The effect of endosperm vitreousness on fermentation characteristics and *in vitro* digestibility of maize

by

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Thesis presented in partial fulfilment of the requirements for the degree Master of Science in Agriculture (Animal Science)
at
Stellenbosch University

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December 2010

DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the authorship owner thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: December 2010

Abstract

Title	:	The effect of endosperm vitreousness on fermentation characteristics and *in vitro* digestibility of maize.
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The purpose of this study was to investigate the variation that exists between maize samples regarding particle size separation, *in vitro* fermentation kinetics and *in vitro* dry matter (DM) disappearance. A second objective was to quantify possible relationships between the Roff Milling Index (RMI) of maize and any of the measured *in vitro* parameters. Three trials were conducted: a particle size distribution trial, a gas production trial and an *in vitro* DM degradability and starch disappearance trial.

Overall, nine maize samples, which differed in terms of cultivar and endosperm type, were collected from different origins for the study. The samples were selected in terms of their Milling Index (MI). Three of the nine samples had a high MI that ranged between 109 and 118, three had a low MI that ranged between 67 and 71 and the other three samples had a medium MI that ranged between 85 and 92. Although the MI is not a direct indication of the hardness or softness of the endosperm, it was believed to be indirectly associated with vitreousness.

In the first trial, the different maize samples were milled through a 1 mm screen and sieved through a series of three sieves 150, 125 and 106 µm, respectively. It was found that RMI was not a reliable indicator to predict particle size distribution, especially in terms of the coarse (>150 µm) and very fine (<106 µm) particles.

In the gas production trial, the nine different maize samples were subjected to a gas production system for a duration of 48 hours. Here gas production and rate of gas production of the different maize types in buffered rumen liquor were measured during incubation. After fitting the gas volume data to the respective models, the non-linear parameters b, c and L were subjected to a main effects ANOVA with the aid of Statistica, version 9 (2009). Main effects were treatment and repetition. Means were separated by means of a Scheffé test and significance was declared at $P < 0.05$. The results were compared to the RMI of the different maize types and it was concluded that RMI was not a reliable predictor of gas production or rate of gas production of different maize types.

In the third trial, *in vitro* DM degradability and starch disappearance of the different maize types were measured. *In vitro* DM degradability was conducted in the Ankom DAISY^{II} incubator apparatus and the

incubation times were 0, 2, 4, 8, 12 and 24 hours. Starch disappearance was measured on residues of the samples incubated for 0, 2 and 4 hours. After fitting the DM disappearance data to the respective models, the non-linear parameters a, b, c and L were subjected to a main effects ANOVA with the aid of Statistica, version 9 (2009). Main effects were treatment and repetition. Means were separated by means of a Scheffé test and significance was declared at $P<0.05$. The results indicated variation between maize samples, especially in terms of the a-, b- and L-values. The RMI did not appear to be a reliable predictor of digestibility parameters.

Uittreksel

Titel	:	Die invloed van endospermtipe op fermentasie-eienskappe en *in vitro*-verteerbaarheid van mielies.
Kandidaat	:	Petro Trudene Burden
Studieleier	:	Prof. C.W. Cruywagen
Instansie	:	Departement Veekundige Wetenskappe, Universiteit van Stellenbosch
Graad	:	MSc (Agric) Veekunde

Die doel van hierdie studie was om die variasie tussen mieliemonsters te ondersoek ten opsigte van die skeiding van partikelgroottes, *in vitro*-fermentasiekinetika en *in vitro*-droëmateriaalverdwyning. ‘n Tweede doel was om te bepaal of daar moontlike verwantskappe tussen die Roff Milling Index (RMI) van mielies en enige van die ander *in vitro*-parameters bestaan. Drie proewe is gedoen: verspreiding van partikelgrootte, ‘n gasproduksieproef en ‘n droëmateriaal degradeerbaarheid- en stysel verdwyningsproef.

Nege mieliemonsters, wat van mekaar verskil ten opsigte van kultivar en endospermtipe, is van verskillende lokaliteite versamel. Die monsters is gekies in terme van hul maal-indeks (MI). Drie van die nege monsters het ‘n hoë MI gehad wat gewissel het tussen 109 en 118, drie het ‘n lae MI gehad wat gewissel het tussen 67 en 71 en die ander drie monsters het ‘n medium MI gehad wat gewissel het tussen 85 en 92. Alhoewel die MI waardes nie ‘n direkte indikasie van ‘n endosperm se hardheid- of sagtheidsgraad is nie, is dit aanvaar dat daar ‘n indirekte verwantskap tussen MI en glasagtigheid van die mielie bestaan.

In die eerste proef is die nege verskillende mieliemonsters deur ‘n 1 mm sif gemaal en daarna deur ‘n reeks van drie siwwe met groottes van onderskeidelik 150, 125 en 106 µm gesif. Daar is bevind dat die RMI nie ‘n betroubare voorspeller is om partikelgrootte-verspreiding aan te dui nie, veral nie ten opsigte van growwe (> 150 µm) en baie fyn (< 106 µm) patikels nie.

Tydens die gasproduksieproef is die nege mieliemonsters vir 48 ure blootgestel aan ‘n gasproduksiesisteem, waar gasdruk outomaties aangeteken is. Gasproduksie en tempo van gasproduksie van die verskillende mieliemonsters is gemeet en aangeteken gedurende inkubasie met ‘n gebufferde rumenvloeistofmedium. Nadat die gasvolumedata met behulp van relevante modelle gepas is, is die nie-linêre parameters b, c en L onderwerp aan ‘n hoof-effek ANOVA met die gebruik van Statistica weergawe 9 (2009). Hoof-effekte was behandeling en herhaling. Gemiddeldes is deur ‘n Scheffé-toets geskei en betekenisvolheid is verklaar by $P<0.05$. Die resultate verkry is vergelyk met die RMI van die verskillende mielietipes. Die gevolgtrekking is gemaak dat Roff MI nie ‘n betroubare voorspeller van totale gasproduksie of gasproduksietempo is nie.

Tydens die derde proef is droëmateriaaldegradeerbaarheid en styselverdwyning van die verskillende mielietipes bepaal. *In vitro* droëmateriaal (DM) degradeerbaarheid is gedoen in die Ankom DAISY^{II} inkubator met inkubasietye van 0, 2, 4, 8, 12 en 24 ure. Styselverdwyning is bepaal deur styselanalises op die residue

van die monsters wat geïnkubeer is vir 0, 2 en 4 ure. Nadat die DM-degradeerbaarheid met behulp van relevante modelle gepas is, is die nie-lineêre parameters a, b, c en L onderwerp aan ‘n hoof-effek ANOVA met die gebruik van Statistica weergawe 9 (2009). Hoof-effekte was behandeling en herhaling. Gemiddeldes is deur ‘n Scheffé toets geskei en die betekenisvolheid is verklaar by $P<0.05$. Die resultate het aangedui dat daar groot variasie tussen mielies bestaan, veral ten opsigte van die a-, b- en L-waardes. Dit het verder geblyk dat die RMI van die verskillende mielietipes nie ‘n betroubare voorspeller van DM-degradeerbaarheid was nie.

Acknowledgements

I would like to express my sincere appreciation and gratitude to the following persons and institutions for the role they have played in completing this thesis. Without any one of the following mentioned people this thesis would not have been possible.

- To God, that granted me the strength, endurance and good health, to complete this work.
- Prof. C.W. Cruywagen, my supervisor for his guidance, support and patience.
- Dr M. Manley, Department of Food Sciences, Stellenbosch University, for assistance and providing the different maize samples that were used in the trials.
- Prof Nel for assistance with the statistical analyses of the data.
- The Animal Sciences Department, Stellenbosch University's technical staff for some of the analyses done.
- The management and staff at Welgevallen Experimental Farm for looking after and managing the animals.
- NRF for the bursary.
- My parents, Eric and Christine Burden, for their encouragement and support during my studies.
- My friend and fellow student, Dawn Botha, for her assistance and support in the trials done.

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CHAPTER 1

1.1 GENERAL INTRODUCTION

Milk, either as milk or products from milk origin, is used every day by people in households, factories and bakeries. In South Africa milk is produced in all of the nine provinces, some more than others. According to Milk Producer's Organisation (2010) of South Africa the Western Cape and the Eastern Cape are the two leading provinces in milk production. Table 1 below gives a brief overview of the number of milk producers found in each province as well as the milk production for each province.

Table 1 Milk producers per province and milk production per producer in South Africa (Milk Producer's Organization, 2010).

Province	Milk producers		% Milk produced	
	2006	2010	1997	2009
Western Cape	878	754	22.9	27.1
Eastern Cape	422	354	13.8	25.0
Northern Cape	39	45	1.2	0.4
KwaZulu-Natal	402	348	15.7	19.8
Free State	1067	835	18.0	14.0
Northwest	649	507	12.6	5.3
Gauteng	275	212	4.4	3.4
Mpumalanga	407	248	11.0	4.5
Limpopo	45	29	0.4	0.3
TOTAL	**4 184**	**3 332**	**100.0**	**100.0**

Due to the high demand for milk for everyday use, the dairy farmer aims to increase milk production per cow. Proper nutrition is one of the most important factors that will increase a cow's milk production and the main expense in a dairy farm is the cost of feed. Furthermore, a large amount of feed needs to be consumed by cows to achieve the amount of milk production that are expected today. Farmers must provide the correct feed in the correct amount to increase the milk production per cow, thus increase the farms profit whilst at the same time keeping feed costs low. Animal nutritionists must formulate dairy rations, using feedstuffs of good nutritional value to meet the cow's nutrient requirements. These rations must increase milk production, while minimising loss in bodyweight and minimising digestive upsets and still save the farmer money, in order for the farm to be an economical operation. What determines a feed's nutritional value is the concentration of its chemical components and the extent and rate of the feed's digestion (Getachew *et al.*, 2004). Good energy sources are very important, because these contain carbohydrates, which will produce the substrates for the synthesis of milk within the cow. An excellent carbohydrate energy source that can be fed to cattle is maize. Carbohydrates are fermented in the rumen and this is why fermentation is of great importance. It is important to try to increase the efficiency of fermentation and degradation of dietary components in the rumen, because by doing so, the efficiency of feed used by cattle will be improved and thus profitability will be increased in modern dairy cattle herds (Zebeli *et al.*, 2010; Eastridge, 2006).

The second largest use for cereals in the world besides for human consumption is for feeding animals. In livestock diets, cereal grains are being used to a larger and larger extent (Evers *et al*., 1999). Of the cereal grains, maize is the largest in size and has a rather large endosperm. Many small starch granules that have an average size of 10 µm occur in the starchy endosperm (Evers *et al.*, 1999). Most of the grain consists of the endosperm. In the endosperm, there can be clearly distinguished between two components, viz. starch and protein. The starch makes up the majority of the endosperm and it consists of cells that are packed with nutrients. These nutrients can be used by the grain at the beginning of germination to support growth of the embryonic axis. Nutrients in the grain are stored in the insoluble form, starch being the major carbohydrate component (Evers *et al*., 1999). Floury endosperm is surrounded by a deep cap, which is the horny endosperm. Among maize types the most significant differences lies in the shape and character of the endosperm (Evers *et al*., 1999).

Like all other cereal grains, maize too has certain limitations as a food source for cattle. Except for the fact that maize is an excellent source of digestible energy, maize is relatively low in protein. The protein present in maize is also of relative poor quality. Maize has a high metabolisable energy value, is low in fibre and contains about 730 g starch/kg DM (McDonald *et al.,* 2002). It is rather difficult, in a high-producing dairy cow's diet, to establish and find the optimal balance between the amount of rumen fermentable carbohydrates and physically effective fibre, but the balance is very important to prevent sub-acute ruminal acidosis, to optimize digestion and nutrient utilization and also to improve the animal's productivity (Zebeli *et al.*, 2010). The advantage of maize is that maize starch digests more slowly in the rumen than other grains. This is an important feature in order to prevent conditions such as acidosis. When maize is fed at high levels a proportion of the starch will pass into the small intestine. Here the starch will be digested and absorbed as glucose (McDonald *et al.*, 2002).

Fermentation in the rumen is largely performed by ruminal bacteria. Fungi and protozoa participate to a lesser extent in ruminal digestive processes (Huntington, 1997). The microbes in the rumen degrade the starch granules starting from the outside whereas α-amylase enzymes attack the granules at particular spots on the surface at first and then begin to degrade the inner part (Cone, 1991; Huhtanen & Sveinbj□rnsson, 2006). Whole maize grains with an intact pericarp are almost completely resistant to ruminal digestion, because bacterial attachment cannot take place on whole kernels. Whole grains are processed by application of combinations of mechanical, moisture, heat and time to improve the ability for the bacteria to attach to the exposed starch granules and thus increase starch digestibility (Huntington, 1997). When maize grain particle sizes are reduced by mechanical processing the ruminal degradability of starch will be increased. This will be because of the larger areas that are being exposed to microbial attack (Zebeli *et al.*, 2010).

The method commonly used for the measurement of starch degradation is the *in vitro* method. Here starch disappearance can be measured directly after incubation for various time intervals or it can be measured indirectly by measuring the amount of gas produced (Menke *et al*., 1979; Huhtanen & Sveinbj□rnsson, 2006). Extensive use has been made of the *in vitro* dry matter digestibility method to evaluate the nutritional

value of ruminant feeds (Mabjeesh *et al.*, 2000). The development of the DAISY^{II} apparatus was a step in the right direction in the search for better labour efficiency. This apparatus allows different feedstuffs, which have been sealed in polyester bags, to be incubated simultaneously within the same incubation vessel for various times. During incubation, the feedstuff that disappears from the sealed polyester bag is considered digestible (Mabjeesh *et al.*, 2000). Starch degradation can be indirectly determined by the measurement of gas production. *In vitro*, when incubation of a feedstuff with rumen fluid takes place the fermentation of carbohydrates will produce gasses (CH_4 and CO_2), short chain fatty acids and microbial cells. The production of gas is a result of carbohydrates that are being fermented to propionate, acetate and butyrate (Getachew *et al.*, 1998). Gas is produced in larger quantities when carbohydrates are fermented to acetate and butyrate. When carbohydrates are fermented to propionate, a relatively small amount of gas will be produced. This is due to gas alone being formed from buffering of the acid (Hungate, 1966; Van Soest, 1994; Getachew *et al.*, 1998). Thus, the gas that is released when propionate is generated is only the indirect gas that is produced from buffering (Getachew *et al.*, 1998).

The diet that an animal receives is the most important factor that influences the microbial fermentation in the rumen (Bergen & Yokoyama, 1977). A high correlation was found in a number of studies between dry matter disappearance and *in vitro* gas production and starch availability in cereal grains (Opatpatanakit *et al.*, 1994; Menke *et al.*, 1979; Xiong *et al.*, 1990; Blummel & Orskov, 1993). Research to-date, where the relationship between dry matter (DM) disappearance or *in situ* starch degradability of maize and maize endosperm vitreousness have been evaluated, have shown that there is a strong negative relationship between DM or *in situ* starch degradability and endosperm vitreousness. This means that the DM degradability as well as *in situ* starch degradability will decrease as the maize endosperm vitreousness increase (Hoffman & Shaver, 2009; Philippeau & Michalet-Doreau, 1997; Correa *et al.,* 2002; Ngonyamo-Majee *et al.,* 2008).

Better knowledge of the relationship between starch or DM digestibility and maize vitreousness may help to select maize hybrids that would result in improvements in the utilization of diets consumed by ruminants (Correa *et al.,* 2002).

A study was done at the Stellenbosch University to investigate the variation that exists among maize samples regarding particle size separation, *in vitro* fermentation kinetics and *in vitro* dry matter disappearance. A second objective was to quantify possible relationships between the Roff Milling index of maize and any of the measured *in vitro* parameters.

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

In the anaerobic environment of the fore stomach of the cow, microbial digestion occurs and this is termed fermentation. Fermentation of carbohydrates in the rumen will produce volatile fatty acids (VFA's) of which the primary ones are acetic acid, propionic acid and butyric acid. These VFA's are absorbed directly across the rumen wall and are the major energy source for the cow (Frandson *et al*., 2006).The primary component of cereal grains is starch. Fermentation of starch in the rumen is determined by the rate at which the starch is fermented and also by the starch retention time in the rumen. Both these two factors will vary by the physical status of the animal, the type of grain that is eaten and also the chemical and physical processing method that the grain has undergone (Knowlton, 2001).

Many grain processing methods have been developed and are used to try and improve ruminal fermentation and feed utilization by dairy cows (Theurer, 1986). The extent of grain processing will play a role in how the animal responds to the type of grain (Foley *et al*., 2006). The efficiency by which cereal grains are utilized is increased by proper processing of the cereal grains, but the potential costs, profit and advantages of processing depends on the method used to process the grain, the ruminant species and also the grain type selected (Theurer, 1986; Beauchemin *et al*., 1994). To increase the extent of starch fermentation and digestion in ruminants, different processing methods such as flaking, steam rolling and fermentation, which includes high moisture storage, will be used rather than fine grinding (Owens & Zinn, 2005). For different processing methods, different grain hybrid characteristics are desired (Owens & Zinn, 2005). For dry rolled and whole maize, very fine grinding of cereal grains with a thin pericarp or loose coat, a floury endosperm and a low amylopectin:amylose ratio would all help to increase starch fermentation and digestion (Owens & Zinn, 2005). Grain processing methods that involve treatment by moist heat and flaking would result in a complete and more rapid fermentation that would also change the VFA ratios (Church, 1971). As dry matter intake increases, there will usually be an increase in the milk yield per cow. Thus, the efficiency of digestibility and ruminal fermentation of the dietary components are very important in improving the efficiency of feed usage (Eastridge, 2006).

Most of the starch of grains is fermented in the rumen. This is very important for microbial protein synthesis and the synthesis of propionic acid (Eastridge, 2006). The efficiency of production of microbial protein and feed intake may decrease with a starch source that is too rapidly fermented (Allen, 2007). The concentration of starch in the diets of dairy cattle must be based on the relative rate of the fermentation of starch and on the effectiveness of the fibre in the diet (Eastridge, 2006). Glucose, sucrose and fructose are readily fermented in the rumen and are known as rapidly fermentable sugars whereas maltose, galactose and lactose are much less efficiently utilized (Barnett & Reid, 1961). How long rumen fermentation will carry on as well as the rate of ruminal fermentation are both affected by the chemical structures of the starch granules

and their links with protein moieties in the cereal grain (Huntington *et al*., 2006). When the fermentation rates of starch is too slow, the total tract digestion of starch will be insufficient (Owens *et al*., 1986; Theurer, 1986; Kotarski *et al*., 1992). On the other hand, when starch fermentation rates are too rapid and there is a high intake of food, the absorptive and buffering capacity of the cow may not counteract for the high amounts of fermentation acids that are produced by the ruminal microflora. This could result in sub-acute rumen acidosis and also a reduction in feed intake (Kotarski *et al*., 1992). The fermentation rate of starch can increase substantially with an increase in the diet's fermentable starch content (Allen, 2007). In the cow's rumen, approximately 85-90% of wheat and barley starch is fermented and about 60% for maize starch (Nocek & Tamminga, 1991; Khorasani *et al*., 2001). Thus, a higher amount of maize starch than barley starch may reach the small intestine (Khorasani *et al*., 2001). Fermentation rates are faster for cereal grains such as wheat and triticale than barley and oats, probably because the latter two cereal grain types have high amounts of non-starch polysaccharides (NSP), including mixed-linked ß-glucans in the endosperm cell walls (Aman & Hesselman, 1984; Salomonsson *et al*., 1984; Henry, 1985; Opatpatanakit *et al*., 1994). Maize, which has a slower fermentability, is preferred to avoid health problems, such as bloat, liver abscesses and acidosis (Ørskov, 1986; Camm, 2008).

Vitreousness of cereals reflects the association between the protein and starch in the endosperm (Kotarski *et al*., 1992; Corona *et al*., 2006). The differences in solubility and amount of endosperm protein in different types of grain such as maize, barley, sorghum and wheat, will have a dramatic effect on the fermentation rate (Allen, 2007). The protein-starch matrix present in the maize horny endosperm is very resistant to rumen fermentation by the microorganisms (McAllister *et al*., 1990b; McAllister *et al*., 1993).

2.2 Fermentation in the rumen

As defined by Pasteur, fermentation is "life without oxygen". Carbohydrates are fermented in the rumen, in the absence of oxygen and will yield high amounts of energy in the form of adenosine triphosphate (ATP) for microbial growth (Webster, 1987). Ruminal carbohydrate fermentation, which includes the conversion of cellulose, hemicelluloses, starch and pectins to VFA's is the main energy source to the animal (Bergen & Yokoyama, 1977). In the cell contents, starches and sugars can be found that will ferment rapidly in the rumen, whereas the other contents will be fermented at a slower rate (Webster, 1987). The main end products are acetate, propionate, butyrate, carbon dioxide (CO_2) and methane (CH_4), resulting in a decrease in rumen pH (Opatpatanakit *et al*., 1994). Hungate (1966) summarized the reactions as follows:

$$1 \text{ hexose} + 2H_2O \rightarrow 2 \text{ acetate} + 2CO_2 + 4H_2,$$

$$1 \text{ hexose} + 2H_2 \rightarrow 2 \text{ propionate} + 2H_2O,$$

$$1 \text{ hexose} \rightarrow 1 \text{ butyrate} + 2CO_2 + 2H_2,$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

These VFA's will serve as a dietary energy source to the cow (Webster, 1987). Unavoidable however, are the heat losses and losses in methane in the rumen when starch is fermented (Hungate, 1966; Ørskov, 1986). Hydrogen (H_2), formed during rumen fermentation are used by methanogenic bacteria to form

methane (Opatpatanakit *et al*., 1994). The VFA's are absorbed through the rumen wall and the gasses that are produced will be lost by eructation (McDonald *et al*., 2002). In dairy cattle the end products, produced from rumen fermentation, plays a very important part in the metabolism of energy. The proportions of propionic acid, acetic acid and butyric acid that are produced will influence milk production, the efficiency of fattening and also the fat percentage of milk (McCullough, 1966; McCullough & Smart, 1968). Thus, the diet given to dairy cattle is the most important in influencing ruminal microbial fermentation (Bergen & Yokoyama, 1977).

For fermentation to result in a maximum rate of degradation controlled conditions are required and these are provided through appropriate temperature, motility and secretions (Reece, 1991). Regurgitation and remastication will also influence and assist fermentation by the provision of a finer material, which will thus have a greater surface area for microbial digestion (Reece, 1991). The environment inside the rumen is very favourable for microbial growth. The pH in the rumen ranges between 5.5 and 7 and the temperature is about 39-40°C which, for many enzyme systems are near the optimum (Church, 1971).The rumen of a cow is adapted for fermentation by the presence of microorganisms in the rumen for example bacteria, protozoa and some fungi (Reece, 1991). The rumen metabolism bacteria account for about 80% and protozoa for about 20% (Reece, 1991). Bacteria and protozoa will both produce VFA's, methane and CO_2 (carbon dioxide) from the fermentation of feed. The proportion of VFA's found in the rumen will usually be about 60-70% acetic acid, 15-20% propionic acid and 10-15% butyric acid (Reece, 1991).

Energy losses are present as a result of ruminal fermentation and attempts must be made to try and decrease these energy losses to the minimum to improve the animal's productive efficiency (Bergen & Yokoyama, 1977). Energy losses from the rumen include methane production and heat produced of fermentation. "The heat of fermentation is the free energy which is dissipated as a result of inefficiencies in microbial metabolic activity (anabolic and catabolic reactions) in the rumen" (Bergen & Yokoyama, 1977). Heat production from fermentation can be reduced by altering the physical form of the diet and its ingredients. This can be achieved by grinding, rolling, flaking or chemical treatment of the feed (Bergen & Yokoyama, 1977). The bacteria in the rumen that is responsible for fermenting starch will produce a larger amount of propionic acid. Propionic acid serves as a hydrogen sink, thus from a fermentation point of view the production of propionic acid is advantageous, because propionic acid will capture the hydrogen in a metabolizable form. In this way, hydrogen is not lost as methane (Hungate, 1966; Ørskov, 1986).

The main precursor of milk fat is acetate. It is thus important to include sufficient amounts of structural or slowly fermented carbohydrates (Webster, 1987). The acetate that is transported to the liver is used for the *de novo* synthesis of cholesterol and long-chain fatty acids (Webster, 1987). Propionate will be extensively metabolized in the liver of which the primary pathway is gluconeogenesis. Thus propionate is the main precursor for the synthesis of glucose with lesser contributions originating from lactate, amino acids and glycerol (Frandson *et al*., 2006). If there is a reduction in milk fat, it can be associated with a high acetate:propionate ratio in the rumen (Russell *et al*., 1992). Figure 1 shows how the energy and protein are

digested in the rumen whereas in Figure 2 the energy metabolism pathways are shown. Table 2 gives us the positive and negative features of rumen fermentation.

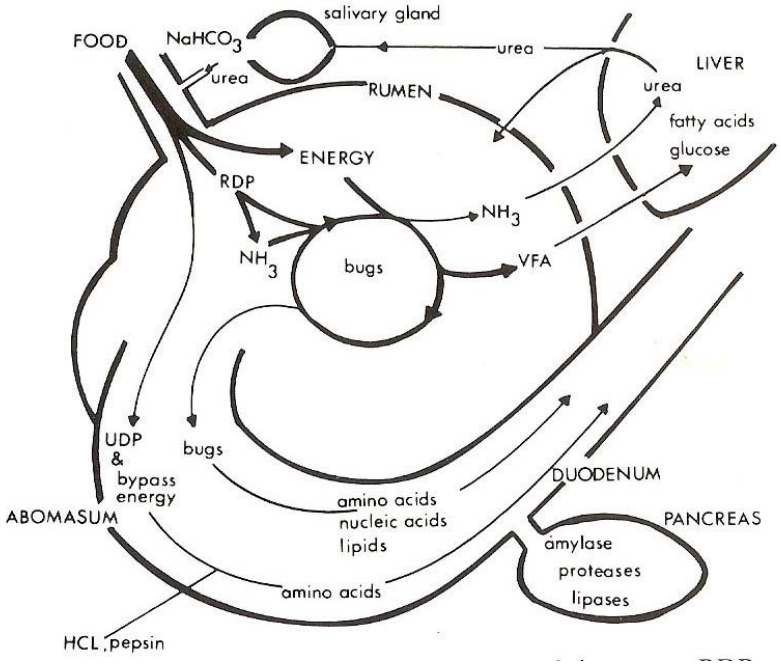


Figure 1 Digestion of protein and energy in the rumen (Webster, 1987). VFA = Volatile fatty acids.

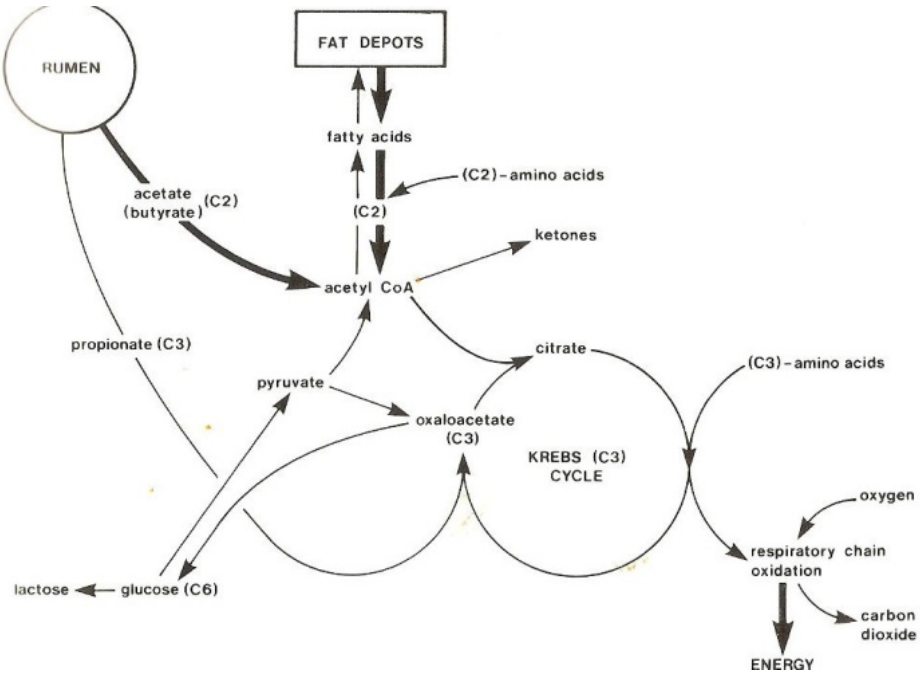


Figure 2 The energy metabolism pathways in the ruminant (Webster, 1987). C2 – two carbon compounds; C3 – three carbon compounds.

Table 2 Benefits and disadvantages of rumen fermentation (Rowe *et al*., 1999).

Positive features	Negative features
Microbial protein and vitamins available for intestinal absorption	Acid accumulation and low pH leads to: risk of acidosis, reduced fibre digestion
VFA absorption provides metabolisable energy	Energy loss through heat, CH_4 and H_2

2.3 Physical properties of grains

When explained in short, the cereal grain contains three components. The pericarp, or outer protective covering, secondly the germ, or embryo, and thirdly the endosperm (Kotarski *et al*., 1992). The endosperm contributes to approximately 70-80% of the maize particle's weight and this is the morphological structure in which the starch is found (Hoffman & Shaver, 2009). Primary components found in the endosperm are starch and protein. Secondary components are small amounts of fat as phospholipids and ash (Hoffman & Shaver, 2009). In cereal grains, the endosperm surrounds the germ (Hoffman & Shaver, 2009). The endosperm serves as the nutrient source for the germ (Hoffman & Shaver, 2009). The pericarp is the structure that protects the endosperm, but hydrophobic proteins, called prolamins, also protect the starch found in the maize endosperm (Hoffman & Shaver, 2009). The combination of proteins, starch and prolamins in the maize endosperm can be referred to as the starch-protein matrix (Hoffman & Shaver, 2009).

When maize is dissected, the differences in the starch-protein matrix can be seen (Hoffman & Shaver, 2009). The visible appearance of the starch-protein matrices in the maize endosperm is given visually illustrative classifications (Hoffman & Shaver, 2009). Soft or floury endosperm is the name given if the starch-protein matrices appear white (Kempten, 1921; Hoffman & Shaver, 2009). Vitreous or horny endosperm is the name given if the starch-protein matrices appear glassy, shiny or yellow (Hoffman & Shaver, 2009). Starch fermentation in the rumen is affected by gelatinization of starch, the size of the particle and the solubility and amount of endosperm proteins (Allen, 2007). When compared to sorghum and maize, barley and wheat have higher fermentation rates because they have low concentrations and higher endosperm protein solubility (Allen, 2007).

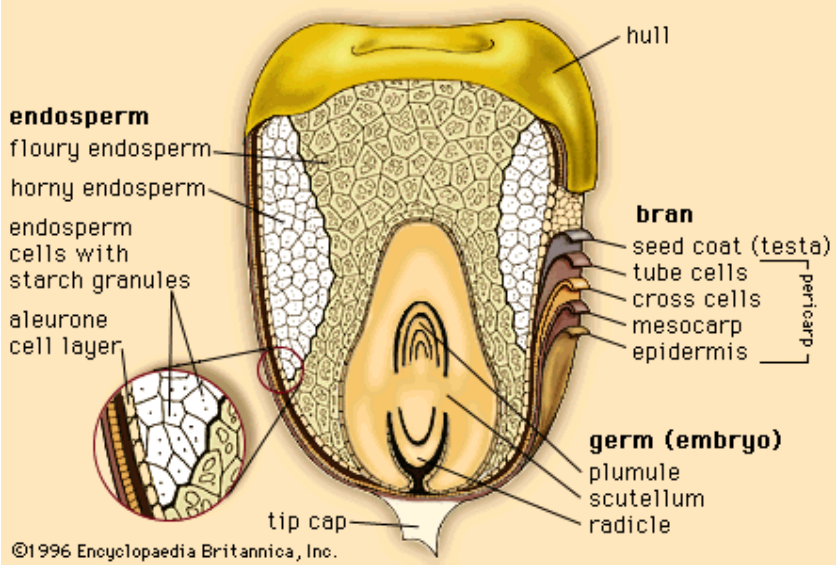


Figure 3 General morphology of maize (Encyclopaedia Britannica, 1996).

2.3.1 Particle size

Cereal grain particle size has an influence on ruminal fermentation (Camm, 2008). For ruminal fermentation to take place it is essential that the microbes attach to the particles (McAllister *et al*., 1994; Camm, 2008). Particles that are large in size will have less exposed area per volume and will thus ferment more slowly. Particles that are smaller in size will ferment faster in the rumen (Camm, 2008). However, particles that are smaller than approximately 1.0 mm will flow out of the rumen unfermented (Walker *et al*., 1973; Galyean *et al*., 1981; Camm, 2008). Whole cereal grains may also flow out of the rumen without being fermented and this can be due to the fact that microbes struggle to penetrate the fibrous outer layer of the grains (Ørskov, 1986; Camm, 2008). Steam treatment of grains before processing can increase the particle size and will reduce the proportion of fine particles that are less than 1.0 mm in diameter (Hironaka *et al*., 1992; Camm, 2008). Grain cultivar, grain species and the growing condition that include year, location, soil fertility and season will influence the starch composition and the starch granule size (Opatpatanakit *et al*., 1994). Small starch granules can be found in maize whereas in barley, wheat and rye two types of granules are present; the predominant ones being large lenticular and small spherical (Opatpatanakit *et al*., 1994).

2.3.2 Seed coat

The seed coat or pericarp will protect the cereal grain from insects, moisture and fungal infections (Owens & Zinn, 2005). In maize and sorghum, for example, the coat makes up about 3-6% of the grain weight whereas in oats it can be as much as 25% of the weight of the grain (Rowe *et al., 1999;* Owens & Zinn, 2005). The coat contains about half of the neutral detergent fibre (NDF) of the kernel (Owens & Zinn, 2005). Neutral detergent fibre is the fraction that contains mostly cell wall constituents of low biological availability, thus mostly cellulose, hemicelluloses and lignin. If the pericarp is hard and thick the fermentation rate will be lower (Owens & Zinn, 2005). Starch ferments at a faster rate than NDF (Allen, 2007). The amount of starch

present is roughly proportional to the availability energy, because of the higher digestibility of starch than other components, especially NDF. Neutral detergent fibre is the biggest component that displaces starch in grain (Owens & Zinn, 2005). The pericarp must be damaged or cracked to give access to the endosperm for fermentation and digestion to take place (Owens & Zinn, 2005).

2.3.3 Germ size

The germ of the cereal grain contains most of the oil. Cereal grains that have smaller germs will have less NDF and ash (Owens & Zinn, 2005). Hybrids that are selected for high oil will have a bigger germ size. Also, "nutrient dense" hybrids contain more oil (Owens & Zinn, 2005). As the oil replaces the starch in grain there will be a reduction in the yield of microbial protein, because ruminal microbes do not ferment oil as an energy source (Owens & Zinn, 2005).

2.3.4 Amylose content

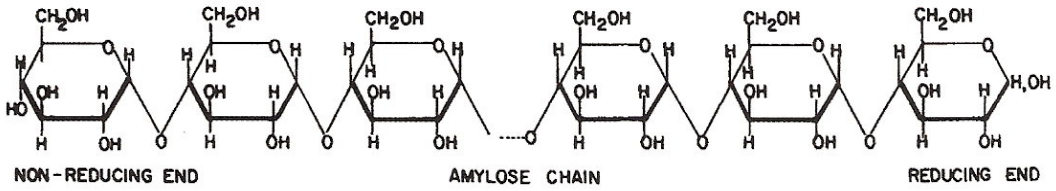
The component starch makes up about 60-80% of cereal grains (Opatpatanakit *et al*., 1994). Within a maize starch granule, the starch is chemically present as either amylose or as amylopectin (Huntington *et al*., 2006). The structures of amylose and amylopectin are shown in Figure 4. Amylose consist of α-D-glucopyranose residues that is linked together by (1-4) bonds and it is a linear polymer, whereas amylopectin consist out of α(1-6) bonds and amylopectin is a branched polymer (Opatpatanakit *et al*., 1994). Amylose is less fermentable than amylopectin and has a linear structure whereas amylopectin has a multi-branched structure (Huntington *et al*., 2006). The tighter intermolecular bindings between the amylose starch molecules make the amylose starch less fermentable (Corona *et al*., 2006). Due to genetic differences, amylose can contribute to as little as 2% or as much as 70% of the total starch component of different cereal grains and hybrids (Owens & Zinn, 2005). The starch that is present as amylose in maize typically ranges between 24-30%. The amylose content is 4-9 units higher in floury than in vitreous starch (Owens & Zinn, 2005). An increase in maturity will also increase the amylopectin to amylose ratio, but the ratio will decrease as the environmental temperature increase (Owens & Zinn, 2005). The fermentation of amylose is restricted to a limited amount of bacterial strains (Owens & Zinn, 2005). The bacterial strain that is able to colonize maize starch granules is primarily, Coccoid bacteria (Camm, 2008). Several species colonize those of other cereal grains (McAllister *et al*., 1990b; Camm, 2008). The proportions of amylose in barley, maize, wheat and rye are similar (Owens & Zinn, 2005).

The cereal grain's starch granules contain amylose and amylopectin in consecutive spheres or rings. Thus, if the degradation of amylose is limited, the starch granules may resist digestion and fermentation (Owens & Zinn, 2005). In addition, the rate of fermentation and digestion can be reduced if the starch granule reducing ends links to phosphorus or lipids (Owens & Zinn, 2005). The waxy maize hybrids contain higher levels, nearly 100%, of amylopectin. Whereas the non-waxy hybrids have less amylopectin, nearly 75%, and more amylose, nearly 25% (Rowe *et al*., 1999). There is a greater extent and higher starch fermentation for waxy maize hybrids than for non-waxy maize hybrids when maize is fed as dry rolled grain (Huntington, 1997;

Corona *et al*., 2006). Waxy cereal grains contain amylopectin, which makes up nearly 100% of the starch and thus a small amount of amylose content is present (Russell *et al*., 1992). For example, waxy barley that contains a high amylopectin content may contain less than 1% amylose, whereas normal or non-waxy barley grain contains 70-75% amylopectin and 20-30% amylose (Bhatty, 1993; Foley *et al*., 2006). When waxy sorghum grains are looked at microscopically, they have a smaller amount of peripheral endosperm and a higher more evenly distributed protein storage bodies (Kotarski *et al*., 1992).

Processing of grain disturbs the starch granule's structure and has been used favourably to upgrade ruminal fermentability of grains (Huntington *et al*., 2006). Due to the fact that amylose is less fermentable in the rumen than amylopectin, maize hybrids that have a larger percentage of amylopectin may have a higher feeding value when the maize is fed dry-processed (Corona *et al*., 2006).

a)



b)

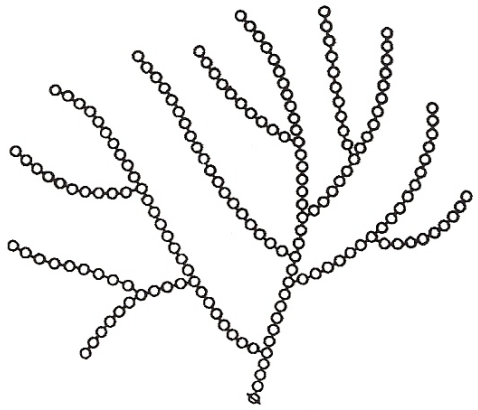


Figure 4 a) Amylose b) Amylopectin (Rowe *et al*., 1999).

The effect of grain type (maize vs. barley) and the amylopectin content of barley on rumen fermentation of dairy cows are presented in Table 3.

Table 3 Effect of grain type and barley amylopectin content on ruminal fermentation in dairy cows (Foley *et al.,* 2006).

Item	Diet		
	Maize	Normal barley (nonwaxy)	Waxy barley (high-amylopectin)
pH	6.2	6.2	6.2
VFA, m*M*	129.2	133.7	133.1
Acetate	87.9	84.8	84.4
Propionate	29.5	26.3	26.7
Acetate:Propionate	2.8	3.2	3.2

In the study done by Foley *et al*. (2006), they found that there were no significant difference in ruminal fermentation between the waxy and non-waxy barley diets, but the waxy barley was a little less fermentable than the normal barley in the rumen. Differences in Table 3 between the grains can be due to the differences in chemical composition, starch characteristics and may also be due to different responses to processing (Foley *et al*., 2006).

2.3.5 Resistant starch

Gelatinization is a process where the starch granules are exposed to moisture and heat (Kotarski *et al*., 1992). The granules will then absorb the water, swell and form gels (Kotarski *et al*., 1992). This can be characterised by a disruption of the matrix that binds the starch cells, because of the expansion of the starch granules (Rowe *et al*., 1999). Before a certain critical temperature is not reached, the starch will not change in physical appearance (Rowe *et al*., 1999). If above the critical temperature, they will lose their characteristic polarisation crosses (Rowe *et al*., 1999). The critical temperature varies for different grains and the term gelatinisation temperature is given when the temperature is reached where they change in appearance (Rowe *et al*., 1999). Amylose will diffuse out of the swollen granules and thus the particles will be enriched in amylopectin (Owens & Zinn, 2005). Swelling of the particles will not take place if the amylose content is very high in the starch granules (Owens & Zinn, 2005). When the gelatinized starch is cooled and stored, the amylose will gel and form retrograde starch, which is an “enzyme–resistant starch” (Owens & Zinn, 2005). “Ruminal microbes must have sufficient capacity to ferment retrograde starch or at least solubilise starch that resists hydrolysis by starch-degrading enzymes” (Owens & Zinn, 2005). For the gelatinisation of starches that contain low levels of amylose, thus high levels of amylopectin, a low temperature is required (Rowe *et al*., 1999). Waxy or non-waxy types do not seem to affect the gelatinisation temperature (Rowe *et al*., 1999).

Maize starch that have a high amylose content shows exceptional behaviour in that even in boiling water it resists gelatinisation (Rowe *et al*., 1999).

2.3.6 Vitreousness

Animal and dairy scientists use the term vitreousness in ruminant nutrition to sub-define maize endosperm types (Hoffman & Shaver, 2009). Maize grain has been divided into five classes according to their kernel characteristics. These classes are as follows, from "hard" to "soft": flint, popcorn, flour, dent and sweet (Corona *et al*., 2006). Flint maize (*Zea indurate*) can also be called, vitreous, horny or corneous and the starch in the endosperm is almost all hard. The starch in the endosperm of the "flour" maize (*Zea indentata*) is soft (Pomeranz *et al*., 1984; Corona *et al*., 2006). The vitreousness of the kernels will also vary depending on the position where they can be found on the ear of the maize and also the growing environment (Corona *et al*., 2006).

The horny to floury ratio (H:F ratio) of the kernels, also termed vitreousness can be estimated by physical dissection of the kernels, or it can be estimated by measuring the absolute density of the grain (Owens, 2005). There is a positive correlation between vitreousness and grain density. Thus, grain density can indirectly be used as a measurement of vitreousness (Correa *et al*., 2002; Pereira *et al*., 2004). To determine the vitreousness of maize by manual dissection is as follows. Firstly, the maize particles are soaked in water and then with a scalpel the pericarp and the germ can be removed (Correa *et al*., 2002; Hoffman & Shaver, 2009). After this, the floury and vitreous endosperm is separated using visual judgement (Hoffman & Shaver, 2009). Then the vitreous endosperm is weighed and the weight of the vitreous endosperm is expressed as a percentage of the total endosperm (Hoffman & Shaver, 2009).

Horny endosperm is extremely dense. On the other hand, floury endosperm is full of void spaces or micro fissures (Philippeau *et al*., 1999). The H:F ratio will be greater for maize grain classified as flint than for maize grain classified as flour (Owens, 2005). Thus, the dent maize has a smaller proportion of vitreous endosperm than the flint maize (Correa *et al*., 2002). The H:F ratio varies genetically and often increases as the grain matures and with nitrogen fertilization (Owens, 2005). The environment as well as genotype influences the horny to floury endosperm ratio (Opatpatanakit *et al*., 1994). The ideal horny to floury ratio will differ for each grain processing method used (Owens, 2005). Digestion and fermentation will be limited for the horny endosperm as the starch granules are surrounded by protein, which are encapsulated in a matrix (Kotarski *et al*., 1992; Johnson *et al*., 1999). Maize has a slower fermentation rate than other cereal species and the major factor responsible for this is the protein-starch matrix, which limits the access for microorganisms to the starch in the rumen (McAllister *et al*., 1993; Opatpatanakit *et al*., 1994). Fermentation of starch is also limited by the compressed nature of starch itself, especially in the kernel's hard endosperm portion that delays the entrance by the amylolytic enzymes and prevents the colonization by microbes (McAllister *et al*., 1990; Corona *et al*., 2006). The protein-matrix incompletely surrounds the starch granules in floury endosperm and it is also thinner than the protein matrix found in horny endosperm (Opatpatanakit *et al*., 1994).

Fermentation in the rumen is faster and maize is fermented to a greater extent if maize grain has a floury endosperm (Taylor & Allen, 2005). Where vitreous maize grain has a slower fermentation rate in the rumen and passed from the rumen faster which results in a decrease in the digestibility of starch in the rumen (Taylor & Allen, 2005). Fermentation in the rumen will be rapid for the fine particles of the floury endosperm and this can increase the risk of acidosis (Owens, 2005). Floury maize results in a lower ruminal pH, rise in total VFA, increased propionate, decreased acetate and decreased branched-chain VFA in dairy cows ruminal fluid when compared to vitreous maize (Huntington *et al*., 2006). The acetate:propionate ratio increased with an increasing vitreousness. The higher molar proportions of acetate and methane and lower molar proportions of propionate can be expected with a hybrid with greater vitreousness (Corona *et al*., 2006). The starch found in oats, barley and wheat is normally fermented and will give rise to a relatively high proportion of propionate to acetate (Webster, 1987).

The characteristics of different cereal grains are presented in Table 4.

Table 4 Characteristics of different cereal grains (Rowe *et al*., 1999).

	Maize	**Sorghum**	**Barley**	**Wheat**	**Oats**
Starch content (% of DM)	76	75	61	76	42
Gas production (mL/g DM after 7h)	138	104	222	251	237
Temperature of gelatinization	62-72	69-75	-	52-63	-
Fermentation in rumen (% of intake)	76	64	87	89	92

2.4 Physical processing

Cereal grains are all rich in starch, which is a good source of energy to the cow (Webster, 1987). The pericarp and germ contains a small amount of starch and this represents a small percentage of the grain. Most of the starch can be found in the endosperm of the grains (Kotarski *et al*., 1992). If cereals are not subjected to processing before they are fed to cows, whole grains can pass rapidly through the gastrointestinal tract and will be found unchanged in the faeces (Webster, 1987). There will be an increase in the rate of starch fermentation with most grain processing methods (Theurer, 1986; Knowlton, 2001). Ruminal fluid pH will decrease when cows are fed cereal grains that have been processed, and this takes place due to the rapid fermentation of the grains starch in the rumen (Hironaka *et al*., 1973; McAllister *et al*., 1991; Beauchemin *et al*., 1994).

Processing of cereal grains has variable effects on the productivity of dairy cattle and ruminal fermentation is also affected by the extent of processing (Dehghan-banadaky *et al*., 2007). If processing is insufficient the ruminal organic matter degradation may not be optimized, whereas in excess processing, fermentation in the rumen will not be optimum as the particles may flow out of the rumen unfermented and acidosis may occur (Dehghan-banadaky *et al*., 2007). Another aspect that can affect the optimum method and the extent of processing cereal grains is the grain quality before processing (Dehghan-banadaky *et al*., 2007). The grain's vitreousness and hardness will affect its response to physical processing (Rowe *et al.,* 1999). The harder grain types are also more prone to shattering and shearing than softer grain types, where the starch granules tend to remain intact (Rowe *et al*., 1999). The physical processing methods include cold physical processing and hot physical processing methods (Dehghan-banadaky *et al*., 2007).

2.4.1 Cold physical processing

During cold physical processing, to decrease the size of the particle and to increase the surface area of the grain particle, a roller or hammer mill is used without the application of steam or heat (Dehghan-banadaky *et al*., 2007). Various cold processing methods are discussed in the following section.

2.4.1.1 Grinding

A hammer mill is used for the grinding of grains into smaller particles, which is a very simple process (Dehghan-banadaky *et al*., 2007). When grain particles undergo the process of grinding, their outer layers will be fractured by the hammer mill and more of the endosperm will be exposed, allowing easier access for the microorganisms (Galyean *et al*., 1981; Dehghan-banadaky *et al*., 2007). Thus, the grinding of cereal grains will increase the surface area by making it more available for microbial attachment and this will in turn increase the rate of fermentation (Dehghan-banadaky *et al.*, 2007). Extremely fine particles can be produced by grinding, which will be rapidly fermented or digested (Rowe *et al*., 1999). Barley grain that is finely ground will ferment more rapidly than barley grain that is cracked and therefore may reduce productivity of cattle (Dehghan-banadaky *et al*., 2007).

2.4.1.2 Dry rolling

Dry rolling is a process where grain particles are passed through rotating rollers that break the particle's pericarp and thereby expose the grain's endosperm to microbial attachment in the rumen (Dehghan-banadaky *et al*., 2007). When compared to grinding, roller mills produce a more even particle size distribution and thus producing less fine particles (Dehghan-banadaky *et al*., 2007). Rolled wheat and barley are fermented more rapidly, because they contain less fibre and more starch and they are thus more prone to cause digestive upsets such as acidosis (Webster, 1987). Ruminal fermentation will be higher for ground, rolled or cracked barley than for similarly processed sorghum or maize (Waldo, 1973; Theurer, 1986; Kotarski *et al*., 1992).

2.4.1.3 Tempering

Tempering is brought about by adding water to the cereal grain, thus increasing the moisture content of the grain and storing it for about 12-24 hours before rolling takes place (Dehghan-banadaky *et al*., 2007). Tempering requires corrosion resistant bins for soaking the cereal grains (Dehghan-banadaky *et al*., 2007). The advantages of tempering include a reduction in the production of very fine particles during rolling and reducing dustiness of grains (Dehghan-banadaky *et al*., 2007). The moisture of the grain particle is restored before rolling, which will help decrease shattering of the particle when being rolled and help maintain the integrity of the grain particle (Yang *et al*., 1996; Dehghan-banadaky *et al*., 2007). When compared to grinding and dry rolling, tempering often reduces the rate of starch degradation (Dehghan-banadaky *et al*., 2007).

2.4.2 Hot physical processing

Methods used during hot processing include moisture, heat, pressure or a combination of these (Dehghan-banadaky *et al*., 2007). Moisture and heat are added to the cereal grain particles during steam flaking and steam rolling. This gelatinizes the starch and may increase degradation in the rumen by microorganisms (Waldo, 1973; Dehghan-banadaky *et al*., 2007). The interactions of pressure, heat and moisture will break down the endosperm structure and this will disrupt the protein matrix that encapsulates the starch granules (Kotarski *et al*., 1992; Knowlton, 2001). Hot physical processing includes steam rolling, steam flaking, pelleting, roasting, extruding and expanding. These processes are discussed in the following section.

2.4.2.1 Steam rolling

The use and application of steam to cereal grains is the most popular method of hot physical processing (Dehghan-banadaky *et al*., 2007). This involves the application of steam to grain particles for 3-5 minutes prior to flaking or rolling in a space above the roller mill (Dehghan-banadaky *et al*., 2007). Advantages of steam processing of cereal grains include the reduction in the amount of small shattered particles that are created during dry processing of cereal grains (Dehghan-banadaky *et al*., 2007). The surface area is increased with steam rolling and starch is gelatinized, which will increase the accessibility by the rumen microbes and the fermentation rate (Allen, 2007). For steam processing, additional equipment is required and the processing costs will be higher (Dehghan-banadaky *et al*., 2007). In the rumen of cattle fed dry rolled barley, the VFA concentration was higher than for the cattle fed steam rolled barley (Dehghan-banadaky *et al*., 2007).

2.4.2.2 Steam flaking

Steam flaking of grain can be done by two methods, which include the application of steam at low pressure or application of steam at high pressure (Dehghan-banadaky *et al*., 2007). In the low-pressure method, grain is exposed to low pressure steam for about 30-60 minutes, until temperatures of 95-99°C is reached, and with an increasing moisture content up to 150-200 g/kg (Dehghan-banadaky *et al*., 2007). In the high-pressure method, a pressure cooker is used to subject the grain to moist steam, for 3 minutes at about 3.5

kg/cm^2 pressure (Dehghan-banadaky *et al*., 2007). Before rolling the heated grain is cooled to 95-99°C (Dehghan-banadaky *et al*., 2007).

Steam flaking of cereal grains causes gelatinization of starch granules and disruption of the protein matrix that engulfs the starch (Dehghan-banadaky *et al*., 2007). This may not always increase ruminal starch digestibility of barley, probably because it is already readily degradable in the rumen without the steam processing (Dehghan-banadaky *et al*., 2007). Barley grain that has undergone the process of moist-heat treatment will produce more VFA's by ruminal microorganisms (Ørskov, 1986). Ruminal degradability of maize increases with steam flaking (Fiems *et al*., 1990; Dehghan-banadaky *et al*., 2007). When cereal grains are steam flaked it causes the starch to gelatinize which will result in an increased ruminal digestibility of starch (Eastridge, 2006). If sorghum undergoes the process of steam flaking the rumen starch digestion will increase (Poore *et al*., 1993; Oliveira *et al*., 1995; Knowlton, 2001).

It was found in a study done by Corona *et al*., (2006) that the volatile fatty acid (VFA) concentration in the rumen will be higher for steam flaked than for dry rolled maize diets. Also the steam flaked maize diets had lower acetate and butyrate concentration and lower acetate:propionate ratio, but the concentration for propionate was higher (Johnson *et al*., 1968; Zinn, 1987; Zinn *et al*., 1995; Corona *et al*., 2006). Approximately 90% of wheat, barley or oats starch are fermented in the rumen when fed as crushed or whole grain. Maize is the exception, because when maize grain is fermented in the rumen about 40% of the maize starch will escape rumen fermentation (Ørskov, 1986). Studies show that the percentage of maize starch that escapes fermentation in the rumen is 10-25% when steam flaked and about 30-45% when dry rolled (Theurer, 1986). Steam flaking will increase the ruminal digestion by microorganisms by approximately threefold than with grinding or rolling (Theurer, 1986).

2.4.2.3 Pelleting

This method is a common commercial process. By making use of a mechanical process in combination with heat, moisture and pressure, small particles are combined into a larger particle (Rowe *et al*., 1999). When ground grain is forced through a thick die, pelleting is accomplished (Dehghan-banadaky *et al*., 2007). This is done by using a roller and steam may or may not be applied in the process (Dehghan-banadaky *et al*., 2007). By increasing the surface area of the grain through gelatinization, starch degradation can be increased through pelleting (Dehghan-banadaky *et al*., 2007).

2.4.2.4 Roasting

When dry heat is applied to the grains, it is called roasting (Dehghan-banadaky *et al*., 2007). There can be a reduction in the rate of starch degradation of barley grain in the rumen if barley is roasted and more of the starch will flow out of the rumen and it will be digested in the small intestine (Dehghan-banadaky *et al*., 2007).

2.4.2.5 Other hot physical processing methods

Other hot physical processing methods that can be applied are extrusion and expansion (Dehghan-banadaky *et al*., 2007). When grains are subjected to extrusion, it involves moisture, pressure and high temperature (Rowe *et al*., 1999). The grain will be ground first and moisture will be added. Next pressure and heat will be added and the grain will be forced through a die (Dehghan-banadaky *et al*., 2007). A long ribbon is formed which is cut into the desired particle lengths (Dehghan-banadaky *et al*., 2007). Extrusion will take place at high temperatures of 125-170°C, but for a short time of about 15-30 seconds (Rowe *et al*., 1999). The process of extrusion cooking will gelatinize the starch and disrupt the grain structure (Rowe *et al*., 1999; Dehghan-banadaky *et al*., 2007).

Table 5 Impact of various processing techniques on grain and its digestion (Owens & Zinn, 2005).

Grain treatment/ processing	Disrupts pericarp or exposes endosperm	Reduces particle size	Disrupts endosperm matrix	Disrupts starch granules	Increases fermentation rate	Increases intestinal digestion
Dry rolling	+++	+	-	-	++	+
Grinding	+++	+++	-	-	++	+
Steam flaking	+++	++	+	+	+++	++
Extrusion	+++	-	++	+	++	++
Pelleting	+++	-	+	?	+	++
Ensiling	+		++	-	++	+
Popping	++	-	+	+++	?	+++
Protease	-	-	?	?	++	?

When different grain processing methods are compared to one another in terms of their effect on fermentation rate in the rumen, it can be seen in Table 5 that steam flaking of grain results in the highest fermentation rate, whereas pelleting results in the slowest fermentation rate (Rowe *et al*., 1999; Owens, 2005).

Grinding, dry rolling and extrusion treatment techniques will have intermediate fermentation rates (Rowe *et al*., 1999; Owens, 2005). Processing methods are selected according to the most economical method, acceptability of the method and to enhance digestibility without affecting ruminal temperature and pH

detrimentally, thereby causing digestive disorders (Rowe *et al*., 1999; Owens, 2005). Table 6 shows the effect of processing of different cereals on rumen pH and proportion of acetic and propionic acid.

Table 6 The effect of processing on different cereals on rumen pH, proportion of acetic and propionic acid (Ørskov, 1979).

			Molar proportion of:	
Cereal	**Form**	**Rumen pH**	**Acetic acid**	**Propionic acid**
Barley	Whole	6.4	52.5	30.1
Barley	Ground pelleted	5.4	45.0	45.3
Maize	Whole	6.1	47.2	38.7
Maize	Ground pelleted	5.2	41.3	43.2
Oats	Whole	6.7	65.0	18.6
Oats	Ground pelleted	6.1	53.2	37.5
Wheat	Whole	5.9	52.3	32.2
Wheat	Ground pelleted	5.0	34.2	42.6

2.5 Chemical processing

During chemical processing, a concentrated chemical solution is applied directly to the grain for a number of hours or a few days prior to feeding (Dehghan-banadaky et al., 2007). Often chemical processing is not combined with a physical processing method (Dehghan-banadaky *et al*., 2007). Chemical processing includes the addition of organic acids or chemical compounds to decrease particle size and to increase fermentation in the rumen (Eastridge, 2006). The use of ammonia or sodium hydroxide in chemical processing has the same effect as crushing or rolling (Dehghan-banadaky *et al*., 2007). This allows access for the microorganisms to the underlying tissue in the grain (Dehghan-banadaky *et al*., 2007). When ensiling high moisture grains, the addition of organic acids, such as propionic acid, and ammonia at the time of ensiling will decrease DM losses and will decrease mould growth. This will increase dry matter intake (DMI) and fermentation in the rumen (Eastridge, 2006). Due to the fact that the costs for mechanical processing continue to increase, the use of chemical processing of cereal grains may become more favourable in the future (Dehghan-banadaky *et al*., 2007).

2.5.1 Sodium hydroxide (NaOH)

Application of sodium hydroxide to cereal grains will destroy the grain's seed coat (Dehghan-banadaky *et al*., 2007). Sodium hydroxide is usually applied at a rate of 30-40 g/kg. When whole barley is treated with sodium

hydroxide, the ruminal starch degradation will be slower, as compared to rolling and grinding of the whole barley grain. The fluctuations in ruminal pH will be decreased and the incidence of ruminal acidosis is lower (Dehghan-banadaky *et al*., 2007). When treated with sodium hydroxide the fermentation of naked oats were not as rapid as for other grains (Ørskov, 1979). There are concerns about making use of sodium hydroxide, because it is a strong chemical and also because over the long term there are incidences of kidney lesions in dairy cattle (Dehghan-banadaky *et al*., 2007).

2.5.2 Ammonia/urea

Several weeks before feeding, the grain is sprayed with ammonia or urea and allowed to soak. Dehghan-banadaky *et al.* (2007) found that the rate of ruminal dry matter digestion decreased with ammonia or urea treatment and that the feed efficiency was improved. The same authors reported that when barley was treated with ammonia the rate of starch degradation decreased in the rumen (Dehghan-banadaky *et al*., 2007).

2.5.3 Other chemical processing methods

When barley grain is treated with propionic acid, whole tract starch digestion will be increased, but only if it is combined with physical processing for example rolling (Ahmed *et al*., 1973; Dehghan-banadaky *et al*., 2007). Sulphur dioxide is used successfully to preserve barley that is high in moisture (Dehghan-banadaky *et al*., 2007).

2.6 Conclusion

Cereal grains are very important in the diets of dairy cattle, because they are the primary contributors of starch (Eastridge, 2006). Starch is important for meeting the cow's energy needs (Eastridge, 2006). "The starch concentration in diets must be based on effectiveness of the fibre in the diet and relative rate of starch fermentation" (Eastridge, 2006). When formulating diets for carbohydrates the aim is to provide low fill, diets that are highly fermentable that result in consistent ruminal fermentation over time (Allen, 2007).

Physical and chemical properties of starch known as intrinsic factors and the way cereal grains are stored or the method used for cereal grain processing known as extrinsic factors affect the digestibility and fermentation of starch (Opatpatanakit *et al*., 1994). Fermentation pattern in the rumen and the fermentation of starch is also influenced by the presence of anti-nutritional factors that includes lectins, phytates, enzyme inhibitors and tannins and the presence of starch-protein matrices (Opatpatanakit *et al*., 1994).

Grain texture plays a significant part in ruminal starch fermentation (Philippeau *et al*., 1999). Rate and extent of ruminal fermentation of starch will change due to grain source and due to cereal processing (Philippeau *et al*., 1999). The use of cereal grains has been improved by processing procedures (Eastridge, 2006). The processing method must make grain very digestible, but must reduce the rate of breakdown, to avoid a

sudden excessive rate of VFA production in the rumen (Ørskov, 1979). For high ruminal starch availability the grain's kernel vitreousness can be used as a useful parameter (Correa *et al*., 2002). For dent maize hybrids, as the maturity increase the kernel density and vitreousness also increase while there will be a decrease in the ruminal starch availability (Correa *et al*., 2002). Thus, an increase in the vitreousness of the grain kernel can be associated with a decrease in ruminal starch degradation (Correa *et al*., 2002). Cereal grains selected for low fermentation rates can possibly help to decrease the interference with the digestion of fibre (Opatpatanakit *et al*., 1994). "Although there are significant differences between grains in the size and characteristics of starch granules it appears that the roles of the protein matrix and NSP (non-starch polysaccharides) in binding the granules together are more important than granule structure *per se* in determining the rate and extent of fermentation" (Rowe *et al*., 1999). For ration balancing to be economically favourable, nutritionists should aim to improve the favourable aspects of ruminal fermentation while at the same time minimizing fermentation losses (Russell *et al*., 1992).

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CHAPTER 3

Characterisation of maize samples

Abstract

Nine maize samples that differed from one another in terms of their endosperm type, as reflected by their milling index (MI) were chosen for the study. According to their MI, three of the 9 samples were regarded as having a hard endosperm type with a MI that ranged between 109 and 118, 3 samples had a medium endosperm type where MI ranged between 85 and 92 and the other three had a soft endosperm type where MI ranged between 67 and 71. The Roff milling index is a method used in South Africa to evaluate maize types in terms of milling quality and is believed to be indirectly related to their amylose, amylopectin and even their protein percentages. For example, a hard type when compared to a soft type, has a smaller amylose content and higher percentage coarse than fine particles. The hardness trait is highly heritable, thus when a certain amount of hardness is required in a maize kernel for the end product, breeders would be able to produce such product. Chemical analyses included moisture, ash, crude fibre, fat, crude protein and starch of the different maize types. The samples were sieved through a series of sieves with mesh sizes of 150, 125 and 106 µm, where the different fractions and weights of the samples were observed and compared to their MI values. In the current study, MI did not appear to be a reliable estimator of particle size distribution.

3.1 Introduction

Maize (*Zea mays*) is a summer grain and falls under the C4 plant category. These C4 species are usually tropical species and are named after the pathway of photosynthesis. C4 species can grow well in soils with low fertility and they tend to store their carbohydrates in the form of starch (McDonald *et al.*, 2002). Maize is a very palatable and digestible feed. It can be used as feed for most domesticated livestock species and rarely causes nutritional problems if used correctly (Kellems & Church, 2002).

In relation to other grain types, the kernels of maize are quite large. The composition is, however, similar to other cereal types, because the starch endosperm is the most dominant component (Fox & Manley, 2009). Maize can be divided into 5 classes, which are based on the characteristics of the kernel. These classes are from hard to soft as follows: flint, popcorn, flour, dent and sweet. In the “flint” or hard maize types, the starch in the endosperm is mostly hard or “vitreous”, whereas the softer types of maize such as “flour” have most of the starch as soft endosperm (Corona *et al.*, 2006). Starch is made up of two parts, amylose and amylopectin. Amylose has a lower digestibility than amylopectin, due to the tighter intermolecular bondages between the starch molecules (Corona *et al.*, 2006).

In the current study, different maize endosperm types were tested for their gas production, dry matter degradability and starch content. Overall, nine maize samples, which differ in cultivars and endosperm type, were collected from different origins for the study. The samples were selected in terms their Milling Index (MI). Three of the nine samples had a high MI that ranged between 109 and 118, three had a low MI that

ranged between 67 and 71 and the other three samples had a medium MI that ranged between 85 and 92. Although the MI is not a direct indication of the hardness or softness of the endosperm, it was believed to be indirectly associated with vitreousness.

The first part of the study, which is described in this chapter, was done to determine if there is any relationship between MI and particle size distribution of maize milled through a 1 mm screen.

3.2 Materials and Methods

3.2.1 Roff Milling Index

The hardness of maize has been tested through numerous methods over the past 50 years. Some of the methods include the measurement of resistance to grinding, yields of grits, abrasion, starch gelatinization properties and the grinding of maize followed by sieving and the amounts of the troughs being measured (Fox & Manley, 2009). The different maize endosperm types used in this study were characterised by the Grain Crop Institute (GCI) in Potchefstroom. The institute used the Roff Milling Index (MI) to characterise the maize samples. The Roff Mill was designed in 1983 and is supplied by Snell Africa Marketing (Pty) Ltd. In the method, maize is put through a roller mill system and the MI is determined from the meal and bran fractions obtained after the milling process. Samples are milled through a set of three rollers with gaps of 0.3, 0.18 and 0.08 mm respectively (Fox & Manley, 2009). The results obtained from the Roff milling index method are robust and can help to differentiate between maize cultivars of varied degrees of hardness and also to identify environmental and cultivar effects on the hardness of maize. Calibrations for a whole grain near-infrared transmission instrument (Foss Infratec 1251) were developed through the use of the MI method (Fox & Manley, 2009). According to the South African Grain Laboratory (SAGL), the ability of white maize cultivars to produce a high percentage of high-quality products would determine the milling performance. The MI of maize provides an indication of that maize expected milling performance for the dry milling industry. Furthermore, it is an indication of the differences between samples that are being tested. A sample with an index of about 95 is considered to have good milling characteristics. Samples with a milling index value of about 55 have a low milling quality whereas a MI of 115 have a very good milling quality.

The industry accepted method for the Roff Mill (SAGL, 2008):

The Roff mill should be pre-set to:

- Break 1: roll nip 0.3 mm
- Break 2: roll nip 0.18 mm
- Break 3: roll nip 0.08 mm

For Break 1, the mill's feed-rate per minute is 500 grams whole maize and then for Break 2 and Break 3, 45 seconds. Thus, the grits from Break 1 are transferred to Break 2 and then to the Break 3 rolls. There are three separations per mill namely germ, grit, and maize meal.

The different fractions are then weighed and documented as follows:

- Break 1 – meal (g)
- Break 2 – meal (g)
- Break 3 – meal (g)
- Break 3 – grits (g)
- Germ and bran from Break 1, 2 and 3 are combined and then weighed in gram (g).

Calculations and reporting of results takes place in percentage (%).

- Break 1, % = a ÷ T1 x 100
- Break 2, % = b ÷ T1 x 100
- Break 3, % = c ÷ T1 x 100
- Grits, % = d ÷ T1 x 100
- Germ & bran, % = e ÷ T1 x 100
- Extraction, % = T2 ÷ T1 x 100

Where: a = Break 1: meal (g)
b = Break 2: meal (g)
c = Break 3: meal (g)
d = Break 3: grits (g)
e = Germ and bran from Break 1, 2 & 3 are combined and then weighed in gram (g).
T1 = a + b + c + d + e
T2 = a + b + c + d

The maize types that were selected for the current study are indicated in Table 7.

Table 7 Different maize types used.

Sample	Cultivar	Origin	Maize type	Roff Milling Index
1	Phb 32 A05 B	Bethlehem	Hard	109.42
2	AFG 4321	Buffelsfontein	Hard	118.71
3	PAN 6223 B	Buffelsfontein	Hard	109.28
4	Phb 30D07	Potchefstroom	Medium	85.22
5	LS 8521 B	Potchefstroom	Medium	85.74
6	PAN 6723	Bethlehem	Medium	92.09
7	Saffier	Bethlehem	Soft	71.00
8	AFG 4411	Buffelsfontein	Soft	71.79
9	KKS 8401	Buffelsfontein	Soft	67.47

3.2.2 Chemical analyses of the maize samples

Dry matter

To determine the dry matter (DM) content of the nine different maize types, 1 gram (g) samples of each maize type were carefully and accurately weighed into pre-dried crucibles on a four-decimal scale. The samples were then dried for 24 hours in a forced air oven at 100°C (AOAC, 2002; Method 934.041).

$$\%\ \text{Moisture} = \frac{\text{Sample weight (g)} - \text{Dry sample weight (g)}}{\text{Sample weight}} \times 100$$

$$\%\ \text{DM} = 100 - \%\ \text{Moisture}$$

Ash

Amounts of 1 g of each of the 9 samples were accurately weighed into 9 crucibles. The crucibles were transferred to a muffle furnace with the temperature set at 500°C. After 6 hours, the crucibles were placed in a desiccator for about 30 minutes to cool and weighed again to determine the ash content (AOAC, 2002; Method 942.05).

$$\%\ \text{Ash} = \frac{\text{Weight of crucible and ash} - \text{Weight of dry empty crucible}}{\text{Sample Weight}} \times 100$$

$$\%\text{Organic matter} = 100 - \%\text{Ash}$$

Crude fibre

Amounts of 1 g of each of the 9 maize types were accurately weighed out in clean 2 pore air-dry glass crucibles. Crucibles were transferred to the extractor unit of the Fibertech apparatus (Fibertech System M, 1020 Hot extractor; SMM Instruments Pty. Ltd. Cape Town, South Africa). The valves of the apparatus were closed. The water tap was opened to cool down the apparatus, followed by checking for any leakage of water. Next, 150 mL of boiling H_2SO_4 solution were added to each crucible. Temperature was set at 100ºC. When the solution reached boiling point the heat was turned down to 65ºC and left for 30 minutes for the sample to gently boil.

After the 30 minute period, the heat was turned off. Filtration was done by opening the vacuum valves and each sample was rinsed with distilled water three times, after which the valves were closed again. Each crucible was then transferred to a drying oven, where it stayed for 24 hours to dry, with the oven temperature set at 100ºC. At the end of the 24 hours, the crucibles were placed in a desiccator for about 30 minutes to cool down. The crucibles' weights were then taken and recorded. Immediately following, the crucibles were transferred to the muffle furnace with the temperature set to 500ºC for 6 hours. After that the crucibles were cooled off in a desiccator for 45 minutes and finally weighed and recorded (AOAC, 2002; Method 962.09).

$$\%\ \text{Crude Fibre} = \frac{\text{Residue in crucible after drying} - \text{Residue in crucible after ashing}}{\text{Sample Weight}} \times 100$$

Crude fat

For the aluminium fat beakers to be moisture free, they were placed in a 100ºC drying oven for 24 hours and then placed in a desiccator for 30 minutes to cool. Beakers were then weighed and the weights were accordingly recorded. Thereafter 2 g of sample were weighed out into the extraction thimbles. In order to prevent the sample from flushing out of the thimbles, a small piece of cotton wool was placed on top of each sample inside the thimble. Now the beakers were filled with about 50 mL of diethyl ether. After that, the water flowing taps were opened for condensation. A Tecator Soxtec System HT 1043 Extraction Unit was the apparatus used for determining the fat content of the different maize types.

The heating, fan and oil bath were turned on and once the ready light flickered, the thimbles were transferred to the extraction tubes. Each thimble in the tube matched the fat beakers on the bottom element. The handle of the apparatus was used to lower the extraction tubes and care was taken to ensure that the tubes are tightly sealed with the beakers. Thimbles were lowered and allowed to boil for 15 minutes in the ether (handle on "boiling" and taps open). Then for 30 minutes, the thimbles were raised and the taps were closed in order to collect the ether (handle on "rinsing"). The collected ether was then boiled for 15 minutes. Beakers were removed from the apparatus and placed into a drying oven (100ºC) for approximately 2 hours

to ensure that all the ether evaporates. The beakers were removed from the oven and placed into a desiccator for 30 minutes to cool down. Beakers were weighed accurately on a 4 decimal scale.

After the beakers were used, they were cleaned out with 10 mL of diethyl ether to remove the fat and then washed out with distilled water. If the beaker does not clean out properly, a small amount of H_2SO_4 can be added to the beaker and left for 1 to 2 hours (AOAC, 2002; Method 920.39).

$$\% \text{ Fat} = \frac{(\text{Weight of Soxhlet beaker} + \text{Fat}) - (\text{Weight of Soxhlet beaker})}{\text{Sample Weight}} \times 100$$

Nitrogen (Crude protein)

The Leco Nitrogen Gas Analyzer FP528, (LECO Africa (Pty) Ltd, Kempton Park), was used for the determination of the total nitrogen content of the maize samples. The Leco FP528 was first standardized according to the manufacturer's instructions. An empty aluminium foil cup was placed on a 4 decimal scale and the scale was zeroed. About 0.1 g of the sample was weighed into the aluminium foil cup and the weight was recorded to the fourth decimal. The cup was then closed by twisting the ends together. The now closed cup was once again weighed and the weight recorded. The closed aluminium foil cup was placed onto the carousel sample tray. This process was repeated for all 9 samples. The Leco was then switched on and the temperature was set at 850ºC. Samples were combusted inside the Leco's furnace and the nitrogen content was recorded in percentage (%). For the determination of the crude protein of each sample, the nitrogen content was multiplied with the factor 6.25 (AOAC, 2002).

Starch

Amounts of 0.2 g sample were weighed and transferred to 100 mL Erlenmeyer flasks. Next 20 mL of distilled water was added and the solution was stirred with a magnetic stir bar. Heat stable α-amylase (0.1 mL) was then added. Square cut aluminium foil pieces were used to cover the flasks and it was placed in a waterbath set at 93ºC for 1 hour. Then the flasks were taken out of the water bath and left to cool for approximately 15 minutes after which it were filtered through funnels that contained glass wool into a volumetric flask (100 mL). Flasks were brought to volume with distilled water.

Thereafter 2 mL of the sample in the 100 mL flask was transferred to a 50 mL volumetric flask. Eight millilitres of 0.1 M sodium acetate buffer were added to each flask, as well as 50 µL amyloglucosidase. Volumetric flasks were then placed into a waterbath set at 60ºC for 30 minutes. When removed from the waterbath the flasks were first brought to volume with distilled water, then 1 mL aliquots were transferred to test tubes. Where 5 mL glucose oxidase-peroxidase reagent was added to each test tube. Tubes were

placed for 15 minutes into a water bath set at 40°C, after which it were left to cool down for 10 minutes in the dark. The absorbance's of each of the samples were then measured and recorded (Karkalas, 1985).

3.2.3 Fractions and particle size separation

Sample preparation

All the maize samples were milled through a hammer mill (Scientec RSA Hammer mill Ser Nr 372; Centrotec) to pass a 2-mm screen. The samples were subsequently milled for a second time through a cyclone mill (Cyclotec 1093 Sample Mill; tecator) to pass a 1-mm screen.

Particle size separation

Of each of the different maize sample types, 10 g were accurately weighed out and sieved through a series of sieves (Kingtest laboratory test sieve, Retsch GmbH, Series AS 200 basic, Germany), with mesh sizes of 150 µm, 125 µm, and 106 µm. The amounts that remained on top of each sieve, as well as in the bottom pan, were accurately weighed and the weights recorded.

3.2.4 Statistical analyses

Data pertaining to the Roff Milling Index, starch content, sieve fractions, gas production parameters, DM disappearance values and starch disappearance were subjected to Statistica version 9 (2009) to Pearson correlations to determine possible relationships between the mentioned parameters.

3.3 Results and Discussion

The MI and nutrient composition of the different maize samples are presented in Table 8.

The maize DM, ash, fibre, fat, protein or starch content did not seem to be related to the MI value of maize. Thus, the MI value of maize appears to be a poor predictor of its chemical composition. The average amount of crude protein was found to be slightly lower for hard endosperm types than for soft endosperm types (8.4 vs. 9.9 %), whereas the average starch content of the different endosperm types did not seem to differ much (60.4 vs. 63.1 %). There was fairly high variation among the maize samples in terms of their ash, crude fibre, crude protein and to a lesser extent, their fat contents, whereas the variation among the maize samples in terms of their DM and starch was very small. The ash and fibre contents of all the maize samples were so low, that the high CV is of no practical concern. The standard error among the maize samples were high for crude protein, and starch compared to DM, ash, crude fibre, crude fat and nitrogen.

In the study done by Philippeau *et al.* (1999), they also found that the starch content was similar for soft and hard endosperm types but the average crude protein content was slightly lower for soft than for hard endosperm maize types.

Table 8 Milling Index (MI) and nutrient composition (g/kg) of maize samples used in the trial. All values are on a DM basis.

Sample[1]	MI	DM	Ash	Crude fibre	Crude fat	Nitrogen	Crude protein	Starch
1	109.4	875.7	10.4	26.2	29.2	12.8	80.0	638.6
2	118.7	883.7	10.7	20.8	35.1	13.4	83.8	627.9
3	109.3	880.3	11.4	20.6	34.2	14.0	87.5	625.9
4	85.2	882.6	12.1	19.4	36.6	17.4	108.8	602.9
5	85.7	884.8	14.5	20.6	39.6	18.9	118.1	594.8
6	92.1	880.3	11.5	23.6	36.4	15.8	98.8	615.4
7	71.0	880.1	9.8	19.4	33.7	15.5	96.9	634.2
8	71.8	879.3	9.8	20.6	33.2	16.3	101.9	626.9
9	67.5	881.6	10.7	23.6	31.8	15.6	97.5	626.0
Mean		880.9	11.2	21.6	34.4	15.5	97.0	621.4
SE		0.9	0.5	0.8	1.0	0.6	4.0	4.8
CV		0.3	13.0	10.6	8.7	12.5	12.5	2.3

[1]Maize samples 1 to 3 had a relatively high Roff Milling Index (MI), samples 4 to 6 had a medium MI and samples 7 to 9 had a relatively low MI.

MI = Milling Index; DM = Dry Matter; SE = Standard error; CV = Coefficient of variation.

The fractions of the samples that remained on top of each sieve were weighed and recorded. Coarse particles were those that remained on top of the 150 µm sieve. The fine particles were represented by those that passed through the 106 µm sieve. The fractions with diameters between 150 and 106 µm were taken as representative of intermediate particles. Three replicates were completed for each of the high, medium and low MI values.

In Table 9 and Figure 5, it can be seen that the majority of the particles were either > 150 µm or < 106 µm in size. The amount that stayed behind on the 125 and 106 µm sieves were the minority. This shows us that the maize samples had a high percentage of hard and relatively large particles after it was milled through 2 mm and 1 mm screens, sequentially. In addition, they had a relatively high percentage of fine (soft) material.

There was a difference between hard and soft maize particle size distribution. Maize types with a lower MI value were characterised with a higher proportion of coarse particles than maize types with a higher MI value (45.9 vs. 41.2 %) and a smaller proportion of fine particles (35.8 vs. 45.3 %). No positive or negative correlation was observed between the vitreousness and particle distribution of the maize types. Some recent research reports that there is well an association between kernel hardness and maize starch. Research found that generally a softer endosperm (expected to have a lower MI value) had a larger starch granules and a higher amylose content, thus a higher percentage of fine particles (Fox & Manley, 2009).

Philippeau *et al.* (1999), found that flint (hard) and dent (soft) maize types differed in their proportion of coarse particles. They also found that the dent types had a smaller percentage of coarse particles (61.9 vs. 69.6 %) and a higher percentage of fine particles (15.6 vs. 9.0 %). In the study done by Philippeau *et al.* (1999), the maize was milled through a hammer mill fitted with a 3 mm screen then 2 g were placed in a Fritsch analysette 28 apparatus (Fritsch GMBH, Idar – Oberstein, Germany), that have a series of four sieves 90, 125, 250 and 500 µm respectively.

Table 9 Sieve separations of milled maize samples in gram and percentage left behind on the different pore size sieves.

Sample	Origin	Maize type	>150 µm		<150 µm; >125 µm		<125 µm; >106 µm		<106 µm	
			gram	%	gram	%	gram	%	gram	%
1	Beth	Hard	3.07	32.70	0.74	7.90	0.34	3.70	5.23	55.70
2	Buff	Hard	4.80	48.40	0.87	8.80	0.44	4.40	3.81	38.40
3	Buff	Hard	4.13	42.50	0.81	8.30	0.71	7.30	4.06	41.90
4	Potch	Medium	5.48	57.30	0.75	7.90	0.64	6.70	2.69	28.20
5	Potch	Medium	5.01	50.30	0.74	7.50	0.81	8.10	3.41	34.20
6	Beth	Medium	2.37	26.20	1.40	15.50	0.75	8.30	4.53	50.10
7	Beth	Soft	5.17	52.90	1.33	13.60	0.72	7.40	2.56	26.10
8	Buff	Soft	3.89	40.10	0.93	9.60	0.64	6.60	4.23	43.70
9	Buff	Soft	4.41	44.60	1.00	10.10	0.76	7.70	3.74	37.70
Mean SE			4.26 0.3	43.89 3.3	0.95 0.1	9.91 0.9	0.64 0.1	6.69 0.5	3.80 0.3	39.56 3.2
CV			417.0	445.2	381.4	354.0	415.4	416.1	448.1	411.9

[1]Maize samples 1 to 3 had a relatively high Roff Milling Index (MI), samples 4 to 6 had a medium MI and samples 7 to 9 had a relatively low MI.
SE = Standard error; CV = Coefficient of variation
Beth = Bethlehem; Buff = Buffelsfontein; Potch = Potchefstroom.

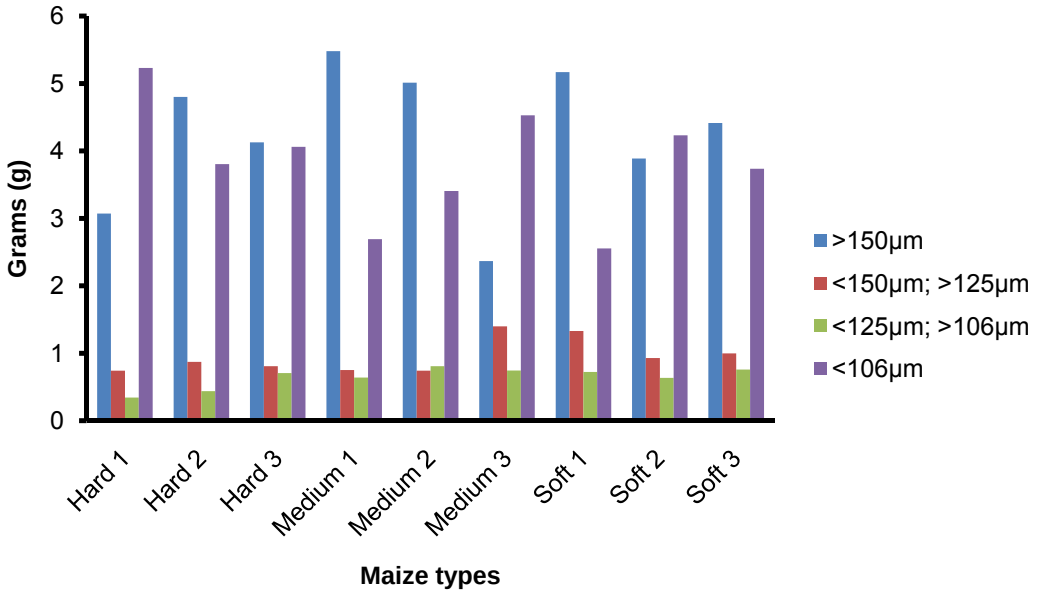

Figure 5 Different weights and fractions of the sieve separations of milled maize samples.

Table 10 and Figure 6, show the particle size distribution of the different samples. From this data and figure, the particle size distribution can be clearly seen within, as well as between, the different maize types. A higher percentage of particles are larger than 106 µm in size and a lower percentage of the maize kernel have a particle size smaller than 106 µm. According to this data, no direct linkage can be made when the different MI values of the maize types are compared to their particle distribution.

The amounts of maize particles that stayed behind on top of each sieve Table 9, whereas in Table 10, the amounts that stayed behind on top of sieves 150, 125 and 106 µm were added together. This was done to show what percentage of the maize kernel can be classified as coarse and what percentage can be classified as fine particles.

Table 10 Particle size distribution of the milled maize samples in gram and percentage.

Sample	Origin	Maize type	> 150 µm		> 125 µm		> 106 µm		< 106 µm	
			gram	%	gram	%	gram	%	gram	%
1	Beth	Hard	3.07	32.70	3.81	40.62	4.16	44.27	5.23	55.70
2	Buff	Hard	4.80	48.40	5.67	57.19	6.11	61.63	3.81	38.40
3	Buff	Hard	4.13	42.50	4.94	50.86	5.64	58.14	4.06	41.90
4	Potch	Medium	5.48	57.30	6.23	65.15	6.87	71.84	2.69	28.20
5	Potch	Medium	5.01	50.30	5.76	57.74	6.56	65.84	3.41	34.20
6	Beth	Medium	2.37	26.20	3.77	41.65	4.51	49.91	4.53	50.10
7	Beth	Soft	5.17	52.90	6.50	66.47	7.22	73.86	2.56	26.10
8	Buff	Soft	3.89	40.10	4.82	49.75	5.45	56.31	4.23	43.70
9	Buff	Soft	4.41	44.60	5.41	54.63	6.17	62.28	3.74	37.70
Mean SE			4.26 0.3	43.89 9.9	5.21 0.3	53.78 3.0	5.85 0.3	60.45 3.2	3.8 0.3	39.56 3.2
CV			417.0	445.2	537.5	590.6	569.3	629.0	448.1	441.9

Beth = Bethlehem; Buff = Buffelsfontein; Potch = Potchefstroom;
SE = Standard error; CV = Coefficient of variation.

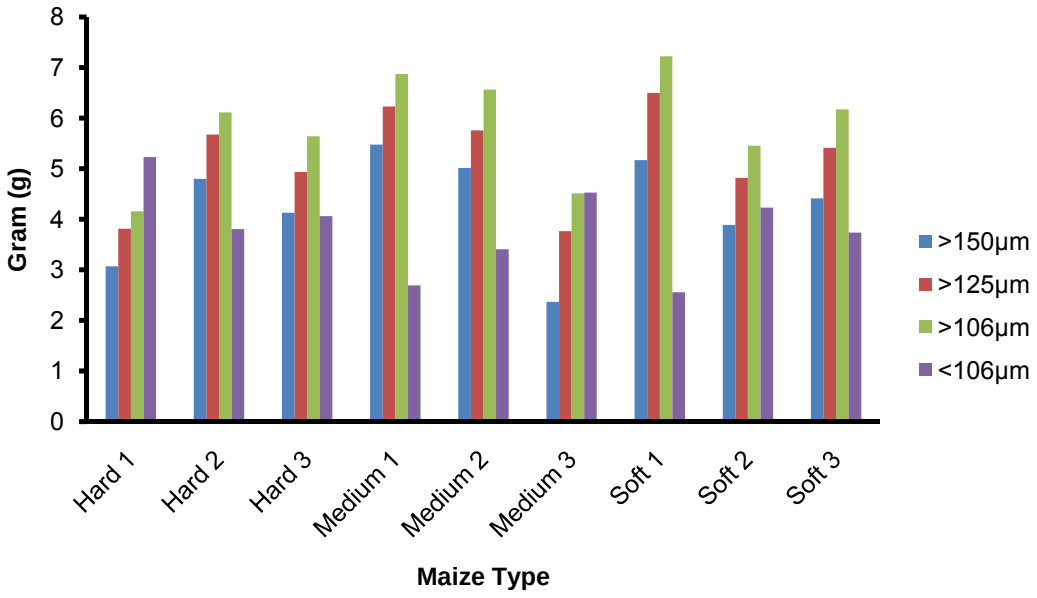

Figure 6 Particle size distribution of maize samples.

3.4 Conclusion

There were no drastic changes in DM, ash, fibre, fat, protein and starch content as the MI value of maize increased or decreased. No significant correlations were observed between maize MI values and the chemical composition of the maize or particle size separation. The Roff Milling Index did not appear to be a reliable indicator of particle size distribution, especially in terms of the coarse (>150 µm) and very fine particles (<106 µm) and also not a reliable indicator of the chemical composition of maize.

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Chapter 4

The effect of different maize endosperm types on gas production

Abstract

The gas production system has been developed successfully and at the present time has become a popular method to evaluate feedstuffs. With the in vitro gas production system, feedstuffs can be estimated for their digestibility in the rumen environment of the cow. With this information, feedstuffs can be characterized and more improved diets can be formulated. In the current study, nine maize samples were subjected to the gas production system. According to their Milling Index (MI) values, three of the nine samples had a hard endosperm type with a MI that ranged between 109 and 118, three samples had a medium endosperm type where MI ranged between 85 and 92 and the other three had a soft endosperm type where MI ranged between 67 and 71. Maize samples were ground through a hammer mill (2 mm) and then ground through a cyclone mill (1 mm). The maize samples were incubated together with the rumen fluid, incubation medium and reducing solution for a time period of 48 hours. During incubation gas production and rate of gas production for the samples were measured and recorded. From these the fermentation kinetics of the different maize endosperm types was determined. On average the total gas production (b) was predicted to be higher for maize types with a low MI value than for maize types with a high MI value (422.3 vs. 400.6 ml g^{-1} DM). The results obtained were compared to the MI values of the different maize types. Roff MI cannot be used as a reliable indicator for gas production of the different maize types.

4.1 Introduction

The correlation between gas production and rumen fermentation parameters has been known for some time. In the late 1930's, an attempt was made to record the gas directly produced from a ruminally cannulated sheep via the cannula, but the technique was too laborious and not reproducible. In 1974, Menke and Steingass (cited by Theodorou *et al*., 1994), who studied the stoichiometry of fermentation in the rumen with a closed syringe system, observed reproducible gas production data. Until today, the same principle applies, although some of the steps have been improved over the years or even simplified (Sallam, 2005).

Expensive resources such as time, labour and feed are needed for conventional production and digestibility trials, which have led to the large scale use of *in situ* digestibility techniques. The reason why the *in situ* techniques have become less attractive nowadays is because of the costs that are associated with cannulating animals, as well as maintaining the surgically modified animals, the small number of samples that can be examined at a time and animal welfare issues (Mauricio *et al*., 1999). It has been established that the *in situ* technique cannot be used for the evaluation of all feedstuffs and, because of this, *in vitro* methods especially the gas production systems, have become popular to evaluate different feedstuffs (Mauricio *et al*., 1999).

Basically, the *in vitro* gas pressure technique provides an estimation of the digestion of insoluble and soluble carbohydrates (Getachew, 2004). When fermentation of different feed ingredients by rumen microorganisms occur, it will largely produce gasses like hydrogen, carbon dioxide (CO_2) and methane (CH_4). The methanogenic bacteria are responsible for the conversion of hydrogen to methane. The CO_2 that will accumulate in the headspace of gas measuring vials generally comes from two sources, viz. acetic and butyric acid production and also from the neutralization of these acids in a bicarbonate buffer. The production of propionic acid does not result in CO_2 formation. Only when neutralization of the propionic acid with the buffer occurs, will CO_2 form. Thus, the extent of gas produced from the incubation of a feed, indirectly reflects the VFA production (Getachew, 2004).

The objective of this study was to determine the fermentation kinetics of different maize endosperm types by total gas production and gas production rate.

4.2 Materials and Methods

4.2.1 Sample preparation

Nine maize samples were used for the study. These nine maize samples differed from one another in terms of endosperm type as reflected by their milling index (MI). According to their MI, three of the nine samples were assumed to have a hard endosperm type with a MI that ranged between 109 and 118, three had a soft endosperm type where MI ranged between 67 and 71 and the other three samples had a medium endosperm type where MI ranged between 85 and 92.

Approximately 100 g of each sample were weighed out and ground in a hammer mill fitted with a 2 mm screen (Scientec RSA Hammer mill Ser Nr 372; Centrotec). After this, the samples were ground with a cyclone mill (Cyclotec 1093 Sample Mill; tecator) to pass through a 1-mm screen.

4.2.2 Preparation of the in vitro medium and reducing solution

The medium that was used for the *in vitro* gas production system, described by Van Soest and Robertson (1985), consisted of distilled water, a macro mineral solution, buffer solution, a micro mineral solution, tryptose and rezasurin. The medium had a pH of approximately 7.27.

The reducing solution used was also described by Van Soest and Robertson (1985). The reducing solution was prepared in two separate beakers, A and B. The content of each was stirred and left until fully dissolved, followed by the gentle addition and mixing of the solution in beaker B to that of beaker A.

The preparation and composition of the media is presented in Table 11.

Table 11 Composition of the media used in the *in vitro* gas production trial.

Reagent	Quantity
1 Litre Buffer solution:	
Distilled water	1000 mL
Ammonium bicarbonate (NH_4HCO_3)	4 g
Sodium bicarbonate ($NaHCO_3$)	35 g
1 Litre Macro-mineral solution:	
Distilled water	1000 mL
Di-sodium hydrogen orthophosphate anhydrous (Na_2HPO_4)	5.7 g
Potassium dihydrogen orthophosphate (KH_2PO_4) (anhydrous)	6.2 g
Magnesium sulphate heptahydrate ($MgSO_4.7H_2O$)	0.59 g
Sodium chloride (NaCl)	2.22 g
100 mL Micromineral solution:	
Calcium chloride dihydrate ($CaCl_2.2H_2O$)	13.2 g
Manganese chloride tetrahydrate ($MnCl_2.4H_2O$)	10 g
Cobalt (II) chloride hexahydrate ($CoCl_26H_2O$)	1 g
Ferric chloride hexahydrate ($FeCl_3.6H_2O$)	8 g
1 Litre Incubation medium:	
Distilled water	500 mL
Tryptose	2.5 g
Micromineral	0.125 mL
Macromineral	250 mL
Buffer	250 mL
Rezasurin	1.25 mL
100 mL Reducing solution:	
Beaker A:	
Distilled water	50 mL
Cysteine hydrochloride ($C_3H_7NO_2$ HCl)	0.625 g
Potassium hydroxide (KOH) pellets	10
Beaker B:	
Distilled water	50 mL
Sodium sulphide nonahydrate	0.625 g

4.2.3 Collection of rumen fluid

The rumen fluid was obtained from two ruminally cannulated, lactating Holstein cows on the Welgevallen Experimental Farm of the Stellenbosch University. The cows received 22 kg of a commercial semi-complete diet per day and had free access to a 50:50 mixture of lucerne hay and oat hay. Cows received the pellets twice daily at 7:00 (11kg) and at 16:00 (11kg).

For the collection of the rumen fluid, pre-warmed thermos flasks, a funnel and two layers of cheesecloth were used. The stopper of the cannula was removed and rumen contents were taken by hand from multiple areas within the rumen and transferred to the double layer cheesecloth. Rumen fluid was then squeezed by hand through the cheesecloth into the pre-warmed thermo flasks. A handful of the solid phase rumen content was also added to each flask and flasks were filled to the brim to exclude air. The flasks were then transported to the laboratory where the content of each flask was pooled in a pre-warmed blender and blended for 20 seconds at a low speed, while gassing continuously with CO_2. The pH of the rumen fluid was subsequently taken, and the blended fluid was then strained through four layers of cheesecloth into pre-warmed beakers, kept in a 39°C water bath and gassed continuously with a gentle stream of CO_2 until used.

4.2.4 In vitro incubation of the maize samples

The gas production method used in the study was based on the Reading Pressure Technique (RPT) (Mauricio *et al*., 1999). The RPT is used for the evaluation of feed *in vitro* and it is based on a semi–automated gas production technique (Mauricio *et al*., 1999).

Glass vials, with a nominal volume of 120 mL, were used for incubation of the maize samples. The exact volume of each vial was determined previously and engraved on the vial. The exact vial volumes are required for the accurate determination of headspace volume. Blank vials, containing buffered rumen inocula, but no substrate, were also prepared to obtain reagent blanks that would be used later to correct for the gas produced by the rumen fluid alone (Mauricio *et al*., 1999). Amounts of 0.25 g of each maize sample were weighed accurately into the vials. A 20 mm magnetic stirrer bar with a volume of 0.2 mL was placed into each vial.

A syringe was used to add 40 mL of the buffered medium into each vial, followed by the addition of 2 mL of the reducing solution. Vials were gassed with a gentle stream of CO_2 and 20 mm rubber stoppers were placed on the vials without pushing them in all the way. The vials were then placed in a pre-warmed (39°C) water bath until the medium was reduced. The buffer solution was reduced when the colour changed from purple-blue to light pink or colourless. After reducing, the vials were reopened and 10 mL of the strained rumen fluid was added to each vial while also gassing with CO_2. This was followed by pushing the rubber stoppers in completely and sealing with 20 mm aluminium crimp caps.

The vials were subsequently transferred to magnetic stirrer plates inside the incubator and the contents were stirred continuously at a low speed. Needles (40 mm, 21 gauge), fitted to pressure transducers, were carefully inserted through the rubber stoppers. The transducers were linked to a pressure logging system and the pressure inside each vial was recorded every 5 minutes for the entire incubation period of 48 hours. The logging system, based on the Reading Pressure Technique (RPT) of Mauricio et al. (1999), was custom built by Eagle Technology (Pty) Ltd. (Cape Town). To avoid excess pressure build-up in the vials, pressure was released from the vials at regular intervals. Throughout the entire incubation period the temperature in the incubator was maintained at 39°C.

4.2.5 The conversion of gas pressure to gas volume

The pressure transducer system recorded the data in terms of pressure, thus psi units. For the conversion of pressure to gas volume, the following linear regression equation was used that was developed in the Department of Animal Sciences' *in vitro* lab:

$$Y = \frac{[1000((0.0977\ X)C)]}{OM}$$

Where: Y = Gas volume (mL/g OM)
X = Gas pressure (psi)
C = Vial head space (mL)
OM= Organic matter (mg)

4.2.6 The estimation of kinetic coefficients

The solver option in Microsoft Office Excel and the non-linear Models 1 and 2 (with or without a lag phase, respectively) were used to calculate the kinetic coefficients from the gas volume data. The models used were based on the modified version that is described by Ørskov and McDonald (1979).

Model 1: $$Y = b(1 - e^{-ct})$$

Model 2: $$Y = b(1 - e^{-c(t-L)})$$

Where: Y = gas volume at time t
b = total gas production (ml g^{-1} DM)
c = rate of gas production (h^{-1})
t = incubation time (hours)
L = lag time (hours)

4.3 Statistical analyses

The non-linear models mentioned above were used to derive potential gas production values (b), gas production rates (c) and lag times (L). These parameters were then subjected to a main effects ANOVA with the aid of Statistica, version 9 (2009). Main effects were treatment and repetition. Treatment means were separated with Scheffé tests. Furthermore, data were subjected to Pearson correlations to determine regressions of RMI on gas production, particle size on gas production and gas production on DM disappearance. A variety of significant correlations were observed, but only those that might have practical application value were further investigated. The Scatterplot procedure of Statistica 9 was used to determine the regressions. In all instances, significance was declared at $P \leq 0.05$.

4.4 Results and Discussion

Gas production of the various maize samples

The data reported in Table 12, represents the data calculated from the non-linear Models 1 and 2. Model 2 with a lag phase and Model 1 without. Where b represents the total gas production, c the rate of gas production and L the lag time.

When calculated through Models 1 and 2, maize samples 3, 4 and 8 had the highest amount of total gas production. On average the total gas production (b) were higher for maize types with a low MI value (7, 8 and 9) than for maize types with a high MI value (1, 2 and 3) (422.3 vs. 400.6). Alternatively when looking at individual maize samples there were no difference ($P > 0.05$) in gas production. It seems that the rate of gas production (c) did not differ ($P > 0.05$) among the samples but it was slightly higher for Model 2 than for Model 1 (0.14 vs. 0.12). The lag time (L) differed among the maize types and was lower for maize types with a high MI value (0.77 vs. 1.04).

For Model 1 (Table 13) the total gas production (b) was the highest for the low MI value maize types and the lowest for the high MI value maize types (388.7 vs. 367.4). This was also true for Model 2, where the gas production (b) for the low MI value maize was on average 383.1 and for the high MI value maize it was on average 363.7.

Table 13, represents the same data as in Table 12, but without the data of samples 3, 4 and 8. When the P-values in Table 12 are compared to that in Table 13, the P-values for b1 and c1 were lower for the nine samples than for the six samples. The P-values for b2, c2 and L2 are also higher for the six samples than for the nine samples.

Table 12 Gas production of the nine different maize samples.

	Maize samples[1]										
Item	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**SEm**	**P**
Model1[2]											
b	370.07	364.72	467.08	476.54	380.21	372.14	391.72	489.65	385.61	41.74	0.21
c	0.14	0.13	0.13	0.11	0.12	0.13	0.11	0.12	0.12	0.009	0.52
Model 2[3]											
b	366.52	360.92	464.83	470.32	374.76	368.2	385.63	483.63	380.61	41.02	0.20
c	0.16	0.15	0.14	0.13	0.15	0.15	0.14	0.14	0.14	0.01	0.67
L	0.84^{ab}	0.84^{ab}	0.63^{a}	1.04^{ab}	1.16^{ab}	0.96^{ab}	1.20^{b}	0.97^{ab}	0.94^{ab}	0.11	0.05

[1]Maize samples 1 to 3 had a relatively high Roff Milling Index (MI), samples 4 to 6 had a medium MI and samples 7 to 9 had a relatively low MI.

[2]Model 1: $Y = b(1 - e^{-ct})$, where b = gas volume at time t; c = rate of gas production; t = incubation time.

[3]Model 2: $Y = b(1 - e^{-c(t-L)})$, where b = gas volume at time t; c = rate of gas production; t = incubation time; L = lag time (h).

[a, b]Values with different superscripts within rows differed significantly ($P<0.05$).

SEm = Standard error of the mean; P = Significance level.

Table 13 Gas production of the six different maize samples.

	Maize samples[1]							
Item	**1**	**2**	**5**	**6**	**7**	**9**	**SEm**	**P**
Model 1[2]								
b	370.07	364.72	380.21	372.14	391.72	385.61	29.61	0.99
c	0.14	0.13	0.12	0.13	0.11	0.12	0.01	0.60
Model 2[3]								
b	366.52	360.92	374.76	368.12	385.63	380.61	28.82	0.99
c	0.16	0.15	0.15	0.15	0.14	0.14	0.01	0.71
L	0.84	0.84	1.16	0.96	1.20	0.94	0.12	0.20

[1]Maize samples 1 and 2 had a relatively high Roff Milling Index (MI), samples 5 and 6 had a medium MI and samples 7 and 9 had a relatively low MI.

[2] Model 1: $Y = b(1 - e^{-ct})$, where b = gas volume at time t; c = rate of gas production; t = incubation time.

[3]Model 2: $Y = b(1 - e^{-c(t-L)})$, where b = gas volume at time t; c = rate of gas production; t = incubation time; L = lag time (h).

SEm = Standard error of the mean; P = Significance level.

Gas production of maize samples incubated in buffered rumen liquor are presented in Figure 7.

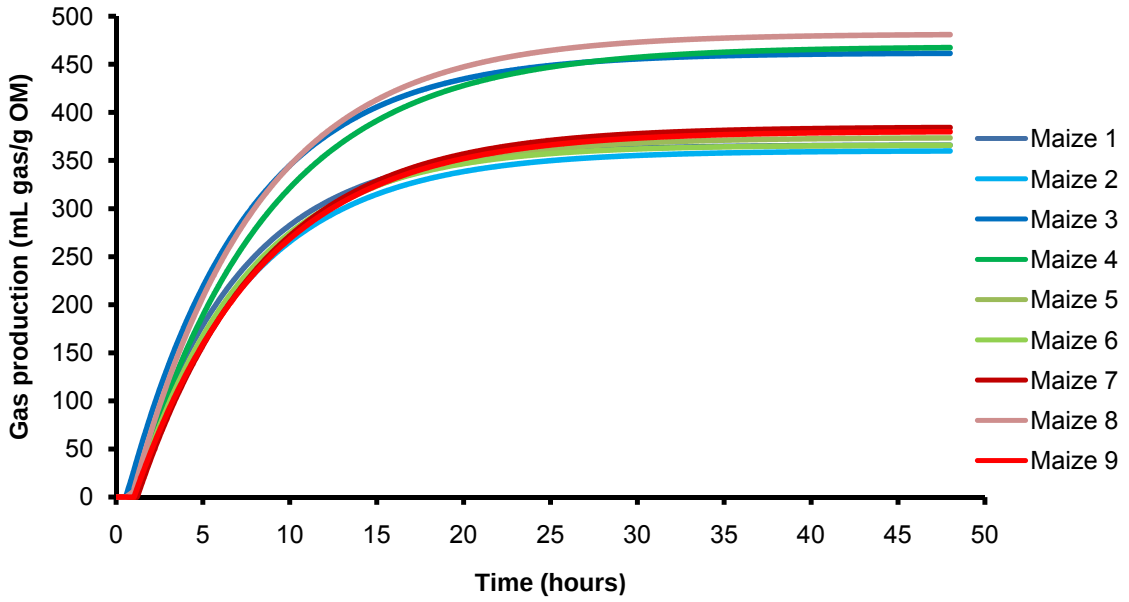

Figure 7 Gas production of maize samples incubated in buffered rumen liquor. The non-linear model used included a lag phase (Model 2: $Y = b(1 - e^{-c(t-L)})$, where b = gas volume at time t; c = rate of gas production; t = incubation time; L = lag time (h)).

Correlation coefficients

Data pertaining to the Roff Milling Index, starch content, sieve fractions, gas production parameters, DM disappearance values and starch disappearance were subjected to Pearson correlations to determine possible relationships between the mentioned parameters. A variety of significant correlations were observed, but only those that might have practical application value were further investigated. The Scatterplot procedure of Statistica 9 was used to determine the regressions indicated in Table 14.

Table 14 Correlations and possible relationships between Roff Milling Index, sieve fractions, gas production parameters and DM disappearance values.

Independent variable X	Dependant variable Y	Regression equation	r	r^2	P
Roff Milling Index	Gas production[1]:				
	c1	Y = 0.087 + 0.0004X	0.749	0.561	0.020
	L2	Y = 1.518 - 0.0063X	-0.676	0.458	0.045
Particles >150µm	Gas production[1]:				
	c1	Y = 0.157 – 0.0008X	-0.753	0.567	0.019
Particles >106 + <106µm	Gas production[1]:				
	c1	Y = 0.080 + 0.0009X	0.851	0.724	0.004
Particles <106µm	Gas production[1]:				
	c1	Y = 0.086 + 0.0009X	0.890	0.803	0.001
Gas production L2	DM disappearance[2]:				
	b1	Y = 49.08 + 5.93X	0.669	0.447	0.049

[1]The non-linear gas production parameters followed by a "1" were derived from a model where a lag phase was omitted, while a "2" indicates that a lag phase was included.

[2]The non-linear DM disappearance values followed by a "1" were derived from a model which predicted the a-value and where a lag phase was included.

As can be seen from Table 14, The Roff Milling Index (RMI) was significantly correlated with a variety of measurements. The positive correlation with gas production rate (c1) is interesting, because a higher RMI index is indirectly associated with vitreousness and one would have thought that a high RMI (harder maize) would rather suppress fermentation rate. Just as interesting is the negative correlation of RMI with the fermentation lag time (L2).

Regarding particle size distribution, the most significant regressions are the finest fractions against gas production rate. The higher the proportion of fine particles (>106 µ plus <106 µ, i.e. <125 µ), the higher the gas production rate can be expected to be. The <106 µ fraction has the highest correlation with gas production rate and it explains almost 90% of the variation.

4.5 Conclusion

For this study maize samples were selected in terms of their Roff Milling Index (MI), to get a variety of endosperm types. Gas production parameters differed among maize samples and maize samples of the high MI types appeared to have a lower gas production (b-value) than maize types with a medium and low MI types. The use of the Roff Milling Index of different maize types was a relatively poor indicator of maize gas production as well as the rate of gas production. Particle size of milled samples, both >106 + <106µm and <106µm was positively correlated with the rate of gas production, it explained 72 and 80 % of the variation observed in gas production rate. To accurately describe and predict gas production kinetics, more research is still needed in the feature.

4.6 References

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CHAPTER 5

In vitro dry matter and starch disappearance of maize samples that differ in endosperm type

Abstract

This study determined the in vitro dry matter digestibility and starch disappearance of maize types that differed in endosperm type. An Ankom DAISY^{II} system was used for the dry matter digestibility determination. Incubation periods were 2, 4, 8, 12, 24 and 48 hours respectively. Starch is important in animal diets as it serves as the energy component for the animal. Amylose and amylopectin are the two major sources of starch of which amylose are the less fermentable one of the two in the rumen. The general steps in a starch analyses procedure are gelatinisation, hydrolysis and then measurement of the end products. Analyses were done on the starch disappearance on the samples incubated for 0, 2 and 4 hours. The DM digestibility for the different maize types were estimated through Models 1 and 2 (with or without a lag phase, respectively). On average, it was predicted that maize types with a high MI value had a higher proportion of soluble fraction (a) compared to the low MI types. Maize samples also differed in terms of the potentially digestible (b). It appeared as if the lower MI types, which are believed to contain more of a soft endosperm, had higher amounts of the potentially digestible fraction than, that of the high MI types. The data obtained from the estimations done showed that there were small, but significant relationships between Roff Milling Index and certain DM digestibility parameters. A relationship was also observed between Roff Milling Index and starch disappearance over time.

5.1 Introduction

In all cereal grains the most abundant carbohydrate is starch and it consist of about 70% of the dry matter of the maize grain (Evers *et al*., 1999). In turn about 80% of the weight of the maize kernel is made up of endosperm, where starch is contained. Although starch is the primary component of the endosperm, the endosperm also contains protein and a small amount of phospholipids and ash (Hoffman & Shaver, 2009). It was observed that a decrease in ruminal starch degradation was associated with an increase in maize vitreousness (Philippeau & Michalet-Doureau, 1997; Correa *et al*., 2002). Maize grains, classified as dent, normally have a higher amount of floury endosperm, whereas maize grains, classified as flint, tend to have a larger amount of vitreous endosperm (Johnson *et al*., 1999). Starch granules present in the vitreous endosperm are encapsulated by a protein matrix, which limits the starch digestion. The dent or floury endosperm is more available for digestion (Kotarski *et al*., 1992). Starch granules that has a diameter of up to 30 µm, are solid and it is optically clear bodies. When seen as a bulk powder they appear white, because of light that scatter at the starch-air interface (Evers *et al*., 1999). Of maize, starch is the major energy-yielding component. It is important in animal diets and is used for the intensive production of milk and beef. This is the reason why starch digestion must be efficient; otherwise, there will be a decrease in the production of milk and beef by cattle (Huhtanen & Sveinbj□rnsson, 2006). Starch granules are degraded from the outside by microbes. Whereas enzymes like α-amylase, degrade the granule from the inside by

firstly attacking the maize granule on the surface at a particular spot and then move inwards to starch digestion inside (Cone, 1991; Huhtanen & Sveinbj□rnsson, 2006). Starch that is digested in the rumen provides volatile fatty acids (VFA), which is directly absorbed or also provides energy for microbial protein synthesis. The only VFA that, in the liver, contributes to gluconeogenesis is propionate (Huhtanen & Sveinbj□rnsson, 2006)

The aim of the study was to determine the dry matter digestibility of the different maize endosperm types with the use of the Ankom DAISY^{II} and to determine the disappearance of starch.

5.2 Materials and Methods

5.2.1 Sample preparation

Nine maize samples were used for the study. The maize differed from one another in terms of endosperm type as reflected by their milling index (MI). According to the MI, three of the nine samples were assumed to have a hard endosperm type with a MI that ranged between 109 and 118, three had a soft endosperm type, where MI ranged between 67 and 71, and the other three samples had a medium endosperm type, where MI ranged between 85 and 92.

Approximately 100 g of each sample were weighed out and ground in a hammer mill fitted with a 2 mm screen (Scientec RSA Hammer mill Ser Nr 372; Centrotec). After this, the samples were ground with a cyclone mill (Cyclotec 1093 Sample Mill; tecator) to pass through a 1- mm screen.

5.2.2 In vitro DM and starch disappearance

For *in vitro* DM and starch disappearance estimations, Dacron bags (5x10 cm Ankom R510 bags, supplied by Bar Diamond, Parma, Idaho) with a mean pore size of 53 µm were used, and the trial was conducted in an Ankom Daisy^{II} Incubator (Ankom Technology, Fairport, NY). First, the Dacron bags were marked with a permanent marker, then dried 24 hours in an oven set at 60°C, and weighed. Two grams of each maize sample were then carefully and accurately weighed into the bags, and the bags were sealed with an impulse sealer (KS-300 POWER 400W (Impulse)) and stored until use.

A buffered, rumen liquor inoculated medium was used for the in vitro incubations. The medium preparation was according to Van Soest and Robertson (1985) and was the same as for the gas production study described in Chapter 4.

Samples were incubated in an Ankom Daisy^{II} Incubator (Ankom Technology, Fairport, NY). The method used was based on that of Goering & Van Soest (1970). Six glass vessels, each with a capacity of 4 L were used for the incubations. Each vessel represented a specific incubation time and contained one bag of each

sample. The sealed bags with the sample were distributed evenly on both sides of the digestion glass vessel divider.

While gassing with CO_2, 1076 mL of incubation medium was added to each jar followed by the addition of 54 ml reducing solution. The vessels were tightly closed with lids fitted with one-way gas releasing valves and placed in the incubator. The heat and agitation switches were turned on and the temperature set at 39°C. Rumen liquor was collected from two cannulated cows, as described in Chapter 4. Once the medium was reduced, the vessels were taken from the incubator and 270 mL of rumen fluid was gently poured into each vessel while gassing with CO_2. The vessels were sealed again and incubation started. The temperature set at 39°C, for the duration of the incubation time.

Incubation times were 0, 2, 4, 8, 12, 24 and 48 hours. The 0 h incubation was done separately by gently washing the bags by hand in a basin filled with luke warm water. The glass vessels were removed from the incubator-sequentially at the designated time intervals. The bags were retrieved and gently washed in a basin filled with tap water. The procedure was repeated with clean water each time until the water remained clear. Bags were subsequently dried in a forced draught oven set at 60°C for 3 days (72 hours). After drying bags were placed in a desiccator for about 30 minutes, to cool and then weighed again.

Starch disappearance was measured using the residues of the 0, 2 and 4 hours incubations. These residues, as well as the original maize samples, were analyzed for starch content. Of each sample for starch analysis, 0.200 mg was weighed and transferred to 100 mL Erlenmeyer flasks, a blank were also included. The samples were then ready for the gelatinization and hydrolysis procedure to begin.

5.2.3 Estimation of DM digestibility

DM digestibility estimation were based on the dry matter disappearance of the samples from the incubated bags. Calculations were done in Microsoft Excel, using the Solver option and the non-linear Moldels 1 and 2 (with or without a lag phase, respectively) as according to Ørskov and McDonald (1979).

Model 1: $$Y = a + b(1 - e^{-ct})$$

Model 2: $$Y = a + b(1 - e^{-c(t-L)})$$

Where:
Y = dry matter disappearance at time t
a = soluble fraction (% of total)
b = potentially digestible fraction (% of total)
c = fractional rate of disappearance of b (% h^{-1})
t = time of incubation (hours)
L = lag time (hours)

The models were used in two different ways to determine the first derivatives. First, the a-values (0 h disappearance) were accepted as predicted by Solver, while in the second instance, the a-value were kept constant as they had been estimated form the 0 h washing procedure. The argument in the second instance was that the a-value is a function of the maize itself and it should thus not be affected by incubation environments.

5.2.4 Starch analysis

Preparation of sodium acetate buffer

The sodium acetate buffer was prepared according to Karkalas (1985). To prepare 1 L of the buffer, 13.61 g of sodium acetate trihydrate was first dissolved in approximately 40 mL of distilled water.

The pH of the solution was measured and carefully adjusted with 1N HCl to pH 4.5 to 4.6, while continuously stirring on a stirrer plate. (When pH is too acidic, 1N NaOH is used to raise it to the desired level). Upon reaching the desired pH distilled water was added to bring the solution to 1 L. Each time before using the solution, the pH was checked to assure a value of 4.5 to 4.6.

Preparation of glucose oxidase–peroxidase reagent

The Glucose oxidase–peroxidase reagent (Table 15) was also prepared according to the method described by Karkalas (1985).

Table 15 Preparation of the glucose oxidase–peroxidase used for starch analysis.

Reagent	Quantity
1 Litre solution:	
Sodium phosphate, dibasic anhydrous (Na_2HPO_4)	9.1 g
Potassium phosphate, monobasic (KH_2PO_4)	5.0 g
Phenol (C_6H_5OH), crystals	1.0 g
4–Aminoantipyrine ($C_{11}H_{13}N_3O$)	0.150 g
Glucose oxidase, Type II: From Aspergillus niger	7000 units
Sigma, G612 – 50KU 24800 units/g solid	0.2822 g
Peroxidase, Type I: From Horseradish	7000 units
Sigma, P8125 – 25KU 342.5 mg solid,	
73 Purpurogalin units/mg solids; 1.6 RZ	0.0958 g
Distilled water	Fill to 1 L

A 1 L volumetric flask was filled 1/3 with distilled water, followed by the addition of sodium phosphate and potassium phosphate. When the latter were dissolved, the phenol (which is light sensitive), was added and

gently mixed until dissolved. Lastly, glucose oxidase and peroxidise were added and dissolved by gentle mixing. The flask was then brought to volume (1 L) with distilled water, mixed and filtered through microfiber glass filter paper into amber bottles. The amber bottles with GOP (glucose oxidase-peroxidase) were stored at 4°C.

Glucose standard solution

The glucose standard solution was prepared as described by Karkalas (1985). An amount of 1.0 g of glucose was weighed out and transferred to a 100 mL volumetric flask. A little distilled water was added to dissolve the glucose completely and the flask was brought to volume by adding distilled water.

Five 100 mL flasks were used, one for each of the different dilutions. Aliquots of the glucose standard solution were then transferred to each of the flasks. Once the aliquots were transferred to each of the different dilution flasks, the 100 mL flasks were carefully and accurately brought to volume with distilled water.

Dilutions for the glucose standard solutions were as follows:
Flask 1: 0 µl/mL = distilled water = standard blank
Flask 2: 25 µl/mL = 0.250 mL stock solution/ 100mL dilution
Flask 3: 50 µl/mL = 0.500 mL stock solution/ 100mL dilution
Flask 4: 75 µl/mL = 0.750 mL stock solution/ 100mL dilution
Flask 5: 100 µl/mL = 1000 mL stock solution/ 100mL dilution

Starch analysis procedure

Erlenmeyer flasks (100 mL) containing 0.2 mg of sample, were placed on magnetic stirr plates. For heat gelatinisation, 20 mL distilled water were added to each flask and stirred with a magnetic stir bar. While stirring, 0.1 mL heat stable α-amylase (Sigma; α-amylase, heat stable, A3306 – 10 mL) were added to the water and sample solution. Small, square cut aluminium foil pieces were used to cap the flasks and the flasks were placed in a water bath with a temperature of 92°C and left for 1 h. The flasks were then removed and cooled at ambient temperature for 15 minutes. After gelatinisation, samples were filtered through funnels, which contained glass wool plugs, into 100 mL volumetric flasks. Filtered solutions were brought to volume with distilled water. A 2 mL aliquot of sample was pipetted into individual 50 mL volumetric flasks.

Of the sodium acetate buffer, 8 mL were added to each flask followed by adding 50 µL amyloglucosidase (Sigma; Amyloglucosidase from Aspergillus niger; A1602 – 100 mg; Suspension in 3.2M $(NH_4)_2SO_4$ solution). The flasks were gently swirled to mix the contents and then transferred to a pre-warmed 60°C water bath for 30 minutes. Flasks were gently swirled every 10 minutes, while in the water bath. When removed after 30 minutes from the water bath, distilled water was added to each flask to bring the flask to 50 mL.

After completion of the starch gelatinization and hydrolysis procedure, the glucose oxidase-peroxidase procedure for glucose analysis was begun. Here, 1.0 mL aliquots of the samples, were transferred from the 50 mL volumetric flasks, to glass test tubes. In addition, 1.0 mL of the standard solutions were also transferred to separate glass test tubes. Next, 5 mL of GOP (glucose oxidase-peroxidase) reagent were added into each tube. A vortex was then used to thoroughly mix the contents of the test tubes.

The tightly sealed glass tubes, placed into a rack, were subsequently placed into a 40℃ water bath for the duration of 45 minutes. The tubes were subsequently cooled to room temperature for 10 minutes in the absence of light, as the continuous exposure to light will influence the colour of the GOP and subsequently the reading obtained from the spectrophotometer. The contents of the tubes were poured into cuvettes (Cuvette Micro PS, 10 x 45 mm, 2 mL; Lasec) which were then taken to the spectrophotometer (Cecil CE 2021 2000 Series Lasec SA (Pty) Ltd) and the absorbance of each sample was measured at λ = 505 nm (nanometer).

Standard curve calculation

To calculate the glucose concentrations, the following calculations were used (Karkalas, 1985).

$$\text{Glucose stock } {}^{\mu g}/_{mL} = \frac{\left[(\text{Glucose gram}) \times (\text{DM\% of Glucose}) \times \left(1\,000\,000\,\frac{\mu g}{g}\right)\right]}{100\text{ mL}}$$

$$\text{Glucose } {}^{\mu g}/_{mL} = \frac{\left[\left(\text{Glucose stock solution}, {}^{\mu g}/_{mL}\right) \times Va\right]}{Vs}$$

Va = aliquot volume of stock solution (0, 0.25, 0.50, 0.75)
Vs = the final dilution volume that the Va is diluted into

5.3 Statistical analyses

For DM disappearance, the non-linear models mentioned earlier were used to derive potential soluble and rapidly degradable values (a), potentially degradable fractions (b) and lag times (L). These parameters, as well as starch disappearance values at 0, 2 and 4h, were then subjected to a main effects ANOVA with the aid of Statistica, version 9 (2009). Main effects were treatment and repetition. Treatment means were separated with Scheffé tests. Furthermore, data were subjected to Pearson correlations to determine regressions of RMI on DM and starch disappearance, and of particcle size on DM disappearance. A variety of significant correlations were observed, but only those that might have practical application value were further investigated. The Scatterplot procedure of Statistica 9 was used to determine the regressions. In all instances, significance was declared at P≤0.05.

5.4 Results and Discussion

As mentioned earlier, the models used to estimate DM disappearance parameters were applied in two ways. In the first instance, the a-values were estimated by the model, while in the second instance, the pre-determined a-value was used as a constant in the model. Table 16 presents that results where the a-value was predicted (Model 2), while Table 17 (Model 1) and Table 18 (Model 2) presents the results where the pre-determined a-value was used.

From Table 16 it is clear that the model predicted a-values differed between maize samples. On average, maize types with a high MI value had a higher proportion of soluble fraction (a) compared to the low MI types. The mean a-value of the high MI types was 49.3 % which was higher (P=0.039) than that of the medium MI types (46.8%) and low MI types (46.4%).

Maize samples also differed in terms of the potentially digestible (b). It appeared as if the lower MI types (Samples 7, 8 and 9), which are believed to contain more of a soft endosperm, had higher amounts of the potentially digestible fraction than Samples 1, 2 and 3, that are of the high MI type. When the types were grouped, a statistical analysis revealed that the mean b-value of Samples 1, 2 and 3 (53.1%) were indeed lower ($P < 0.005$) than that of the medium MI types (55.2%) and the low MI types (55.9%). It is hypothesized that, because maize types with a hard endosperm, it is more difficult for enzymes to penetrate the protein matrix, thus digestion is retarded (Corona *et al.*, 2006).

Although the rate of digestion differed between maize samples, it did not appear to follow a clear pattern in terms of maize type. A grouped statistical analysis indicate no difference in apparent digestion rate between types. Results in Table 16 indicate that maize samples differed in terms of the lag phase. Some of the lag values are, however, unrealistically high for maize and lag results obtained from the alternative application of the model will instead be discussed.

Table 16 Non-linear parameters obtained when the a-values were predicted by the model.

	Maize samples										
	High MI			Medium MI			Low MI				
Item	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**SEm**	**P**
Model 2[1]											
a	49.7^{ab}	52.3^{a}	46.01^{b}	48.8^{ab}	44.0^{ab}	47.5^{ab}	48.9^{ab}	45.4^{ab}	44.9^{b}	1.16	0.0003
a (for type)	49.3^{a}			46.8^{b}			46.4^{b}			0.86	0.0390
b	53.9^{ab}	54.0^{ab}	51.4^{a}	56.2^{ab}	54.2^{ab}	55.2^{ab}	55.5^{ab}	55.8^{ab}	56.3^{b}	0.97	0.0290
c	0.04^{ab}	0.04^{a}	0.06^{c}	0.05^{bde}	0.06^{c}	0.05^{bc}	0.04^{ad}	0.05^{bcd}	0.06^{ce}	0.003	0.0000
L	2.3^{a}	1.8^{abc}	0.4^{b}	4.3^{d}	2.1^{abc}	4.4^{cd}	3.4^{acd}	4.2^{acd}	4.7^{d}	0.49	0.0000

[1]Model 2: $Y = a + b(1 - e^{-c(t-L)})$, where a = soluble fraction (%); b = potentially digestible fraction (%); c = rate at which b is digested (%/h); L = lag time (h).

[a, b, c, d, e] Values with different superscripts within rows differed significantly (P<0.05).

SEm = Standard error of the mean; P = Significance level.

Because a-values were kept constant within maize samples for the different *in vitro* runs, there was no variation within sample and the data were omitted from the statistical analysis. However, when pooled per type, it became evident that the high MI type of maize had higher a-values, as was also observed in the first application of the model. The mean a-value of the high MI types was 49.3%, which was higher (P=0.039) than that of the medium MI types (46.8%) and the low MI type (46.4%). It thus appears that the higher the MI, i.e. the higher the vitreousness, the more soluble the maize was. This phenomenon is not easy to explain, but it is speculated that the ratios of amylopectin to amylose may differ among types and that it could affect solubility.

The potentially digestible endosperm (fraction b) differed among maize samples. When data were pooled for endosperm type it was found that the low MI value maize types had a higher proportion of fraction b than the maize types with the high MI value. The effect appeared to be somewhat more magnified in Model 1 compared to Model 2. Although significant differences were observed among maize samples in terms of

digestion rate, no clear pattern was observed. When data were pooled, there were no differences in digestion rate among high MI and low MI maize types.

The lag time (Model 2), differed among the different maize samples. Maize type did not, however, appear to have a clear effect on lag time in the current study. It seems that there are a correlation between maize MI value and its slowly digestible fraction for Model 1 and 2.

In a study done by Philipeau *et al.* (1999), they found that maize texture affected *in vitro* dry matter degradation. Dent types had a higher proportion of rapidly degradable fraction (a) when compared to flint types. Flint types on the other hand had a higher percentage of slowly degradable fraction (b) when compared to dent types (Philipeau *et al.,* 1999). It has been reported that there is a strong negative correlation between maize endosperm type and starch degradability or dry matter degradability. Thus as the vitreousness of the maize endosperm increases the dry matter and starch degradability would decrease (Philippeau & Michalet-Doreau, 1997; Correa *et al*., 2002; Ngonyamo-Majee, *et al*., 2008; Hoffman & Shaver, 2009). In the current study, all the maize samples were from the dent type and the difference in vitreousness among samples were not as high as between dent and flint corn, for example.

Table 17 Non-linear parameters obtained when pre-determined a-values were used as constants in the model (Model 1).

	Maize samples[1]										
	High MI			Medium MI			Low MI				
Item	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**SEm**	**P**
Model 1[2]											
a (for type)		49.2^{a}			39.3^{b}			37.8^{b}		1.249	<0.001
b	61.4^{ab}	63.7^{ab}	51.7^{a}	69.6^{b}	59.9^{ab}	69.4^{b}	70.6^{b}	69.3^{b}	72.8^{b}	2.790	0.00012
b (for type)		58.9^{a}			66.3^{b}			70.9^{b}		1.873	0.0003
c	0.03^{ab}	0.03^{ac}	0.05^{d}	0.04^{be}	0.05^{d}	0.05^{de}	0.02^{c}	0.04^{bd}	0.05^{d}	0.003	0.0000
c (for type)		0.038			0.044			0.042		0.003	0.4070

[1]Maize samples 1 to 3 had a relatively high Roff Milling Index (MI), samples 4 to 6 had a medium MI and samples 7 to 9 had a relatively low MI.

[2]Model 1: $Y = a + b(1 - e^{-ct})$, where a = soluble fraction (%); b = potentially digestible fraction (%); c = rate at which b is digested (%/h).

[a, b, c, d, e] Values with different superscripts within rows differed significantly (P<0.05).

SEm = Standard error of the mean; P = Significance level.

Table 18 Non-linear parameters obtained when pre-determined a-values were used as constants in the model (Model 2).

	Maize samples[1]										
	High MI			Medium MI			Low MI				
Item	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**SEm**	**P**
Model 2[2]											
a (for type)		49.2^{a}			39.3^{b}			37.8^{b}		1.249	<0.001
b	55.3^{a}	54.5^{ab}	50.1^{b}	64.4^{c}	56.9^{abd}	66.7^{ce}	57.2^{a}	64.9^{cde}	71.1^{e}	1.72	<0.001
b (for type)		53.3^{a}			62.7^{b}			64.4^{b}		1.510	0.00001
c	0.04^{ab}	0.04^{a}	0.06^{c}	0.05^{bde}	0.06^{c}	0.05^{bc}	0.04^{ad}	0.05^{bc}	0.06^{ce}	0.003	<0.001
c (for type)		0.047			0.53			0.52		0.003	0.230
L	1.7^{a}	1.6^{ab}	0.8^{bc}	1.5^{ad}	1.2^{ab}	0.7^{bcd}	2.7^{e}	1.2^{abc}	0.7^{c}	0.18	<0.001
L (for type)		1.33			1.15			1.54		0.186	0.331

[1]Maize samples 1 to 3 had a relatively high Roff Milling Index (MI), samples 4 to 6 had a medium MI and samples 7 to 9 had a relatively low MI.

[2]Model 2: $Y = a + b(1 - e^{-c(t-L)})$, where a = soluble fraction (%); b = potentially digestible fraction (%); c = rate at which b is digested (%/h); L = lag time (h).

$^{a, b, c, d, e}$ Values with different superscripts within rows differed significantly (P<0.05).

SEm = Standard error of the mean; P = Significance level.

DM disappearance of the different maize samples where the a-values were estimated by the model is presented in Figures 8 and 9. Data derived from Model 2 were used to construct Figure 8 (all maize samples) and Figure 9 (three MI types).

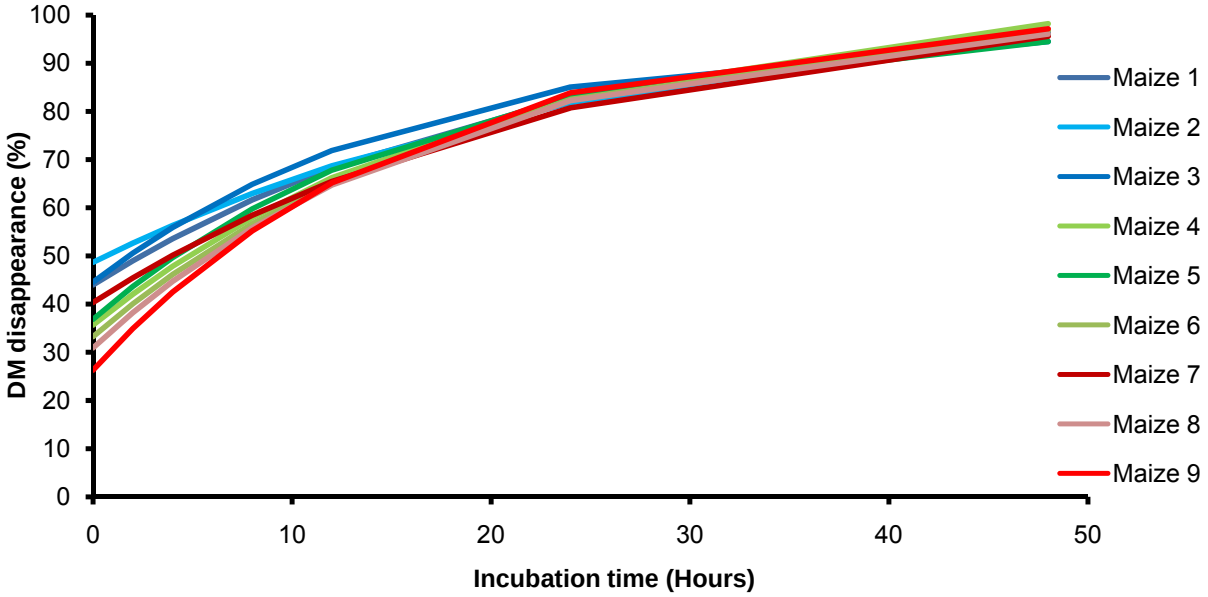

Figure 8 DM disappearance of all the maize samples where the a-values were predicted by the model.

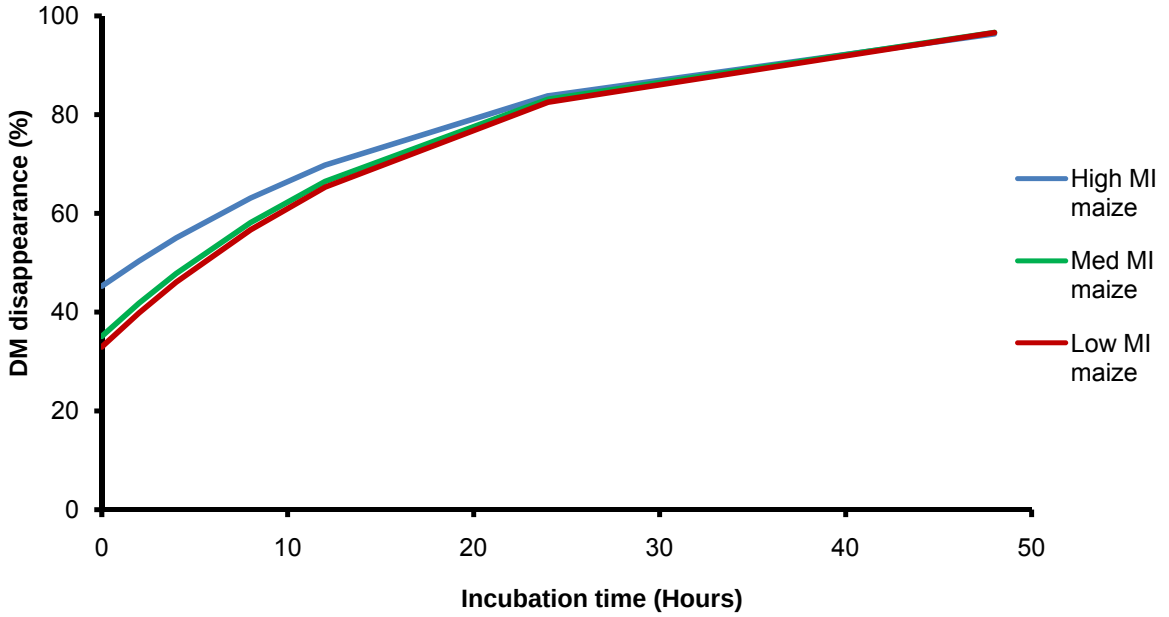

Figure 9 DM disappearance of the three Milling Index types where the a-values were predicted by the model.

Dry matter disappearance of the different maize samples where pre-determined a-values were used as a constant in the model is also presented in Figures 10 and 11. Data derived from Model 2 were used to

construct Figure 10 (all maize samples) and Figure 11 (three MI types). The higher a-value in the high MI types is apparent in Figure 11.

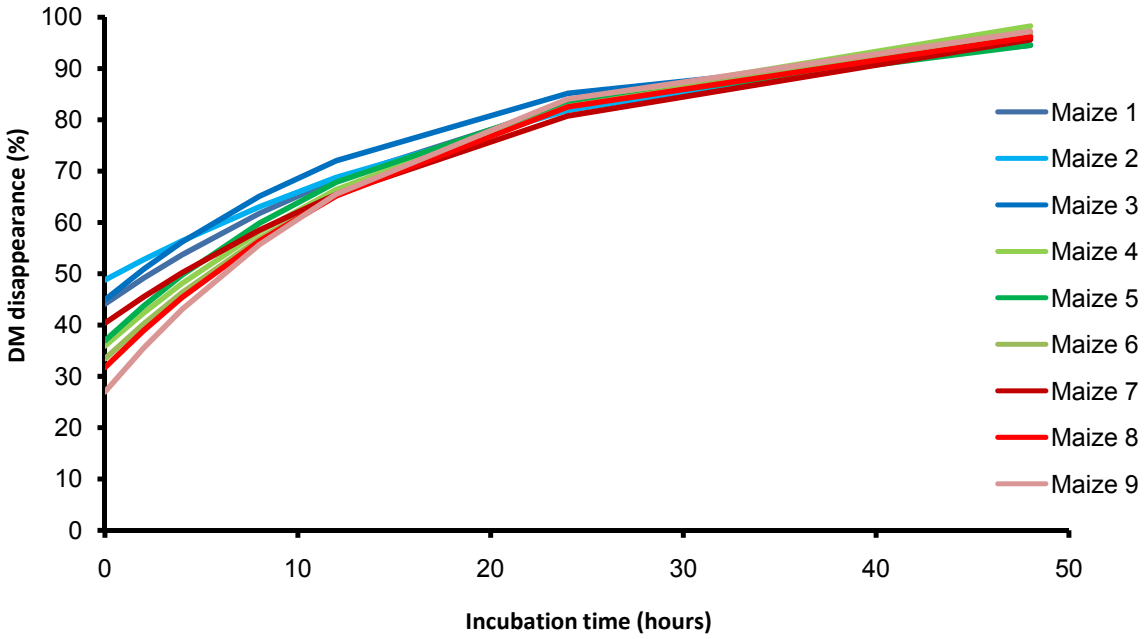

Figure 10 DM disappearance of the different maize samples where pre-determined a-values were used as a constant in the model (Model2).

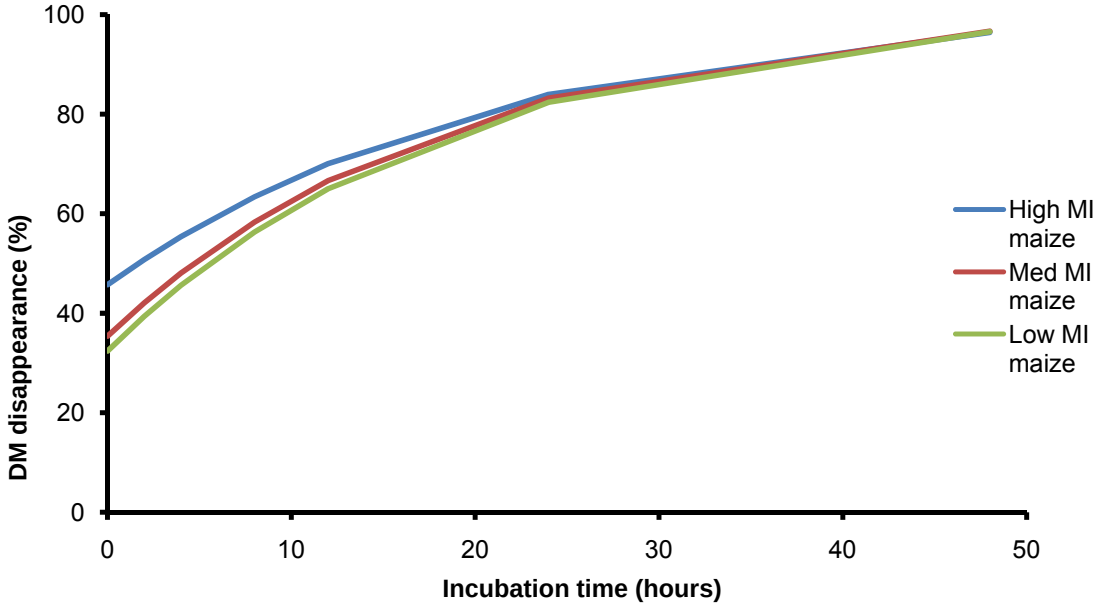

Figure 11 DM disappearance of the three MI types where pre-determined a-values were used as a constant in the model (Model 2).

Figure 12 and 13, represents the starch disappearance off all the samples over time after incubation in rumen fluid. It shows that, as the incubation time increased the percentage of starch disappearance also increased. When the disappearance of starch is compared between the different endosperm types, it appears as if the hard types had a higher percentage of starch disappearance when incubated for 4 hours than the soft types.

In a study by Philippeau *et al*. (1999), the rapidly degradable fraction of starch was slightly higher for dent corn than for flint corn types (26.6 vs. 19.4 %). Alternatively the flint types had a higher percentage of slowly degradable fraction of starch than dent types (80.6 vs. 73.4 %). They found that starch degradability was lower for flint than for dent maize types, averaging 46.2 and 61.9 % respectively (Philippeau *et al*., 1999). Ezeogu *et al*. (2005), found that starch digestibility is influenced by maize vitreousness and that as maize vitreousness increases the digestion of starch decreases. This depression in the digestion of starch can be due to the limitation of *α*-amylase to access the starch of the vitreous maize, because of the more complex protein matrix found in vitreous types (Ezeogu *et al*., 2005).

Amylopectin in starch is more fermentable than amylose. The starch that is present as amylose in maize typically ranges between 24-30%. The amylose content is usually 4 to 9 percentage units higher in floury starch than in vitreous starch (Owens & Zinn, 2005). An increase in maturity will also increase the amylopectin to amylose ratio, but the ratio will decrease as the environmental temperature increase (Owens & Zinn, 2005).

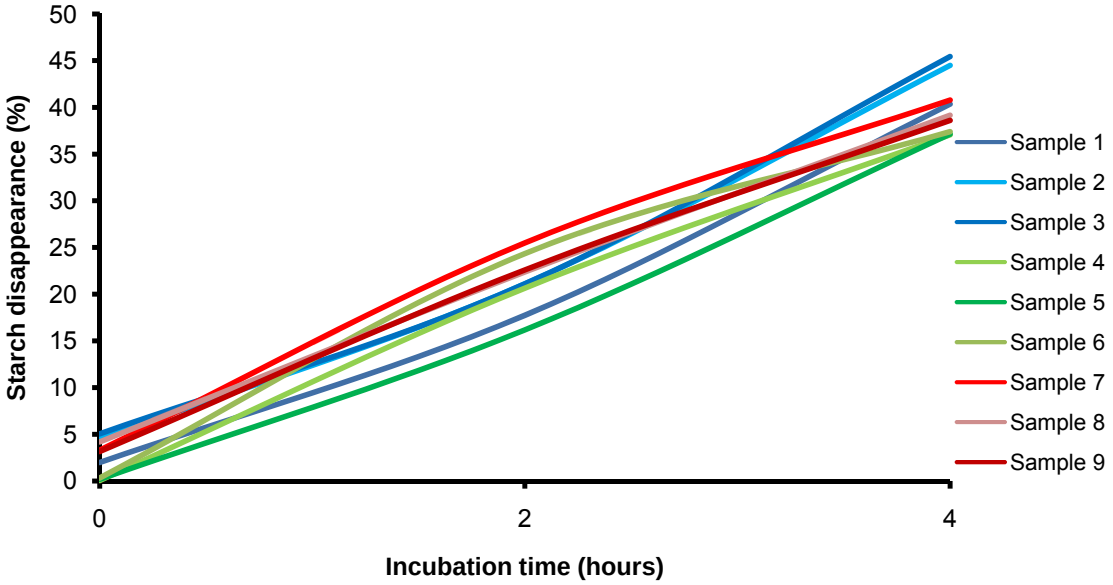

Figure 12 Starch disappearance over time.

Due to the high cost of starch analysis and the limited post-incubation residues, starch analysis were done on all the original maize samples and on the *in vitro* residues of one of the five *in vitro* runs. Starch

disappearance data were thus not analyzed statistically. The graphs were constructed by using the mean values of duplicate analyses. Based on these values, it appears as if the starch in the three low MI maize types disappeared more rapidly over the first two hours than that of the other maize samples, but that the rate tended to decrease towards 4 h of incubation. When data of all the maize samples were pooled together, it can be seen in Figure 13 that just over 20% of the starch had disappeared by 2 h, while 40% of the starch was digested by 4 h of incubation.

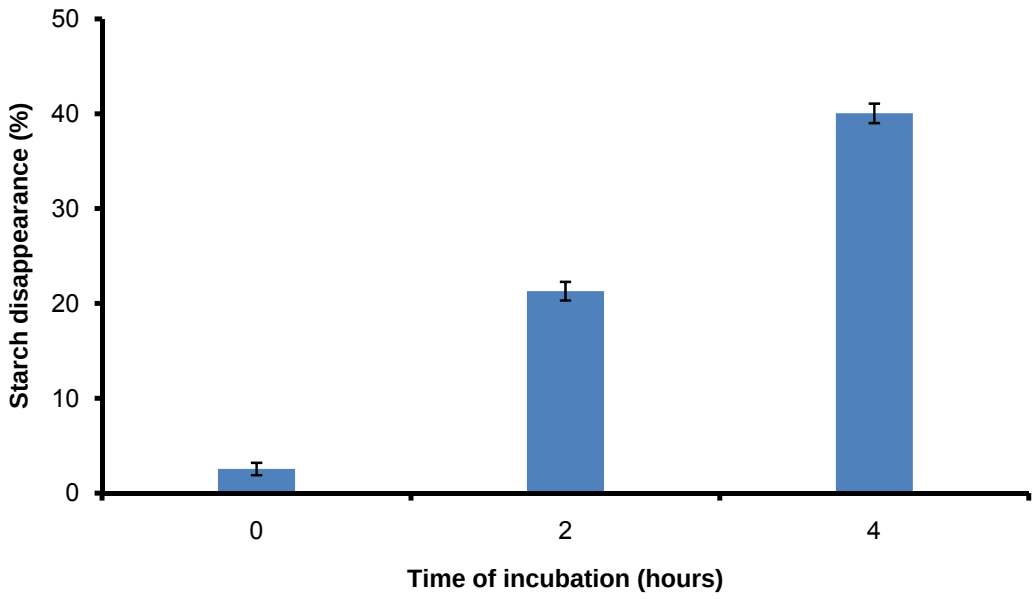

Figure 13 Starch disappearance over time, all samples.

Figure 14, shows the starch content of the maize samples before and after incubation (the latter on residues of one *in vitro* run only. The starch content was higher in the 0 h residue than in the original maize samples, suggested that most of the soluble non-starch contents of the samples had been washed out. In most cases (except for Samples 2 and 3), the starch content of the residues were higher after 4 h of incubation than in the original samples, which would mean that the other nutrients probably digested faster in the first four hours than starch. Regarding Samples 2 and 3 that were of the high MI types, the starch appeared to digest more rapidly that the others, which is in agreement with Figure 13.

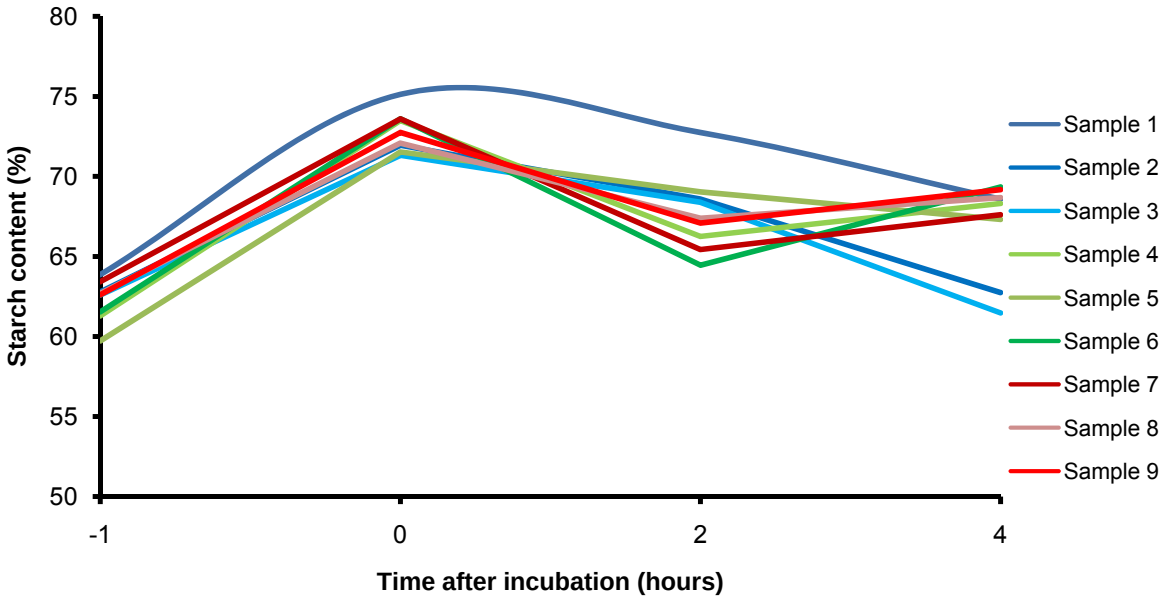

Figure 14 Starch content of maize samples before and after incubation.

The effect that starch granule size and type have on starch digestion, degradability and performance of the dairy cow is still not very well defined (Hoffman & Shaver, 2009). Akay and Jackson (2001), reported that when amylopectin (maize starch) rather than amylose (maize starch) was fed to dairy cows they observed an increase in starch digestibility as well as in the cows lactation performance. Alternatively Foley *et al*. (2006), reported that when barley amylopectin were fed rather than barley amylose, starch digestibility in lactating dairy cows was lower.

Correlation coefficients

Data pertaining to the Roff Milling Index, starch content, sieve fractions, gas production parameters, DM disappearance values and starch disappearance were subjected to Pearson correlations to determine possible relationships between the mentioned parameters. A variety of significant correlations were observed, but only those that might have practical application value were further investigated. The Scatterplot procedure of Statistica 9 was used to determine the regressions indicated in Table 19.

Table 19 Correlations and possible relationships between Roff Milling Index, starch content, sieve fractions, DM disappearance values and starch disappearance.

Independent variable X	Dependant variable Y	Regression equation	r	r^2	P
Roff Milling Index	DM disappearance[2]:				
	b1	Y = 60.21 – 0.061X	-0.740	0.548	0.023
	L1	Y = 8.355 - 0.059X	-0.740	0.548	0.023
	b2	Y = 88.21 – 0.254X	-0.702	0.493	0.035
	b3	Y = 83.07 – 0.255X	-6.696	0.484	0.037
	Starch disappearance:				
	0h	Y = -10.08 + 0.387X	0.727	0.528	0.027
	2h	Y = 13.46 + 0.208X	0.792	0.628	0.011
	4h	Y = -0.87 + 0.385X	0.809	0.655	0.008
Particles >106µm	DM disappearance[2]:				
	a1	Y = 55.55 – 1.206X	-0.724	0.525	0.027
	c1	Y = 0.025 + 0.0037X	0.683	0.466	0.044
	c3	Y = 0.025 + 0.0037X	0.683	0.466	0.044

[2]The non-linear DM disappearance values followed by a "1" were derived from a model which predicted the a-value and where a lag phase was included. Where the parameter is followed by a "2", the a-value was kept constant and included in the model as it was actually determined at 0 hours and a lag phase was omitted. Where the parameter is followed by a "3", the a-value was also kept constant, but a lag phase was included in the model.

As can be seen from Table 19, The Roff Milling Index (RMI) was significantly correlated with a variety of measurements. All the DM disappearance parameters were negatively correlated with RMI. This would appear to be as one would expect, except for lag time (L1). It should be kept in mind, however, that although the regressions are significant, they explain less than 55% of the variation.

The most significant and probably the most usable RMI regressions were against starch disappearance after 2 and 4 h of incubation. In these cases, RMI explained > 60% of the variation, which is still not very much, but it would at least warrant further research on the matter as the number of samples used and the *in vitro* incubation repetitions in the current study were somewhat limited.

5.5 Conclusion

Maize samples for this study were selected in terms of their Roff Milling Index (MI) to get a variety of endosperm types. *In vitro* DM digestibility parameters differed among maize samples and maize of the high MI types appeared to have a higher soluble fraction (a-value) than maize of the medium and low MI types. On the other hand, the high MI types had a lower potentially digestible fraction (b-value) than the medium and low MI types. The Roff Milling Index *per se*, however, did not appear to be a reliable predictor of digestibility parameters. The best correlation between MI and other measured parameters pertained to starch disappearance, where MI explained more than 60 and 65% of the variation of starch disappearance. Particle size of milled samples (<125>106 µm) was positively correlated with the rate of DM digestibility, although it only explained 47% of the variation observed in digestion rate. More research is needed to accurately describe and predict maize DM and starch digestion kinetics.

5.6 References

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Chapter 6

General Conclusion

Maize samples for the current study were selected in terms of their Roff Milling Index (MI) to get a variety of endosperm types. Several characteristics of the maize samples that were ground and sieved were correlated to their Roff Milling Index. Chemical analyses were also done on the different maize types. There were no changes in DM, ash, fibre, fat, protein and starch content as the MI value of maize increased or decreased. No correlations were observed between maize MI values and the chemical composition of the maize or particle size separation. From this study, it was concluded that the Roff Milling Index appear to be a relatively poor indicator of particle size distribution and chemical composition of maize.

In the second part of the study, the gas production potential of the different maize types in buffered rumen liquor was measured and the results correlated with the Roff MI of the different maize types. The results showed that gas production parameters differed among maize samples and maize samples of the high MI types appeared to have a lower gas production (b-value) than maize types with a medium and low MI types. It was concluded that the Roff Milling Index cannot be used as a reliable indicator for total gas production or rate of gas production of different maize types. Particle size of ground samples was positively correlated with the rate of gas production and it explained 72% and 80% of the variation observed in gas production rate of the >106 + <106 µm fraction and the <106 µm, respectively.

In the third part of the study, *in vitro* DM degradability and starch disappearance of the different maize types were measured. Results showed that *in vitro* DM digestibility parameters differed between the maize types. Also, maize types of the high MI types appeared to have a higher soluble fraction (a-value) than maize of the medium and low MI types. In addition, the maize types with high MI values had a lower potentially digestible fraction (b-value) than the maize types with medium and low MI values. The results concluded that the Roff Milling Index, however, did not appear to be a reliable predictor of digestibility parameters. The best correlation between MI and other measured parameters pertained to starch disappearance, where MI explained more than 60% and 65% of the variation of starch disappearance. Particle size of ground samples (<125 and >106 µm) was positively correlated with the rate of DM digestibility, although it only explained 47% of the variation observed in digestion rate.

In the future, more research is needed where different maize types are compared to one another in terms of their vitreousness, gas production, starch content, starch disappearance and DM digestibility. Maize can make up a high percentage of a formulated animal feed. The type of maize used is important to formulate a good quality feed. Including maize from the right type, in terms of starch content and vitreousness, in the diet of dairy cows, may increase milk production and in turn increase the profit of the farm.