diets. Based on rumen NH_3-N concentration results, protein degradation was low across all diets. The optimal rumen NH_3-N concentration is 5mg/100ml when based on microbial growth

estimates (Satter and Slyter, 1974). However, Mehrez *et al.* (1977) suggested that the estimate of optimal rumen NH_3-N concentration was dependent on whether the rate of bacterial protein synthesis or rate of dry matter degradation.

The extent and rate of degradability of dry matter determines the degradable part available for the microbes (Ørskov and McDonald, 1979). This study revealed that the MS SOM diet has a higher effective degradability than the MS DDGS and WDGSMS diets at 2% rumen outflow rate. At the 5% rumen outflow rate, MS SOM diet was also more degradable than the MS DDGS diet, but comparable to the WDGSMS diet. High DMD of the MS SOM diet indicated a potentially higher intake when fed to sheep. Therefore, it could be recommended to conduct further nutritional degradability studies for other nutrients such as nitrogen, to come up with a more accurate conclusion about feeding value of the three diets. However, degradability in the rumen does not necessarily mean that the feed will be available to the animal (McDonald *et al.*, 1990), so it is also necessary to conduct intestinal digestibility studies to quantify the nutrients available to the animal.

The growth performance of merino lambs revealed that animals offered the MS SOM and MS DDGS diets had a greater ADI (g/kg$W^{0.75}$/day), than those fed the WDGSMS diet, which resulted in higher final weights for lambs offered the MS SOM and MS DDGS diets. The lower DMI and final weights for lambs offered WDGSMS diet was possibly a result of the reduced DM concentration in the diet.

Average daily gain (ADG) was lower for lambs offered the WDGSMS diet treatment as a result of a lower DMI. However, average values for FCR were similar for all diet treatments, which therefore indicated that WDGS could be preserved as a blend with whole maize plants and fed to ruminants without compromising the conversion rate of feed to animal product, even though week-on-week data showed a drop in FCR towards the end of the experimental period. There was, however, a need to supplement the WDGSMS diet with maize meal and sunflower oil cake meal to improve DM concentration of the diet, DMI and ADG. The DM can also be improved by ensiling WDGS with wilted whole maize plants. Therefore, lamb performance was

better when the MS SOM and MS DDGS diets were fed compared to the WDGSMS diet.

Chapter 6

Critical Evaluation

The trend to include distillers grains in animal feeds depends on the further development of the ethanol industry as well as other renewable sources of energy. These agricultural by-products present challenges in diet formulation due to excessive or deficient nutrients compared to the nutrient requirements of ruminant animals. Combination of these feeds seems to be a logical approach to improve the efficiency of utilization in ruminant diets as was demonstrated in the present study. The results of this study are important especially to those farmers with access to acquisition of distillers grains (dry or wet), either near or further way from the distillers grain producing plant.

Wet distillers grains with solubles (WDGS) were blended with chopped whole maize plants harvested at dough stage of maturity, and preserved through ensiling in bottle silos. The WDGS already contain sulphuric acid, which was expected to preserve the silage well due to its low initial pH. According to results obtained in the present study, blending 40% WDGS resulted in a lower DM concentration. However, this silage was well preserved when judged on pH, NH_3-N, and N losses which were low. Acetic acid concentration was high and lactic acid was low at the 40% WDGS inclusion level. So it was going to be informative to include more treatments higher than 40% WDGS inclusion level to determine the threshold for WDGS inclusion, but there were constraints of limited funding.

There were major limitations when analysing silage samples for fermentation characteristics. During each time of bottle opening, the plant material was immediately frozen. At time of analysis, the frozen silage material was left to thaw overnight and this lengthy period could have affected the silage composition. Room temperature variation between days of silage analysis could have also affected the results of fermentation characteristics. Further analysis for aerobic stability should have been conducted as it is an important characteristic for WDGS-maize silage blend.

Future studies could look at determining the sulphuric acid concentration in different inclusion levels of WDGS and quantify its involvement in the preservation of silage. It is also important to consider ensiling different inclusion levels of WDGS, as was in this study, but using bulk silos and then test the silage on feedlot performance of ruminants. There are huge differences in silage fermentation characteristics between results obtained from mini-silos and bulk ensilage.

The remainder of the study involved feeding formulated silage diets to rumen cannulated sheep and lambs. The bulk ensiling of WDGS-maize blend used in the diet formulation took in to consideration the fat, CP and P concentration in WDGS which led to the inclusion of 27% of WDGS in the silage blend. However, higher amounts of WDGS should also have been considered for increased utilisation of the by-product as it continues to be readily available due to the increased demand for cleaner renewable energy sources.

Experimental diets were formulated on an iso-nutrient basis for ME, CP, NDF, Ca and P to meet the nutritional requirements for growing lambs. Therefore, other ingredients like maize meal, limestone and monocalcium were included in the experimental diets. This meant that nutrient concentrations in the diets were not going to influence results of parameters measured for rumen fermentation and growth performance. Therefore, results could only have been affected by variations in dry matter intake, degradability and nutrient absorption.

One of the major constraints in the rumen fermentation trial was the more frequent opening of the sheep cannulae during incubation and removal of nylon bags. The two trials were conducted concurrently due to limited time and feed available. The ideal situation was to conduct the two trials separately which limits disturbances in the rumen fermentation process.

The growth performance trial was well designed and conducted throughout the experimental period. It is possible that the differences observed in growth performance in the current study reflect responses associated with the fibre concentration of WDGS and DDGS, but more research is required in this area. Given

potential cost and water use advantages for maize ethanol production, it would be desirable to quantify an ideal dietary concentration and optimal ratio for maize and WDGS blended silage. Future research should also consider *in situ* fibre digestion changes in rate of passage, and ruminal pH and VFA proportions in response to by-product source and level.

References

Aina, A.B.J., Ohiwasanmi, O.R., Oyesanya, N.K., Farinde, A.O., Akambi., O.E., Fadipe, T.A. and Sowande, O.S., 2004. Comparative rumen degradability of forages, agricultural byproducts and baobab bark in sheep. Arch. Zootec. 53: 321-324.

Aines, G., Klopfenstein, T. and Stock, R., 1997. Distillers grains. Nebraska Agricultural Research Division. Nebraska. USA.

Akayeza, J., Linn, J.G., Harty, S.R. and Cassady, J.M., 1998. Use of distillers grains and co-products in ruminant diets. Cargill Animal Nutrition Center. Minnesota. USA.

Akbar, M.A., Lebzien, P. and Flachowsky, G., 2001. Influence of maturity stage on in situ dry matter degradability of six maize varieties in fistulated sheep. Scottish Agricultural College. Aberdeen, UK.

Al-Suwaiegh, S.K., Fanning, C.K., Grant, J.R., Milton, T.C. and Klopfenstein, T.J., 2002. Utilisation of distillers grains from fermentation of sorghum or corn in diets for finishing beef and lactating dairy cattle. J. Anim. Sci. 80: 1105-1111.

Anderson, J.L., Schingoethe, D.J., Kalscheur, K.F. and Hippen, A.R., 2006. Evaluation of drier and wet distillers grains included at two concentrations in the diets of lactating dairy cows. J. Dairy Sci. 89: 3133-3142.

Anderson, J.L., Kalscheur, K.F., Garcia, A.D., Schingoethe, D.J. and Hippen, A.R., 2009. Ensiling characteristics of wet distiller's grains mixed with soybean hulls and evaluation of the feeding value for growing Holstein heifers. J. Anim. Sci. 87 (6):2113-2123.

AOAC, 2000. AOAC Method 965, 17. Photometric method. CAS-7723-14-0.

AOAC, 1990. Official methods of analysis of the Association of Official Analytical Chemists. 15th ed. AOAC, Washington, DC. USA.

ARC, 1978. Forage Maize: Production and utilization. Agricultural Research Council. London. UK. Pp346-68.

Bach, A., Calsamiglia, S. and Stern, M.D., 2005. Nitrogen metabolism in the rumen. J. Dairy Sci. 88 (Suppl.): 9-21.

Bechaz, F.M., 2000. The influence of growth stage on the nutritional value of *Panicum maximum* and *Digitaria eriantha* species silage for sheep. MSc Dissertation. University of Pretoria. Pretoria, South Africa.

Bell, B., 1997. Feeding sheep corn silage. Ministry of Agriculture, Food and Rural Affairs. Ontario.Canada.

Belyea, R.L., Rausch, K.M. and Tumbleson, M.E., 2004. Composition of corn and distillers dried grains with solubles from dry grind ethanol processing. Bioresourse Technology. 94 (3): 293-298.

Bergen, W.G., Cash, E.H. and Henderson, H.E., 1994. Changes in nitrogenous compounds of the whole corn plants during ensiling and subsequent effects on dry matter intake in sheep. J. Anim. Sci. 39: 629-637.

Black, J.L., Pearce, G.R. and Tribe, D.E., 1973. Protein requirements of growing lambs. Br. J. Nutr. 30: 45-52.

Bothast, R.J. and Schlicher, M.A., 2005. Biotechnological processes for conversion of corn into ethanol. Appl. Microbiol. Biotechnol. 67 (1): 19-25.

Bremer, V.R., Erickson, G.E. and Klopfenstein, T.J., 2008. Meta-Analysis of UNL feedlot trials replacing corn in WDGS. Nebraska Beef Cattle Reports. University of Nebraska. Lincoln, Nebraska, USA.

Broderick, G.A and Kang, J.H., 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. J. Dairy Sci. 63: 64-75.

Byers, F.M., Goodrich, R.D. and Meiske, J.C. 1982. Influence of acetic acid, lactic acid and ethanol on the fermentation of corn silage. J. Anim. Sci. 54: 640-648.

Castro, F.G.F., Nussio, L.G., Haddad, C.M., de Camos, F.P.,Coello, R.M., Mari, L.J. and Toledo, P.A., 2006. Fermentation characteristics and chemical composition of Tifton 85 silage with different dry matter contents. R. Bras. Zootec. 35:7-20.

Charmley, E., 2004. Making good corn silage. Agriculcure and Agri-Food Canada. Nappan. Nova Scotia. Available at www.farmwest.com/index.cfm?method=library.

Chillard, X., 1993. Dietary fat and adipose tissue metabolisim in ruminants, pigs and rodents. J. Dairy Sci. 76: 3897-3931.

Christiansen, R.R. and Wagner, D.G., 1974. Reconstituted wheat: Influence of physical form on *in vitro* digestibility. J. Anim. Sci. 38: 463-466.

Church, D.C., 1991. Roughages, Ch6. In: Livestock feeds and feeding. 3rd Edition. Prentice-Hall International Editions. USA.

Colenbrander, V.F., Miller, D.C., Wasson, J.A. and Cunningham, M.D., 1971. Corn stover silage supplemented with varying increments of energy for growing dairy heifers. J. Anim. Sci. 33: 1306-1309.

Collins, K., 2006. Economic issues related to biofuels. Agricultural Research Services. Northern Plains Research Lab. Sydney. Montana, USA.

Corrigan, M.E., Erickson, G.E., Klopfenstein, T. J. and Meyer, N.E., 2008. Effects of corn processing and wet distillers grains on nutrient metabolism. Beef Cattle Report. University of Nebraska. Lincoln, USA.

Dale, N. and Batal, A., 2005. Distillers grains: Focusing on quality control. University of Georgia. Georgia, USA.

Dermarquilly, C., 1988. Variation factors in the nutritive value of maize silage. In: Quality of maize silage, digestibility and zootechnical performance. Proceedings of an International Seminar at Gembloux. Belgium. pp 85-104.

Dijkstra, J., 1994. Production and absorption of volatile fatty acids in the rumen. Livestock Production Science. 39: 61-69.

Downing, T.W., Buyserie, A., Gamroth, M. and French, P., 2008. Effect of water soluble carbohydrates on fermentation characteristics of ensiled perennial Rygrass. Proffesional Animal Scientist. http//findarticles.com/p/articles /mi_qa 4035/.

Drouillard, J.S., Klopfenstein, T.J. Britton, R.A. Bauer, M.L Gramlich, S.M., Wester, T.J and Ferrell, C.L., 1991. Growth, body composition, and visceral organ mass and metabolism in lambs during and after metabolisable protein or net energy restrictions. J. Anim. Sci. 69: 3357-3375.

Engels, E.A.N. and Van der Merwe, F.J., 1967. Application of an *in vitro* technique to South African forages with special reference to the effect to certain factors on the results. S. Afr. J. Agric. Sci. 10: 983-992.

Erfle, J.D., Boila, R.J., Teather, R.M., Mahadevan, S. and Sauer, F.D., 1982. Effect of pH on fermentation characteristics and protein degradation by rumen microorganisms in vitro. J. Dairy Sci. 65: 1457-1464.

Feedinfo News Services, 2007. Debate the ethanol boom: What impact on Synthetic methionine usage. Available at http/www.feedinfo.com/console PageViewer.aspx?page=527623&source.

Ferreira, G. and Mertens, D.R., 2005. Chemical and physical characteristics of corn silages and their effects on *in vitro* disappearance. J. Dairy Sci. 88: 4414-4425.

Forouzmand, M.A., Gharbani, G.R and Alikhani, M., 2005. Influence of hybrid and maturity on nutritional value of corn silage for lactating dairy cows. 1: Intake, milk production and component yield. Pak. J. Nutr. 4 (6): 435-441.

Freer, M. and Dove, H., 2002. Sheep nutrition. CABI Publishing. New York. USA pp 143-163.

Ganesan, V., Kurt, A., Rosentrater, R. and Muthukumarappan, K., 2005. Effect of moisture content and soluble levels on the physical and chemical properties of DDGS. American Society of Agricultural and Biological Engineers. Michigan, USA.

Garcia, A.D., Thex, N., Kalscheur, K.F. and Tjardes, K., 2003. Interpreting corn silage analysis. Cooperative Extension Services. South Dakota, USA.

Garcia, A.D and Kalscheur, K.F., 2004. Ensiling wet distillers grains with other feeds. Cooperative Extension Services. South Dakota, USA.

Garcia, A.D. and Kalscheur, K.F., 2006. Storage of wet distillers grains. Cooperative Extension Services. South Dakota, USA.

Giron, H.C., 1973. Atomic Absorption Newsletter. 12. Pp 28-30.

Givens, D.I., Cottyn, B.G., Deway, P.J.S., and Steg, A., 1995. A comparison of the NDF – cellulose method with other laboratory methods for predicting the digestibility *in vivo* of maize silage from three European countries. Anim. Feed Sci. Tech. 54: 55-64.

Gordon, F.J., 1989. Effects of silage additives and wilting on animal performance, Ch. 8. In: Recent Advances in Animal Nutrition. (Eds W. Haresign and D.J.A Cole). Butterworth. London. Pp159-173.

Ham, G.A., Stock, R.A., Klopfenstein, T.J., Larson, E.M., Shain, D.H. and Huffman, R.P., 1994. Wet corn distillers byproducts compared with dry corn distillers grains with solubles as a source of protein and energy for ruminants. J. Anim. Sci. 72: 3246-3257.

Harris, J. and Wishart-Smith, L., 1984. Silage Management in Queensland. Queensland Govt. Brisbane, Australia.

Harris, L.E., 1970. Nutrition research techniques for domestic and wild animals. Vol. 1. Animal Science Department. Utah State University, Logaus, USA.

Hippen, A.R., Linke, K.N., Kalscheur, K.F., Schingoethe, D.J. and Garcia, A.D., 2003. Increased concentration of wet corn distillers grains in dairy cow diets. J. Dairy Sci. 86: 340. (Abstr.).

Hippen, A.R., Kalscheur, K.F., Schingoethe, D.J. and Garcia, A.A., 2004. Increasing inclusion of dried corn distillers grains in dairy cow diets. J. Dairy Sci. 87: 1965 (Abstr.).

Hoover, W.H., 1986. Chemical factors involved in ruminal fiber digestion. J. Dairy Sci. 69: 2755-2766.

Horvey, R., 2003. Making good silage. Ag-Info Centre. Alberta Agriculture and rural Development. Available at http://www.agric.gov.ab.ac.

Huls, T.J., Bartosh, A.J., Daniel, J.A., Zelinsky, R.D., Held, J. and Wertz-Lutz, A.E., 2006. Efficiency of dried distillers grain with solubles as a replacement for soyabeans meal and a portion of the corn in a finishing lamb diet. Sheep and Goat Research Journal. 21: 30-34.

Johnson, J.C. and Gates, R.N., 1999. Silage test results. Selection of corn hybrids for use as silage. University of Georgia. Goergia, USA. Available at http://www.pubs.caes.uga.edu/caespubs/ES-pubs/RR661-silage.htm.

Jonsson, A., 1991. Growth of *Clostridium tyrobutyricum* during fermentation and aerobic deterioration of grass silage. J. Agric. Sci. 54: 557-568.

Kaiser, R.M., 2005. Variation in composition of distillers wet grains with solubles. Mid west Plan Service. Wisconsin. USA.

Kaiser, R.M., 2006. Utilizing the growing supply of distillers grains. University of Wisconsin Extension Services. Wisconsin. USA.

Kalscheur, K.F., 2005. Impact of feeding distillers grains on milk fat, protein and yield. Processing Distillers Grains Technology Council. 10th Annual Symposium. Louisville. Kentucky. USA.

Kalscheur, K.F., Garcia, A.D., Hippen, A.R. and Schingoethe, D.J., 2003. Fermentation characteristics of ensiling wet corn distillers grains in combination with corn silage. J. Dairy Sci. 86 (suppl.1): 211

Kamalak, A., Erol, A., Guibuz, Y., Ozay, O., Canbolat, O. and Tumer, R., 2004. Evaluation of DM yield, chemical composition and IVDMD of silage from different maize hybrids. Bursa Uladag University, Turkey. Livestock Research for Rural Development 15 (11): 1-5

Kempton, K.J., 1980. The use of Nylon bags to characterize the potential degradability of feeds for ruminants. Trop. Anim. Prod. 5 (2): 107-116.

Kennelly, J.J., Robinson, B. and Khorasani, G.R., 1999. Influence of carbohydrates source and buffer on rumen fermentation characteristics, milk yield and milk composition in early lactating Holstein cows. J. Dairy Sci. 82: 2486-2496.

Kim, S.C. and Adesogan, A.T., 2006. Influence of ensiling temperature, simulated rainfall, and delayed sealing on fermentation characteristics and aerobic stability of corn silage. J. Dairy Sci. 89: 3122-3132.

Kleinschmit, D.H., Schingoethe, D.J., Kalscheur, K.F. and Hippen, A.R., 2006. Evaluation of various sources of corn distillers dried grains plus solubles for lactating dairy cattle. J. Dairy Sci. 89: 4784-4794.

Kononoff, P.J and Christiansen, R.R., 2007. Feeding dried distillers grains to dairy cattle. Western Nutrition Conference. University of Saskatchewan. Saskatchewan. Canada.

Köster, H., 2007. Dry milling ethanol by-products for animal feed (Part 1). AFMA Matrix. Special AFMA Forum 2007 Edition. Vol. 16 (1): pp 32-40.

Kung, L., 2000. Silage fermentation and additives. Miller Publishing Co. Minnesota, USA.

Kung, L. and Shaver, R., 2001. Interpretation and use of silage fermentation analysis reports. Forage Research Centre. Madison. Wisconsin, USA.

Kung, L. and Stokes, M.R., 2005. Analyzing silages for fermentation end products. University of Delaware. Delaware, USA. Available at http://www.ag.udel.edu/departments/faculty/kung/articles/analyzing_silage_for_fermentation htm.

Lahr, D.A., Otterby, D.E., Johnson, D.G., Linn, J.G. and Lundquist, R.G., 1983. Effect of moisture content of complete diets on feed intake and milk production by cows. J. Dairy Sci. 66: 1891-1900.

Larson, E.M., Stock, R.A., Klopfenstein, T.J., Sindt, M.H. and Huffman, R.P., 1993. Feeding value of wet distillers byproducts for finishing ruminants. J. Anim. Sci. 71: 2228-2238.

Linington, M.J., Meyer, J.H.F. and van der Walt, J.G., 1998. Ruminal VFA production rates, whole body metabolite kinetics and blood hormone

concentration in sheep fed high- and low-fibre diets. S. Afr. J. Anim. Sci. 28(2): 82-98

Lodge, S.L., Stock, R.A., Klopfenstein, T.J., Shain, D.H. and Herold, D.W., 1997. Evaluation of corn and sorghum distillers byproducts. J Anim, Sci. 75: 37-43.

Loy, D.D., 2006. Reducing energy costs in ethanol production through improved on-farm storage methods. Iowa Energy Center. Iowa, USA.

Loy, D.D., Strohbelm, D.R. and Martin, R.E., 2005. Factors affecting the economic of corn co-products in cattle feed. Ethanol Co-Products for Cattle. Iowa State University Extension. Iowa, USA.

May, L.M., 2007. The effect of processing method, wet and dry distiller's grains with solubles and roughage level on performance and carcass characteristics of finishing cattle. Masters Thesis. Kansa State University. Kansas, USA.

May, L.M., Quinn, M.J., Depenbush, B.E. and Drouillard, J. S., 2007. Uses of distillers dry grains in steam-flaked corn finishing diets with reduced roughage levels. J. Anim. Sci. (Suppl. 2): 411-412.

McCullough, M.E., Sisk, L.R. and Sell, O.E., 1963. Influence of corn distillers dried grains with solubles on the feeding value of wheat silage. J. Dairy Sci. 46: 43-45.

McDonald, I., 1981. A revised model for the estimation of protein degradability in the rumen. J. Agric. Sci. 96: 251-252.

McDonald, P., Edwards, R.A. and Greenhalgh, J.F.D., 1990. Animal Nutrition. 4th Edition. Longman Scientific and Technical. NY, USA.

McDonald, P., Henderson, A.R. and Heron, S.J.E., 1991.The Biochemistry of silage. Chalcombe Publications. Marlow, UK.

Meeske, H., Basson, H.M., Pienaar, J.P. and Crywagon, C.W., 2000. A comparison of the yield, nutritional value and predicted production potential of different maize hybrids for silage production. S. Afr. J. Anim. Sci. 30(1): 18-21.

Mehrez, A.Z., Ørskov, E.R. and McDonald, I., 1977. Rates of rumen fermentation in relation to ammonia nitrogen concentration. Br. J. Nutr. 38: 437-443.

Muck, R.E., 1987. Dry Matter level effects on alfalfa silage quality. 1. Nitrogen transformantions. Trans. Am. Soc. Agric. 30:7-14.

Muck, R.E., 1988. Factors influencing silage quality and their implications for management. J. Dairy Sci. 71: 2992-3002.

Muntifering. R.B., Burch, T.J., Miller, B.G., and Ely, D.G., 1983. Digestibility and metabolism of mature tall fescue hay reconstituted and ensiled with whole stillage. J. Anim. Sci. 57:1286-1293.

Nguessan, G.Y., 2007. Economical impact of ethanol production on US livestock sector: A spatial analysis on corn and distillers grains shipment. Ph.D Diss., Kansas State University. Manhattan, Kansas, USA.

NRC, 1985. Nutrient requirements of sheep. 6th Ed. National Academy of Science. Washington D.C, USA.

NRC, 2001. Nutrient requirements of dairy cattle. 7th Ed. National Academy of Science. Washington D.C, USA.

Neylon, M.J. and Kung Jr, L., 2003. Effects of cutting height and maturity on the nutritive value of corn silage for lactating cows. J. Dairy. Sci. 86: 2163-2169.

Norton, B.W., 1994. The nutritive value of tree legumes. Forage Tree Legumes in Tropical Agriculture. CAB International. Oxon, UK. pp 178-191.

Osuji, P.O., Nsahlai, I.V. and Khalili, H., 1993. Feed evaluation. ILCA Manual 5. International Livestock Centre for Africa, Addis Ababa, Ethopia. 40pp.

Owens, F.N., Secrist, D.S., Hill, W.J. and Gill, D.R., 1998. Acidosis in cattle: A review. J. Anim. Sci. 76: 275-286.

Ørskov, E.R. and Fraser, C., 1975. The effects of processing of barley-based supplements on rumen pH, rate of digestion and voluntary intake of dried grass in sheep. Br. J. Nutri. 34 (3): 493-500.

Ørskov, E.R. and McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. 92: 499-503.

Playne, M.J. and McDonald, P.M., 1966. The buffering constituents of herbage and silage. J. Sci. Food Agric. 17: 264-268.

Peter, C.M., Faulkner, D.B., Merchen, N.R., Parrett, D.F., Nash., T.G. and Dahlquist, J.M., 2000. The effect of corn milling on growth performance and diet digestibility by beef cattle. J. Anim. Sci. 78: 1-6.

Promkot, C., Wanapat, M. and ROwlinson, P., 2007. Estimation of ruminal degradation and intestinal digestion to tropical protein resources using the nylon bag technique and the 3step *in vitro* procedure in dairy cattle on rice straw diets. Asia-Australasian J. Anim. Sci. 12: 5-20.

Pryce, J.D., 1969. A modification of the Barker-Summerson method for the determination of lactic acid. Analyst. 94 (1125): 1151-1152.

Renewable Fuels Association, 2009. How ethanol is made. Available at http://www.ethanolrfa.org/resource/made.

Rinker, C.B. and Berger, L.L., 2003. Optimizing the use of distillers grains for dairy-beef production. University of Illinois. Illinois, USA.

Robertson, J.B. and VAN Soest, P.J., 1981. The detergent system of analysis and its application to human foods. In: The Analysis of Dietary Fibre in Food. Eds. James, WP.T. and Theander, O. Marcel Dekker. New York, USA. pp 123-158.

Robinson, P.H., 2003. Estimating the energy value of dairy feeds: Evaluating UC Davis and NRC (2001) equations. Cooperative Extension. University of California. Davis. California, USA.

Roffler, R.E. and Setter, L.D., 1975. Relationship between ruminal ammonia nitrogen and non-protein nitrogen utilization by ruminants. 1: Development of a model of predicting non-protein nitrogen utilization by cattle. J. Dairy Sci. 58: 1880-1889.

Samuels, M.L., 1989. Statistics for the life sciences. Collier MacMillan Publishers. London, UK.

SAS., 1990. SAS User's Guide; Statistics (Version 6 Ed.). SAS Institute Inc., Cary. North Carolina, USA.

Satter, L.D and Slyter, L.L., 1974. Effect of ammonia concentration on rumen microbial production in vitro. Br. J. Nutr. 32: 199-208.

Schingoethe, D.J., 2001. Using distillers grains in the dairy rations. National Corn Growers Association. Ethanol Co-products. Lincoln. Nevada, USA.

Schingoethe, D.J., 2004. Distillers grains for dairy cattle. South Dakota State University. Extension Service Extension Extra. ExEx 4022. South Dakota, USA.

Schingoethe, D.J., Brouk, M.J. and Birkelo, C.P., 1999. Milk production and composition from cows fed wet corn distillers grains. J. Dairy Sci. 82:574-580.

Schingoethe, D.J., Kalscheur, K.F and Garcia, A.D., 2002. Distillers grains for dairy cattle. Cooperative Extension Services. South Dakota State University. South Dakota, USA.

Schroeder, J.W., 2003. Distillers grains as a protein and energy supplement for dairy cattle. NDSU Extension Service. North Dakota, USA.

Schroeder, J.W., 2004. Silage fermentation and preservation. NDSU Extension Services. North Dakota, USA.

Schutz, M.M., Johnson, T.R. and Donkin, S.S., 2006. Issues surrounding the use of distillers grains by the Indiana Dairy industry. Purdue University Extension. Indiana, USA.

Seglar, B., 2003. Fermentation analysis and silage quality testing. Global Agronomy and Nutritional Sciences. Proceedings of the Minnesota Dairy Health Conference. College of Veterinary Medicine. University of Minnesota, USA.

Shaver, R.D., 2003. Practical application of new forage quality tests. University of Wisconsin-Extension. Wisconsin, USA.

Shaver, R.D., Erdman, R.A., O'Connor, A.M. and Vandersall, J.H., 1985. Effects of silage pH on voluntary intake of corn silage and alfalfa haylage. University of Wisconsin-Extension. Wisconsin, USA.

Shurson, J., 2006. What's new in feeding distillers byproducts to swine? 67th Minnesota Nutrition Conference. St Paul. Minnesota, USA.

Smith, O.B., Idowu, O.A., Asaolu, V.O. and Odunlabi, O., 1989. Comparative rumen degradability of forages, browse, crop residues and agricultural by-products. Proc. Conf. African small Ruminant Researchand Development. Bamenda, Cameroom. pp 205-215.

Spiehs, M.J., Whitney, M.H. and Shurson, G.C., 2002. Nutrient database for distillers dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. J. Anim. Sci. 80:2639-2645.

Spiehs, M.J., Ferrell, C.L., Nienaber, J. A., Brown-Brandl, T.M. and Shackelford, S. D. 2008. Heat production and efficiency of energy utilization in finishing steers fed diets containing wet distillers grains with solubles (WDGS). J. Anim. Sci. E-Suppl. 2: 583 (Abstr.).

Stern, M.D., Bach, A. and Calsamiglia, S., 2006. New concepts in protein nutrition of ruminants. 21st Aminal Southwest Nutrition and Management Conference.

Storm, E. and Ørskov, E.R., 1983. The nutritive value of rumen microorganisms in ruminants. 1. Large-scale isolation and chemical composition of rumen microorganisms. Br. J. Nutr. 50: 463-470.

Susmel, P., Stefanon, B., Mills, C.R and Spanghere, M., 1990. Rumen degradability of organic matter, nitrogen and fibre fractions in forages. Anim. Prod. 51: 515-536.

Tilley, J.M.A. and Terry, R.A., 1963. A two-stage technique for the *in vitro* digestion of forage crops. J. Brit. Grassland Soc. 18: 104-111.

Tjardes, K. and Wright, C., 2002. Feeding corn distillers co-products to beef cattle. Animal and Range Science. ExEx 2036. South Dakota, USA.

Trenkle, A., 2004. The advantages of using corn distillers grains with solubles in dairy-beef diets. Iowa State University. Iowa Corn Growers Association Brochure. Iowa, USA.

Tuah, A.K., Okai, D.B., Orskov, E.R., Shand, W., Greenhalgh, J.F.D., Obese, F.Y. and Kankari, P.K., 1996. In Sacco dry matter degradability and *in vitro* gas production characteristics of some Ghanian feeds. Livestock Research for Rural Development. 8 (2): 23-33.

Tylutki, T.P., 2006. Raw material processing and quality. AFMA Symposium. Pretoria, South Africa.

Umberger, S.H., 1996. Feeding Sheep. Extension Animal Scientists. Virginia Tech. Virginia, USA.

Vander Pol, K., Erickson, G., Kloptenstein, T. and Greenquist, M., 2005. Effect of level of wet distillers grains on feedlot performance of finishing cattle and energy value relative to corn. J. Anim. Sci. 83 (Suppl. 2): 55 (Abstr.)

Wahlberg, M.L., 2004. Treating corn silage to enhance its protein content. Virginia State University. Virginia, USA.

Webb, E.C., 1994. Synthesis of Long chain fatty acids in ruminants and their effects on meat quality. Chapter 3. pp 58-59.

Wise, M.B., Ordeveza, A.L. and Barrick, E.R., 1963. Influence of variations in dietary calcium and phosphorus ratio on performance and blood constituents on calves. J. Nutr. 1:79-84.

White, P.J and Johnson, L.A., 2003. Corn: Chemistry and Technology. 2nd Edition. American Association.

Wilkens, M., Mader, T.L., Erickson, G.E. and Johnson, L.J., 2009. Comparison of dry distillers or modified wet distillers grains plus solubles in wet of dry forage

based diets. Nebraska Beef Cattle Reports. University of Nebraska. Lincoln, USA.

Wilson, J.K. and Webb, H.J., 1937. Water soluble carbohydrates in forage crops and their relation to the production of silage. J. Anim. Sci. 20 (5): 257-268.

Zimmerman, C., 2002. Silage fermentation analysis. Available at www.blueseal.com.