# Chemical and physical factors affecting starch digestibility *in vitro* and interactions with fibre.

by

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DECLARATION

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# Chemical and physical factors affecting starch digestibility *in vitro* and interactions with fibre.

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## ABSTRACT

Maize is a valuable and expensive resource in the dairy industry. It is routinely used in ruminants' diets as an energy concentrate to ensure that the high energy demands of top performing animals are met. The purpose of this study was to investigate chemical and physical factors affecting starch digestibility *in vitro* as well as possible interactions with fibre digestibility. Milling of grains is considered to have a great impact on the rate and extent of starch digestion, however differences in milling conditions lead to variation in particle and often an inconsistent product. In our first experiment hard and soft maize produced a NGMPS of 274.58 ± 0.87 and 470.91 ± 0.87 respectively when milled at 3mm; and a NGMPS of 396.64 ± 0.87 and 576.66 ± 0.87 respectively. There was significant interaction between the type of maize and screen size used. In the second experiment ground maize was divided into five different fractions and combined with a forage (lucerne or oat hay) to create combinations of either high or low starch-to-neutral detergent fibre (NDF) ratios. The chemical constituents were determined for the different maize fractions as well as the forages. Subsequently the individual ingredients as well as the combinations were analysed for 24 hour *in vitro* starch digestibility, rate of starch digestion, 48 hour *in vitro* NDF digestion (NDFd), and rate of NDF digestion ($K_{NDF}$). Starch digestibility for the maize fractions Very fine, Fine, Medium, Coarse, and Cracked maize were 64.33, 62.28, 59.84, 47.58, and 42.15%, respectively, and rate of starch digestion was 18.24, 13.48, 10.02, 7.16, and 3.77 %/h, respectively, when pooled for forages and starch-to-NDF ratio. Fibre digestion was influenced by particle size, starch level

and forage, resulting in NDF digestibility being the highest when combined with coarse or cracked maize, 43.15% and 44.15% respectively, and lowest with fine maize, 32.99%. The rate of NDFd for oat hay and lucerne was 3.11 and 5.11 %/h, respectively and it was influenced by particle size, with very fine maize reducing the rate. In our second experiment, we investigated how different proportions of starch type (amylose/amylopectin) impact the rumen digestion of grains. Hylon VII (74% amylose starch) and Amioca (98% amylopectin starch) were combined with forages (lucerne or oat hay) in order to create combinations of either high or low starch-to-NDF ratios. The chemical constituents of Amioca, Hylon, oat hay and lucerne were determined. Consequently, the individual ingredients as well as the combinations were analysed for 24 hour *in vitro* starch digestibility, rate of starch digestion, 48 hour *in vitro* NDF digestion, and rate of NDF digestion. Amioca had the greatest starch digestibility and the addition of forages increased starch digestion. Rate of starch digestion was 12.55 %/h and 6.13 %/h for Amioca and Hylon respectively and the rate was influenced forage type, but not by starch level. The $K_{NDF}$ was 7.35%/h for lucerne and 3.87%/h for oat hay (when pooled for starch type and starch level). The rate of NDFd for oat hay was, 3.15 %/h when combined with Amioca and 3.30 %/h with Hylon, but the difference between the control and the starch types was not significant. For lucerne the rate of NDF digestion was reduced by the addition of starch, 7.07 %/h when combined with Amioca and 5.88 %/h with Hylon. Enhanced characterization of grains', with regards to particle sizes and starch type, has the potential to better describe a specific feed's starch digestibility, the possible interactions with cell wall digestion and to more effectively satisfy the nutritional requirements of animals in different physiological stages.

## Notes

The language and style used in this thesis are in accordance with the requirements of the Journal of Dairy Science. This thesis represents a compilation of manuscripts, where each chapter is an individual entity and some repetition between chapters is therefore unavoidable.

Die style en taal gebruik in hierdie tesis is volgens die vereistes van die "Journal of Dairy Science". Hierdie thesis is 'n samevatting van manuskripte, waar elke hoofstuk as 'n enkele entiteid bestaan, en dus is herhaling van inligting tussen hoofstukke is onvermydelik.

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# LIST OF ABBREVIATIONS

ADF – Acid detergent fibre

ADL – Acid detergent Lignin

AOAC Association of Official Analytical Chemist

ARA – acute rumen acidosis

CP-Crude protein

CF- Crude fat

DM – Dry matter

DMI – Dry matter intake

EE - Ether extract

FA – Fatty acids

GMPS - Geometric mean particle size

HOT – Hepatic oxidation theory

iNDF – Indigestible NDF

ivNDFd – *In vitro* neutral detergent fibre digestibility

ivSd – *in vitro* Starch digestibility

$K_d$ – Rate of digestion (1/h)

$K_s$ – Rate of starch digestion (1/h)

$K_{NDF}$ – Rate of NDF digestion (1/h)

ME – Metabolisable energy

NDF – Neutral detergent fibre

NGMPS - Nominal geometric mean particle size

NIR - Near-inferred reflectance

NSC – Non-structural carbohydrates

OM – Organic matter

peNDF – Physically effective NDF

RDS – Rumen degradable starch

RRS – Rumen resistant starch

RVA - Rapid visco analyser

SARA – Sub-acute rumen acidosis

VFA – Volatile fatty acids

# CHAPTER 1

## Introduction

Maize is a valuable and expensive resource in the dairy industry. It is routinely used in ruminants' diets as an energy concentrate to ensure that the energy demands of high performance animals are met, especially during lactation. During 2016 maize prices reached a record high and due to droughts in 2015 and 2016, the milk to feed price ratio in South Africa is now at the lowest since 2007 (Bureau for Food and Agricultural Policy - BFAP, 2016). Compared to the beef industry, the beef to maize price ratio has been able to remain relatively stable due to increased exports. For the dairy industry, the importance of having a diet that is accurately formulated and fine-tuned to stage of lactation has never been more evident.

Differences in the digestibility of grains are often attributed to differences in nutritional value, genetics, variety, geographical locations, year, climatic conditions and agronomic practices (Huntington, 1997; Offner *et al.*, 2003). Among the various factors particle size of milled grains and type of starch (i.e. amylose or amylopectin) contained within the starch granules of the endosperm of grains are recognized as having a major influence on digestibility (Huntington *et al*., 2006).

In South Africa, feed companies such as Meadow feeds (Roodepoort, South Africa) and Afgri (Centurion, South Africa) standardly mill grains at the theoretical size of either 2 or 4 mm (B. van Zyl and P. Henning, personal communications). Various factors can influence the resulting particle size distribution during milling, such as type of grain and endosperm type (hard or soft; Greffeuille *et al.*, 2006). This may lead to a variable distribution in particle size and an inconsistent product.

With regards to experimental procedure, when preparing samples to be analysed it is routine practice to mill all feed ingredients at the same theoretical size. It is then assumed that

any difference in digestibility is due to treatment effect or intrinsic characteristics of the sample. However, there exists considerable differences in the starch digestibility between whole, cracked, ground and finely ground maize. Thus, if different grains react differently to milling, resulting in different particle size distributions, and it is known that particle sizes interact with digestion, it is possible that differences seen in digestibility within and amongst various studies could in part be due to size differences and respective digestibility. The difference between two kinds of maize may therefore be augmented by milling, with higher quality maize resulting in finer and more digestible particles and vice-versa for lower quality maize, assuming soft maize being of higher quality than hard maize (Almeida-Dominguez *et al.*, 1997).

Therefore, in order to better define the effect of particle size on the digestibility of maize, ground maize was divided into five different fractions based on particle size, very fine (<250µm), fine (250-500 µm), medium (500-1180 µm), coarse (1180-2000 µm), and cracked (2000-3350 µm). The different fractions were analysed for chemical composition, and 24-hour *in vitro* starch digestibility and rate of starch digestibility. Furthermore, the effect of particle size and starch digestibility on neutral detergent fibre (NDF) digestibility was examined by combining maize with forage (lucerne or oat hay) in order to create starch-to-NDF ratio of either high starch or low starch. The combinations were then analysed for 24-hour *in vitro* starch digestibility and rate of starch digestibility, as well as 48-hour *in vitro* NDF digestibility and rate of NDF digestibility.

The ratio of amylose to amylopectin has been proven to influence the digestibility of grains (Sajilata *et al.*, 2006). When ground grains with different amylose content were compared, the *in vitro* rumen digestibility increased as amylose content decreased (Stevnebø *et al.*, 2006). The reason that amylopectin is more readily digested then amylose is because amylose has tighter intermolecular bonding between starch molecules (Buléon *et al.*, 1998a).

This leads to a more compacted structure of the starch granules in the endosperm. Therefore, grains with greater proportions of amylopectin have greater rumen starch and total tract starch digestion.

However, the results from previous studies may be confounded by other factors such as chemical composition and particle size of grains. The direct effect of amylose and amylopectin on starch digestion thus needs clarification.

Thus, in order to determine the direct effect that amylose-to-amylopectin ratio has on digestibility, high amylose (Hylon) and high amylopectin (Amioca) starch were analysed for chemical composition, and 24-hour *in vitro* starch digestibility and rate of starch digestibility. Furthermore, it is known that starch digestion negatively affects fibre digestion (Grant and Mertens, 1992; Oba and Allen, 2003). However, the majority of research does not distinguish between amylose and amylopectin starch on fibre digestion. Therefore, the effect of starch type and starch digestibility on NDF digestibility was examined by combining starch with forages (lucerne or oat hay) in order to create starch-to-NDF ratio of either high starch or low starch. The combinations were then analysed for 24-hour *in vitro* starch digestibility and rate of starch digestibility, as well as 48-hour *in vitro* NDF digestibility and rate of NDF digestibility.

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# CHAPTER 2

## Literature Review: Starch characteristics affecting ruminal and post-ruminal digestion in dairy cows and interactions with fibre digestion: a review

### 2.1 Introduction

Traditionally, the main objective in feeding dairy cows has been to maximise dry matter intake (DMI), in order to increase milk production. Many factors play a role in influencing DMI, such as feeding management, feed quality, palatability, fibre content, feeding conditions, and environmental climate, to name a few.

The methods whereby feed intake is regulated in ruminants can be divided into two categories: physical (gut distention and fill) and chemical (nutrients, metabolites, and hormones stimulating or suppressing appetite). It is theorised that gut distention is the primary factor regulating feed intake when ruminants consume a low-energy diet or when energy requirements are high, but when excess energy is consumed chemostatic factors regulate feed intake (Allen, 2014). Therefore, in dry cows that typically consume a low-energy dense diet a few weeks before calving, feed intake will be limited by gut distention. However, as dairy cows are moved onto the more energy dense diets typically supplied during the transition phase (previously known as "steam-up diets") feed intake is regulated by various chemical and metabolic factors.

One of these chemostatic factors is the hepatic oxidation of fuels. In a review by Allen e*t al.* (2009) the hepatic oxidation theory (HOT) is comprehensively discussed. According to the theory, feed intake would be controlled by a signal from the liver to the brain, in response to the oxidation of fuels in the liver. Fermentation in the rumen results in volatile fatty acids (VFA; mainly propionate, butyrate, and acetate) which are metabolised by the liver and thus

can cause appetite suppression. Furthermore, it was found that propionate is more hypophagic than butyrate and acetate (Anil and Forbes, 1980).

Recently, there has been interest in the ratio of starch digested in the rumen vs. post-ruminally, and how this influences dry matter intake (DMI) (Reynolds, 2006). The digestion of starch in the rumen favours the production of propionate and, as discussed previously, hepatic oxidation of propionate suppresses DMI. During the transition phase, as cows are switched to a more energy dense diet, cows become more sensitive to the effect of propionate metabolism in terms of satiety (Allen e*t al.*, 2009). Excessive starch digestion in the rumen can therefore be detrimental to maintain a positive energy balance during this phase. Theoretically, if the major site of starch digestion were to be shifted to the small intestine it would reduce the production of propionate to some extent, thereby preventing any loss of appetite while maintaining a positive energy balance during early lactation. Also, altering the degradability of starch would be more desirable than replacing starch with fibre for animals with high energy demands (Allen e*t al.*, 2009). It is important to note that starch infusions into the abomasum have no effect on DMI (Knowlton e*t al.*, 1998a; Reynolds e*t al.*, 2001a). This is in agreement with Allen e*t al.* (2009) who stated that it is starch digestion in the rumen that depresses DMI, not starch supply to the small intestine. Starch digestion in the small intestine produces glucose that can be oxidised in the liver and in accordance with HOT would cause intake levels to drop. However, in reality ruminants hardly oxidised any glucose in the liver. Most of the glucose entering the lower digestive tract is oxidised by the enterocytes and a satiety signal from the gut wall is unlikely (Allen e*t al.*, 2009).

Increasing the supply of starch post-ruminally has been investigated for its potential to increase milk production. Milk production is dependent on glucose supply to the mammary gland (Nocek and Tamminga, 1991b). Increasing the supply of glucose to the mammary gland

could be achieved by either increasing the supply of glucogenic substrates to the liver from rumen fermentation, or by increasing the amount of glucose absorbed from digestion (Nocek and Tamminga, 1991b). As starch digestion in the small intestine produces glucose, increasing the post-ruminal supply of starch could theoretically increase milk production. Disappointingly this has never been proven in practice (Nocek and Tamminga, 1991b; Iqbal e*t al.*, 2009). Several studies have come to the same conclusion that no net glucose absorption is evident from hepatic drained viscera in dairy cattle (Huntington, 1984; Reynolds e*t al.*, 1988; Reynolds and Huntington, 1988; Arieli e*t al.*, 2001). Reynolds e*t al.* (2001) concluded that any glucose obtained from the diet was primarily used for intestinal metabolism and is either oxidised or stored as omental fat.

Furthermore, shifting the site of starch digestion to the small intestine could provide an energetic advantage. Starch that is digested in the small intestine to produce glucose has a greater efficiency of metabolizable energy (ME) utilization compared to starch that is fermented in the rumen to produce VFA (Reynolds, 2006). Owens e*t al.* (1986) determined starch digestion in the rumen to be only 70% as efficient as starch digested in the small intestine. This is because starch digestion in the small intestine does not incur losses in the form of methane or heat of fermentation (Black, 1971; Harmon and McLeod, 2001).

The utilization of starch by ruminants has been extensively reviewed in the past (Theurer, 1986; Owens e*t al.*, 1986; Nocek and Tamminga, 1991b; Pflugfelder, 1986; Huntington e*t al.*, 2006). The purpose of this review is to provide insights into the most important factors that influence the rumen degradability and post-ruminal digestibility of starch in ruminants. It will also provide an overview of starch digestion in ruminants, review new research, investigate specific aspects that influence site of starch digestion, and how this affects fibre digestion.

## 2.2 Ruminal and post-ruminal starch digestion and absorption

Starch digestion in the rumen takes place with the aid of microorganisms such as bacteria. They ferment starch to produce volatile fatty acids (VFA), which in turn are utilized by the animal as well as the rumen microbes themselves, as energy and protein substrate, respectively. Competition within the rumen for an easily digestible and energy rich substrate is fierce and usually only a limited portion of starch escapes rumen fermentation (Harmon e*t al.*, 2004). Ruminal starch digestion ranges from 51 to 93% of starch intake (Nocek and Tamminga, 1991b). Digestion in the rumen is dependent on several intricate and often interconnected factors, such as feed intake, ration composition, processing, particle size, animal factors (breed, age, physiological stage, and body weight), and adaptation to diet (Huntington, 1997). Increasing ruminal supply of starch is linked with increased output of organic acids and microbial protein, decreased fibre digestion, ammonia concentration, and acetate to propionate ratio (Huntington, 1997). Rapid and excessive rumen fermentation of starch can lead to metabolic conditions such as acute rumen acidosis (ARA) or sub-acute rumen acidosis (SARA) (Kleen e*t al.*, 2003).

Post-ruminally starch breakdown occurs similarly to that of a simple-stomached animal. Starch that reaches the small intestine is digested enzymatically to produce glucose which is then absorbed by enterocytes (Huntington, 1997). When high forage diets are fed, all of the starch present in the small intestine is from microbial polysaccharides and can account for up to 10% of the duodenal digesta (Owens e*t al.*, 1986). However, with high concentrate diets it is possible for starch to reach the small intestine by escaping rumen fermentation (Owens e*t al.*, 1986).

Unfortunately, not all starch that enters the small intestine is digested there. It was found that infusing starch into the abomasum had failed to increase blood glucose levels to the same

degree as infusion with glucose, maltose and lactose (Larsen e*t al.*, 1956; Huber, 1969). It was later discovered that on average 47-88% of the starch that enters the small intestine is digested (Owens e*t al.*, 1986). This is significant when compared to starch digestion in monogastric animals where nearly all of the ingested starch is digested and absorbed. This suggests that the small intestine of ruminants may have a limited capacity to digest starch. Various theories have been developed to explain this, for instance limited activity of amylase, maltase or isomaltase due to inadequate production, inadequate working conditions or presence of enzyme inhibitors; low capacity for glucose absorption from the small intestine by enterocytes; insufficient time for complete starch digestion; and inadequate access of enzymes to starch granules (Owens e*t al.*, 1986).

Studies on high carbohydrate diets and post-ruminally infused starch in cattle have consistently reduced pancreatic α-amylase production (Kreikemeier e*t al.*, 1990; Branco e*t al.*, 1999; Swanson e*t al.*, 2002). For instance, Swanson e*t al.* (2002) infused glucose (20g/hour, 40g/hour) and partially hydrolysed starch (20g/hour, 40g/hour) into the abomasum of five steers over a period of eight days. A pancreatic pouch which drained the main pancreatic duct was used to determine the enzyme secretions. Increasing postruminal glucose and starch decreased pancreatic α-amylase secretion (Swanson e*t al.*, 2002). Most of these studies do not take into account long term adaption to high starch diets and research on longer adaption periods are limiting. In monogastric animals the signal for amylase secretion is blood glucose and insulin, and in ruminant's levels are typically low. This could mean that ruminants need longer adaption periods than typically given in these trials (Owens e*t al.*, 1986).

Low capacity for glucose absorption is unlikely to limit starch digestion. Glucose absorption occurs mainly through the activity of SGLT1 transporters (Harmon e*t al.*, 2004). Shirazi-Beechey e*t al.* (1989) found that the presence of increased amounts of glucose in the

intestinal lumen upregulated the expression of glucose transporters in the brush-border membranes of ruminants and is therefore unlikely to limit starch digestion in the small intestine.

It is important to note that the quality of starch supplied to the small intestine also influences its capacity to be digested. Before starch reaches the intestinal lumen it must first pass though the rumen. Therefore, ruminal degradation influences not only the quantity but also the quality of starch reaching the small intestine (Owens e*t al.*, 1986). Starch that reaches the small intestine are devoid of easily digestible starch and only the more resistant starch remains. Therefore, the digestibility values obtained for various starch sources may not be a true representation of the small intestines capacity to digest starch (Owens e*t al.*, 1986). If the starches were somehow protected from ruminal fermentation, higher digestibility could be expected.

Starch that remains undigested after passing the ileo-caecal valve will be exposed to hindgut fermentation (Ørskov e*t al.*, 1970). The modes of degradation in the caecum and colon resemble that of the rumen and produce similar end products such as VFA and methane. However, these end products are largely unavailable to the animal and hindgut fermentation is largely viewed as unfavourable because of the risk of hindgut acidosis (Gressley e*t al.*, 2011).

## 2.3 Grain factors that influence rumen degradability and post-ruminal delivery and digestion of starch

### *Composition of grains*

The basic structure of grain kernels has three morphologic parts: pericarp, germ and endosperm. The pericarp functions to protect the endosperm and embryo from moisture, insects, and fungal infections (Huntington, 1997). Before starch within the grain kernel can be digested, the pericarp or seed coating must first be broken, this is achieved either through chewing or processing. Once this has been achieved the seed coating has little effect on subsequent digestion, other than diluting the amount of starch in the diet (Rowe *et al.*, 1999). However, in grains such as sorghum the pericarp represents only 6-7% of the grain weight and as long as the grain is effectively cracked it will have little effect on the nutritional value. The pericarp and embryo contain minimal amounts of starch (Kotarski *et al.*, 1992a). The embryo has the highest lipid and lipid soluble vitamin content (Evers and Millar, 2002). The principal fatty acids found in grains lipids are C16:0, C18:0, C18:1, C18:2, and C18:3, with slight differences seen between species but typically C16:0 and C18:2 make up the largest percentage (Morrison *et al.*, 1984a).

The endosperm makes up the largest component of grains (Evers and Millar, 2002) and it contains the majority of the starch which is enclosed within structures called starch granules (Kotarski *et al.*, 1992a). The endosperm consists of four layers: aleurone layer, sub-aleurone layer (peripheral endosperm), corneous endosperm and the inner floury endosperm (Kotarski *et al.*, 1992a). The cells of the aleurone layer are block-like, thick walled and occur in a continuous layer around the endosperm and embryo (Evers and Millar, 2002). It has high concentrations of proteins, lipids, vitamins, and minerals (Evers and Millar, 2002). The

peripheral and corneous endosperm is comprised of starch granules embedded in a matrix of storage proteins (Evers and Millar, 2002).

The amount of vitreous endosperm compared to floury endosperm determines the vitreousness of grains (Lopes *et al.*, 2009). Haddad *et al.* (1999) also describes vitreousness as an optical property that is defined by two possible states of the endosperm namely glassy or mealy. Vitreousness of grains has been positively linked to decreased ruminal starch degradation (Corona *et al.*, 2006).

The degree of vitreousness is strongly related to the agro-climatic conditions of growth, such as climate and soil conditions (Haddad *et al.*, 1999). Vitreousness is also related to genetic factors such as the type of maize cultivar (Corona *et al.*, 2006). Based on the characteristics of the grain kernel, maize can be divided into five classes: flint, popcorn, floury, dent, and sweet. The endosperm of flint maize is almost completely vitreous. Floury maize, as the name implies, has an almost entirely floury endosperm (Kotarski et al., 1992b). Dent maize is a hybrid that contains different ratios of floury and vitreous endosperm depending on the type of cultivar (Corona et al., 2006). These structural differences are also responsible for some of the differences seen in *in vitro* and *in vivo* digestion among grain sources (Deckardt et al., 2013). Several methods have been developed to estimate the vitreousness of grains, including manual dissection, grain density and Near-Infrared Reflectance Microscopy (NIRS). Manual dissection is the predominant method used to quantify vitreousness in maize (Correa et al., 2002; Ngonyamo-Majee et al., 2008). Whole maize kernels are soaked in distilled water. The germ and pericarp are removed, and the vitreous and floury endosperm are separated using a scalpel. After drying, the endosperm is weighed and expressed as a percentage of the total endosperm. Manual dissection can only be performed on whole intact kernels and not on ground feed samples. It also has the disadvantage of destroying the sample (Ngonyamo-Majee et al., 2008;

Hoffman et al., 2010). The reliability of this method is dependent on the skill and experience of the technician (Louis-Alexandre et al., 1991). Grain density is another method that can be used to estimate vitreousness. Correa e*t al.* (2002) found a correlation between grain density and ruminal starch availability, and a correlation between vitreousness and ruminal starch availability. Grain density is therefore an indirect measure of vitreousness. Grain density is less labour intensive than manual dissection and can be used to screen large amounts of grain (Correa et al., 2002). Near-Infrared Reflectance Microscopy could provide a rapid and non-destructive way to measure virtuousness, even for ground samples (Perez et al., 2001).

Differences between samples are generated by the endosperm colour, protein and starch concentration, particle distribution, density and hardness (Ngonyamo-Majee et al., 2008). Ngonyamo-Majee e*t al.* (2008) conducted an experiment to determine the correlation between the endosperm properties and digestibility's of 33 different maize cultivars with measurements of endosperm properties obtained either manually or by NIRS. They concluded that NIRS had the potential to become an effective screening tool for maize vitreousness, density and hardness. Additionally, NIRS can be conducted without the use of expensive reagents or production of potentially hazardous chemical residues (Perez et al., 2001).

It was found that increasing vitreousness leads to a decrease in the rumen digestibility of maize. Phillippeau e*t al.* (1998) studied the difference between flint and dent maize, as well as the amylose content of different cultivars on ruminal starch digestion. Their studies confirmed that flint maize is more vitreous than dent maize. Furthermore, rumen starch degradability *in situ* averaged 58% for flint maize and 71% for dent maize. Maize with high amylose content tended to have a higher ruminal starch degradability, independent of flint- or dent-endosperm type.

Similarly, Correa e*t al.* (2002) examined the relationship between vitreousness and *in situ* ruminal starch digestibility of maize. They determined the vitreousness of 14 different dent-endosperm cultivars and five different flint-endosperm cultivars at different stages of maturity. Manual dissection was used to determine kernel vitreousness. Three lactating Holstein cows fitted with rumen cannula were used to determine *in situ* starch digestibility. Again, flint cultivars had higher vitreousness than the dent cultivars and vitreousness tended to increase with maturity and decreased ruminal starch availability. The correlations between kernel density and vitreousness was found to be 0.87. The correlation between kernel vitreousness and ruminal starch availability to be -0.97, and kernel density and ruminal starch availability to be -0.87. Stage of maturity did not influence starch content. They observed that both kernel density and vitreousness increased with age and therefore came to the conclusion that kernel density could become an indirect measurement of starch digestibility (Correa et al., 2002).

As mentioned earlier the endosperm consists of four layers: aleurone layer, sub-aleurone layer (peripheral endosperm), corneous endosperm and the inner floury endosperm (Kotarski et al., 1992a). The corneous endosperm is tightly compacted and translucent. While the floury endosperm has a more “open” structure and is not covered by a protein matrix and is therefore much more susceptible to external attack such as digestion and grain processing (Kotarski et al., 1992a). The floury endosperm also contains the majority of the starch granules (Huntington, 1997). The protein matrix consists of mostly protein and non-starch carbohydrates and is resistant to water and hydrolytic enzymes (Kotarski et al., 1992a). The matrix consists of four different types of proteins: albumins, globulins, glutelins and prolamins (Shewry and Halford, 2002b). Prolamins are considered to be the principal storage protein of the endosperm of grains (Shewry and Halford, 2002b). Maize prolamin is called zein, in barley

it is called hardein, in wheat it is called gliadin, and in sorghum it is called kafarin (Shewry and Tatham, 1990). The zein content of maize makes up 50-60% of protein (Shewry, 2007), hardein content of barley protein is 50%, gliadin content of wheat protein is 33%, and kafarin content of sorghum protein is 42-45% (Taylor and Schüssler, 1986). There are four types of prolamins: ά, β, γ, and δ (Shewry and Halford, 2002b). One of the major amino acids that make up prolamins is proline which is hydrophobic and explains why prolamins are not soluble in water or rumen fluid (Shewry and Halford, 2002a). Rumen starch degradation is negatively correlated with the prolamin content (Philippeau et al., 2000). The poor rumen starch availability of flint maize could possibly be explained by the presence of prolamins within the protein matrix (Corona et al., 2006). Phillippeau e*t al.* (2000) studied the protein distribution of maize endosperm and its consequence on rumen starch degradation. They determined the protein content of eight dent cultivars and six flint cultivars. Flint cultivars had a higher crude protein content than dent cultivars. The (α, β, δ)-prolamins and true glutelins were found to be the predominant proteins in the endosperm. Rumen starch degradability was negatively correlated with prolamins and positively correlated with glutelin content. Likewise, prolamins were positively correlated with vitreousness and glutelins were negatively correlated with vitreousness. The decrease in ruminal starch degradation of flint maize can be explained by presence of protein storage bodies surrounding the starch granules of the vitreous endosperm (Philippeau et al., 2000). Prolamin is the major storage protein, thus explaining why flint maize has a higher prolamin content than dent corn (Shewry and Halford, 2002b). The protein storage bodies prevent the rumen microbes from accessing the starch granules and thereby decrease starch availability (Philippeau et al., 2000).

Starch is a polysaccharide molecule comprised of α–D-glucose units (Tester e*t al.*, 2004). Two distinct populations of starch exist, amylose and amylopectin. Amylose is a linear

molecule consisting of (1-4) linked α-D-glucopyranosyl units (Buléon e*t al.*, 1998a). Amylopectin is a highly branched molecule and is formed through chains of α-D-copyranosyl residues linked by (1-4) linkages and (1-6) linkages (Buléon e*t al.*, 1998a). Amylose has few branch points, less than 20 per molecule, in contrast amylopectin is characterised by many branch points, on average one branch point for every 20 glucose units (Svihus e*t al.*, 2005a). Amylose has a molecular weight of 105- 106g.mol-1 and amylopectin has a molecular weight of 108 g.mol-1 (Parker and Ring, 2001). Most starches contain between 20 and 25% amylose (Svihus e*t al.*, 2005a). However, grain species exist with more or considerably less amylose, such as certain waxy species that contain less than 1% amylose; and Amylomaize that contains up to 65% amylose (Parker and Ring, 2001). Amylose, as a percentage of total starch, was found to be 3-46% in barley (Åkerberg e*t al.*, 1998), 0-70% in maize (Morrison e*t al.*, 1984b), 3-31% in wheat, and 0-30% in sorghum (Beta e*t al.*, 2001; Sang e*t al.*, 2008). Dombrink-Kurtzman and Knutson (1997) measured the differences in amylose content of vitreous and floury endosperm of maize and discovered a small but significant difference. Floury endosperm contains less amylose than vitreous endosperm (Dombrink-Kurtzman and Knutson, 1997). Similarly, El-Khayat e*t al.* (2003) found that the amylose content in wheat was slightly higher in cultivars with more vitreous endosperm. Cagampang e*t al.* (1984) determined the correlation between vitreousness and amylose content in sorghum to be 0.52. Furthermore, it is known that high amylose levels decrease digestibility of grains (Sajilata e*t al.*, 2006). Stevnebø e*t al.* (2006) investigated the effect of amylose level of barley starches on *in vitro* rumen digestibility. They found that cultivars with low amylose levels had higher starch digestion than normal or high amylose cultivars, for both isolated starch and ground samples (Stevnebø e*t al.*, 2006). The reason that amylopectin is more readily digested than amylose is because amylose has tighter intermolecular bonding between starch molecules (Buléon e*t al.*, 1998a). This leads to

a more compacted structure of the starch granules in the endosperm. Therefore, grains with greater proportions of amylopectin have greater rumen starch and total tract starch digestion. Because waxy species contain more amylopectin than amylose, they swell faster in heated water and are digested faster than non-waxy species (Buléon e*t al.*, 1998b; Deckardt e*t al.*, 2014).

***Grain processing***

Processing involves any process that improves the efficiency of nutrient utilization in the rumen or post-ruminal tract. The types of processing are routinely divided into two types: physical and chemical. Physical processing includes grinding, cracking, rolling, or pelleting; and heat treatments such as steam flaking, extrusion, roasting, popping, reconstituting, and micronizing (Nocek and Tamminga, 1991a). Chemical treatments include aldehydes, alkalines, ammoniation, acetic acid, tannins, mild acids, lactic acid, or organic acids (fumaric, malic, aspartic acids).

*Physical Processing*

When feeding grains to cows the minimum amount of processing required for efficient digestion is cracking, this is because whole grains have been known to pass through the digestive tract unchanged. Cracking breaks open the pericarp and exposes the endosperm. Taking this concept a step further, grinding and rolling can be used to further decrease particle sizes and expose more surface area for the attachment of microbes and digestive enzymes. Particle size will also influence the amount of starch granules freed from the protein matrix of the endosperm.

*Grain particles size*

Reducing particle size predominantly leads to increased starch digestion in the rumen. Callison e*t al.* (2001) used 5 cannulated Holstein cows to determine the starch digestibility of

maize at different particle sizes. The diet comprised of 50% lucerne silage and 36.6% of either coarsely ground, medium ground, or finely ground maize with mean particle sizes of 1.3 mm, 2.6 mm, and 4.8 mm, respectively. Decreasing particle size increased true ruminal digestibility of non-structural carbohydrates from 49.8%, to 46.5 and 87.0 (Table 2.1). The apparent total tract digestibility increased from 91.3, 92.2, to 98%, indicating that starch digestion in the small intestine was higher for larger particle sizes (Callison e*t al.*, 2001). In contrast to this, Remond e*t al.* (2004) conducted a similar experiment using semi-flint maize with mean particle sizes of 0.730, 1.807, and 3.668 mm (Table 2.1). Apparent total tract digestibility was 91.4, 86.0, and 69.5% respectively for the different particle sizes, which is lower than expected compared to the study by Callison e*t al.* (2001). Possibly indicating that the vitreous nature of semi-flint maize had a marked effect on starch digestibility. Unfortunately, the study included no information about the nature of the maize used.

Similarly, reducing the particle size of barley increases rumen starch digestion. Yang e*t al.* (2001) utilized eight rumen and duodenal cannulated dairy cows to examine the digestibility of coarse and finely ground barley. The rumen digestibility of starch increased from 37.8% for coarse ground to 50.1% for finely ground barley, and apparent total tract digestibility also increased from 81.7% to 90.2%. Post-ruminal starch digestion (as a percentage of intake) decreased from 43.9% for coarsely ground to 40.1% for finely ground barley (Yang e*t al.*, 2001c). It can be assumed that other grains such as wheat and sorghum will produce similar results. However, research comparing the starch digestibility of different particle sizes for other grains are limiting.

Particle size also influences the density and specific gravity of particles, which in turn influences the retention time in the rumen (Hyslop e*t al.*, 1989). Smaller particles have a higher density and tend to sink to the bottom of the rumen where it can pass on to the lower digestive

tract thereby decreasing digestion in the rumen (Hooper and Welch, 1985a). This principle is well established with forages (Poppi e*t al.*, 1980; Hooper and Welch, 1985a; Hooper and Welch, 1985b; Nocek and Kohn, 1987), but not in grains. The majority of the research indicates that decreasing particle size in grains increases rumen starch degradation (Table 2.1; Galyean e*t al.*, 1979; Galyean e*t al.*, 1981; Yu e*t al.*, 1998; Knowlton e*t al.*, 1998b; San Emeterio e*t al.*, 2000; Callison e*t al.*, 2001; Remond e*t al.*, 2004). However, these studies merely examine the differences between whole, cracked, coarse and finely ground grains. Not enough is known about the effects of the individual particle size fractions on starch digestion.

Some inference can be made on the effects of particle size on starch digestion. For instance, Ewing e*t al.* (1986) used four ruminally cannulated steers fed whole and cracked maize to determine the effects of particle size on rumen passage rates and particle size reduction rates. The cracked and ground maize were separated into four different particle size classes: <1.19mm, 1.19mm-4.76, 4.76-8mm, and >8.0mm. Each particle class was assigned to a different steer and 1kg administered daily through rumen cannulas and repeated for 7 days. As particle size decreased, mean pool passage rates increased from 0.024 to 0.046h-1 (Ewing e*t al.*, 1986). This study did not include any information on rumen and post-ruminal starch digestion, however we can assume that if ruminal passage rates increase less starch will be broken down in the rumen and thus increase starch supply to the small intestine.

*Heat Treatment*

Physical processing aims to break open grains in order to expose starch to microbes and digestive enzymes. However, even the smallest particle sizes can still contain whole starch granules protected from digestion in the endosperm matrix (Rowe e*t al.*, 1999). Using high temperatures, with or without the use of water, it is possible to further disrupt the protein matrix and expose starch to digestion.

Starch occurs naturally in highly organised water insoluble granules contained within the endosperm of grain kernels (Pflugfelder, 1986; Huntington, 1997). Starch granules are created by depositing starch in layers containing various amounts of amylose and amylopectin held together by hydrogen bonds. The layers alternate between semi-crystalline and amorphous in nature (Buléon e*t al.*, 1998b). The crystalline regions are quite impervious to water, while the amorphous region allow free movement of water (Pflugfelder, 1986; Nocek and Tamminga, 1991a).

Considerable variation exists in the starch granule structure of different plant species with regards to granule size (1-100µm in diameter), shape (round, lenticular, polygonal), size distribution (uni- or bi-modal), association as individual (simple) of granule clusters (compound) and composition (α-glucan, lipid, moisture, protein and mineral content) (Tester e*t al.*, 2004). Additionally, environmental factors during development such as temperature can influence both granule size and starch distribution (Svihus e*t al.*, 2005a).

Various non-starch compounds are also associated with starch granules. The most important of these are lipids, not only because it is the most abundant non-starch component, ranging from 5 to 10%, but also because lipid-starch complexes that form influence starch digestion (Evers and Millar, 2002). Lipids are found in the form of free fatty acids and lysophospholipids and are associated with the amylose fraction (Morrison e*t al.*, 1984a; Pérez and Bertoft, 2010). These amylose-lipid complexes play an important role during gelatinization and can restrict swelling, dispersion of starch granules, and solubilisation of amylase (Buléon e*t al.*, 1998a).

When starch granules are placed in excess water and slowly heated (55°C) they undergo swelling (Nocek and Tamminga, 1991a). During this process starch granules can absorb water up to 50% of their weight, however this process is reversible after cooling and drying (Nocek

and Tamminga, 1991a). If the temperature is increased (60-80°C), irreversible swelling occurs called gelatinization (Parker and Ring, 2001). Swelling occurs primarily in the amorphous region but not the crystalline regions. This imposes stress on the bonds between the amylopectin in the crystalline regions and the amylose in the amorphous regions (Donald, 2001; Svihus e*t al.*, 2005b). At a certain point the crystalline regions are irreversibly broken and gelatinization occurs. Amylose in the starch granule leaches out making it available for amylase digestion (Pflugfelder, 1986). Starch molecules are gelatinized during processes such as steam-flaking, extrusion, and rolling. Mechanical 'gelatinization' also occurs during milling or grinding of grains, the crystalline regions are damaged through compressing, impact, shear or attrition, making starch within the granules vulnerable to enzyme attack (Pflugfelder, 1986). The granules will also undergo swelling when they come into contact with water causing starch to leach out (Karkalas e*t al.*, 1992).

In a review by Theurer e*t al.* (1999) which summarises nineteen lactation trials involving 43 grain processing comparisons, the starch digestion of dry-rolling and steam-flaking was compared. Ruminal starch digestion of maize increased from 35% to 52% when steam flaked, while sorghum increased from 54% to 76%. Post-ruminally, starch digestion (as a percentage of entry) increased from 77.5% to 96.6% for maize, sorghum increased from 74% to 90% (Table 2.1; Theurer e*t al.*, 1999). Similar results are seen with barley, ruminal and post-ruminal starch digestibility are greatly improved by steam flaking over dry-rolling (Plascencia and Zinn, 1996). Malcom and Kieslin (1993) compared the *in situ* digestibility of steam flaked barley to dry ground barley through a 3.2mm screen and found little benefit in steam flaking. They concluded that steam flaking and grinding were equally effective at increasing rumen starch degradation and exposing starch to microbes (Malcolm and Kiesling, 1993).

Retrogradation is the reassociation of the starch molecules after gelatinization through the reestablishment of hydrogen bonds between amylose and amylopectin (Nocek and Tamminga, 1991a). The resultant bonds are very strong, causing a glue-like hardening of the affected starch, decreased porosity of the internal starch matrix, and limits rehydration and enzyme penetration (Zinn e*t al.*, 2002). Consequently, retrogardation decreases rumen starch digestibility (Pflugfelder, 1986). Ward and Galyean (1999) found that enzymatic starch digestion was lowered by 40% after steam-flaked maize was allowed to retrograde.

***Chemical Processing***

Chemical methods of grain processing involve the addition of substances such as Formaldehyde (CHCO), Sodium hydroxide (NaOH), or ammonia (NH3) in order to alter the starch structure and ultimately its digestion. The site of starch digestion will depend on the type of process and the degree of processing.

Aldehydes, especially formaldehyde, are sometimes used to treat grains and it has been used effectively to decrease rumen digestion of starch. Formaldehyde enters the starch granule and forms a complex with the hydroxyl groups which then form cross-linkages with hydroxyl groups on other starch granules (Fluharty and Loerch, 1989). The amorphous, amylose rich regions of the starch granule are primarily affected (Pflugfelder, 1986). This causes the starch granule to be tightly bound and prevents it from swelling and thereby increasing RRS. Once the grain reaches the acidic environment of the abomasum the formaldehyde is released and the starch is free to be digested in the small intestine (Fluharty and Loerch, 1989).

In a study by Fluharty and Loerch (1989) formaldehyde treatment of grain reduced rumen degradation of starch while maintaining whole tract starch digestion (Fluharty and Loerch, 1989). The addition of 1 and 2% formaldehyde decreased ruminal starch digestion of maize 30 and 41.5%, respectively, in sheep. However, total tract starch digestion was not

affected, indicating that the rumen resistant starch (RRS) was digested in the small and large intestine (Deckardt e*t al.*, 2013). Formaldehyde was also effective in decreasing ruminal degradation of wheat, Shcmidt e*t al.* (2006) compared untreated ground wheat with ground wheat treated with 2% formaldehyde in Holstein steers. The amount of starch entering the duodenum increased by 75% when treated (Schmidt e*t al.*, 2006). Additionally, the small intestinal digestibility of starch increased from 67.36% to 73.12% indicating that the cow's amylase secretion can adapt to the increase in starch reaching the small intestine. This is of particular interest because pancreatic amylase secretion is considered to be one of the limiting factors in small intestine starch digestion (Owens e*t al.*, 1986).

However, Ortega-Cerrilla e*t al.* (1999) found no evidence that treating barley with formaldehyde could reduce rumen starch digestion *in vivo*. The author suggests that the difference seen between barley and other grains is due to structural differences of the starch granule.

Alkaline treatment, such as sodium hydroxide, has been observed to slow down ruminal degradation of starch and decrease susceptibility to rumen acidosis (McNiven e*t al.*, 1995). Shmidt e*t al.* (2006) found that treating ground wheat increased the amount of starch entering the small intestine by 57% and increased the small intestinal digestibility of starch from 67.36% to 77.5%. O'Mara e*t al.* (1997) also found sodium hydroxide treatment of wheat effective in protecting starch from rumen degradation in dairy cows. Barley also shows a positive response to treatment with sodium hydroxide. When coarsely milled barley grain was treated with 35gNaOH/kg the total track starch digestibility increased and the post-abomasal tract starch disappearance increased from 37% in the control to 79% (Dehghan-Banadaky e*t al.*, 2008). However, sorghum treated with sodium hydroxide had reduced total tract starch digestibility (Miron e*t al.*, 1997).

Another alkaline treatment, ammonia, has been proven to increase RRS in barley. Robinson e*t al.* (1988) examined 4 levels of ammonia treatment (0%, 0.65%, 1.3% and 1.95% as a percentage DM) of barley grain in dairy cows. Ammonia decreased *in situ* ruminal starch degradation rates without decreasing whole tract digestibility (Robinson and Kennelly, 1988). Interestingly, milk yield increased with higher ammonia levels (Robinson and Kennelly, 1989).

## 2.4 Starch digestion and its subsequent effect on forage NDF digestion

**S**tarch is one of the main factors negatively influencing fibre digestion in the rumen (Hoover, 1986; Firkins e*t al.*, 2001). The effect of feeding diets with different starch levels to lactating dairy cows were investigated by Gencoglu e*t al.* (2010). Cows were fed diets differing in the starch content of the concentrate, 33% vs. 20.1%. Dry matter intake was slightly higher for the reduced starch diet, 4.16% vs. 3.88% of body weight. The total tract NDF digestibility was higher for the reduced starch diet, 54.1% compared to 39.4% for the higher starch diet. Similarly, in an experiment conducted by Valadares e*t al.* (2000) the nutrient digestibility's of forages was examined at different concentrate ratios. As the level of starchy concentrates increased the NDF total tract digestibility suffered (Valadares Filho e*t al.*, 2000).

Therefore, any factor that influences the digestibility of starch in grains, will also influences the digestibility of fibre. For instance, the type of endosperm in maize, vitreous vs. floury, affects the digestibility of NDF. As discussed previously, vitreous maize is more resistant to rumen degradation than floury maize (Philippeau e*t al.*, 1998). Lopes e*t al.* (2009) conducted an experiment to determine if the type of endosperm influences the digestibility of nutrients in lactating dairy cows. Three different diets were formulated with similar starch and NDF content, differing only in vitreous content. The less vitreous maize had higher rumen

starch and total tract starch digestibility, and NDF digestibility was higher for the vitreous maize (Lopes e*t al.*, 2009).

Processing of grains in order to improve digestibility also has a subsequent effect on NDF digestion of forages. Joy e*t al.* (1997) carried out an experiment to determine the effect of processing maize on nutrients' digestibility. Lactating dairy cows were fed diets consisting of 40% forages and 60% concentrates. The starch content of the different diets were similar and the diets differed only in the processing methods used on maize, steam-flaking vs. dry-rolled. Steam-flaked maize had the highest rumen digestibility of starch, but also the lowest NDF digestibility (Table 2.1). Poore e*t al.* (1993) investigated the relationship between fibre and rumen starch digestion in rumen cannulated Holstein cows (Table 2.1). Diets were compiled using wheat straw and either steam flaked or dry-rolled sorghum, in order to produce a forage NDF (FNDF) to rumen degradable starch (RDS) ratios of either 0.8 or 1.35. Increasing RDS decreased fibre digestion, especially cellulose, as well as lowered DMI, milk fat percentage, and fat corrected milk. The authors suggest that the ratio of FNDF and RDS to be at least 1:1 in order to minimize these negative effects (Poore e*t al.*, 1993). Similar results were obtained for maize by Sarwar e*t al.* (1992) when the NDF to NSC ratio was lower than 1 there was a reduction in DMI, milk and milk fat production.

Enhancing rumen degradability of starch through particle size also decreases NDF digestibility. In an experiment by Callison e*t al.* (2001) the effect of particle size on maize was examined. As the particle size decreased, from 4.8, 2.6, to 1.2mm, the rumen digestibility of starch increased from 49.8, 46.5, to 87% (Table 2.1). Simultaneously, NDF digestibility (as a percentage of intake) decreased linearly, from 52.7, 51.5, and 45.6% (Callison e*t al.*, 2001).

Although it is commonly acknowledged that starch digestion adversely affects NDF digestion in the rumen, Armentano and Pereira (1997) suggests that there are a few factors that

might confound these results. Increasing the inclusion of either NFC or NDF in the diet, inadvertently leads to a decrease in the other. This makes it hard to determine which dietary component is responsible for any changes in the response seen. When forages in the diet are increased at the expense of concentrates, not only is NDF increased but the proportion of NDF from forages is increased (Armentano and Pereira, 1997). Furthermore, when NDF in the diet is reduced by increasing the concentrate content of the diet the DMI of cows' increase. It was also found in a study by Tine e*t al.* (2001) that increased DMI decreases NDF digestibility. Therefore, it is difficult to deduce whether the decrease in NDF digestibility is due to an increase in starch in the diet or to higher DMI.

To resolve this issue, Beckman and Weiss (2005) ascribed treatments effects as different NDF to starch ratios rather than changes in starch to NDF concentrations. They thereby hypothesized that any changes in the response would not be confounded by DMI, and NDF digestibility will be less sensitive to decreases in the NDF to starch ratio (Beckman and Weiss, 2005). Six Holstein cows were fed one of three different diets with NDF to starch ratio equal to 0.74, 0.95, or 1.27. The diets were designed to have the same *in situ* NDF digestibility. All the diets had 18% forage NDF, but starch concentration and NDF varied. This was achieved by using a mixture of soy hulls and cottonseed hulls with the same *in situ* NDF digestibility as the forages. They found that intake tended to increase as NDF to starch ratio increased, however intake of digestible energy remained constant despite treatment differences. Total tract digestibility of DM and energy decreased linearly as the NDF to starch ratio increased. The overall NDF digestibility was not affected by starch concentration. However, the digestibility of the forage was reduced by high concentrate diet.

NDF is vital in the diets of dairy cows, it aids healthy rumen function and normal milk fat percentages (Sarwar e*t al.*, 1992). The predominant theory as to why rumen starch

fermentation depresses NDF digestibility is because it decreases rumen pH (McCarthy e*t al.*, 1989). Fermentation of starch in the rumen results in the production of VFA which cause the acidity of the rumen fluid to increase. The optimal pH for cellulolytic bacteria is 6.8 and once the pH drops below this their activity decreases along with fibre digestion (McCarthy e*t al.*, 1989). Thus, shifting the site of starch digestion to the small intestine could have potential benefits as relates to fibre digestion. Furthermore, forages influence DMI through gut fill (Oba and Allen, 1999). Improving the digestibility of forages can therefore increase passage of forages and potentially improve DMI.

## 2.5 Conclusion

In recent years, starch has been described as a hot topic in dairy cattle nutrition for various reasons. The transition period is hallmarked by poor feed intake, often resulting in negative energy balance. Negative energy balance during this period can have several short and long term health risks, such as milk fever, mastitis, displaced abomasum, laminitis, and poor fertility (Hayirli e*t al.*, 2002; Butler, 2003; Esposito e*t al.*, 2014). Starch during this phase can be a tool to mitigate these risks. Furthermore, starch digested in the small intestine has an energetic advantage over starch digested in the rumen and it also lowers the risk of rumen acidosis, and may improve DMI and energy balance of transition cows, according to recent theories (Reynolds, 2006; Allen e*t al.*, 2009). Although more research is needed to develop ways to improve starch nutrition during the transition period, the benefits of better fine-tuning starch during this time are evident.

Total tract starch digestion in ruminants can exceed 95%. However, ruminal digestion ranges between 51 and 93% (Nocek and Tamminga, 1991a); and, of the starch reaching the small intestine 47 to 88% is digested there (Owens e*t al.*, 1986). The composition of the diet

and starch characteristics are considered to be the primary factors influencing the rate and extent of starch fermentation in the rumen. Many are the published works of at least the last 20 years, analysing, for example, the effects of species, vitreousness, amylose-amylopectin ratio, protein and starch interactions, endosperm type, prolamins, degree of maturity, processing. In the small intestine, instead, capacity of ruminants at digesting starch, more than starch characteristics, seem to affect amount of starch digested. Despite of the many published works, starch still remains a hot topic and all the models that are daily used by nutritionists would probably benefit from a better starch and grain characterization, similarly to what is done for fibre.

**Table 2.1** Summary of starch and NDF digestibility

| Reference | | Starch digestibility | | | | | | | | | NDF Digestibility | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Grain | Treatment | Particle size µm | DMI kg/day | Starch intake (kg/day) | Rumen (%intake) | SI (% passage) | SI (% intake) | Post-ruminal (% intake) | Total tract (% intake ) | Intake (kg/day) | ruminal | total tract |
| Knowlton et *al.* (1998b) | *In vivo* | Dry maize | Ground | 618 | 23.4 | 7.93 | 60.9 | 13.2 | 9.11 | | 88.9 | 6.552 | 57.0 | 30.4 |
| | | | Rolled | 1725 | 23.4 | 7.94 | 69.2 | - | - | | 76.4 | 6.4818 | 57.3 | 33 |
| | | High moisture Maize | Ground | 489 | 24.4 | 8.8 | 86.8 | 58.8 | 58.9 | | 98.2 | 6.5392 | 64.7 | 26.3 |
| | | | Rolled | 1789 | 23.7 | 8.3 | 81.2 | 63.3 | 56.6 | | 95.7 | 6.3753 | 60.2 | 25.7 |
| Remond *et al.* (2004) | *In vivo* | Dry Semi-flint Maize | Ground | 730 | 16 | 4.33 | 58.6 | 67.5 | 28.9 | | 91.4 | | | |
| | | | Medium rolling | 1807 | 15.9 | 4.33 | 49.8 | 61.1 | 31.5 | | 86 | | | |
| | | | Coarse rolling | 3668 | 15.9 | 4.27 | 35.5 | 47 | 30.6 | | 69.5 | | | |
| | | Dent Maize | Ground | 568 | 18 | 4.73 | 69.8 | 77.8 | 23.4 | | 97.3 | | | |
| | | | Coarse rolling | 3458 | 18.1 | 4.66 | 53.5 | 68.3 | 31.9 | | 89.2 | | | |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Galyean et *al.* (1981) | *In vivo* | Maize | Dry rolled | 3000 | | | 19.9 | | | | | | | |
| | | | | 1500 | | | 17.2 | | | | | | | |
| | | | | 750 | | | 26.6 | | | | | | | |
| | | | Steam flaked | 3000 | | | 30.7 | | | | | | | |
| | | | | 1500 | | | 36.9 | | | | | | | |
| | | | | 750 | | | 40.8 | | | | | | | |
| Firkins et *al.* (2001) | *In vivo* | Maize | Steam rolled | | 26.5 | | 35 | | | 42 | 77.5 | | | |
| | | | Steam flaked | | 26.5 | | 52 | | | 44 | 96.6 | | | |
| | | Sorghum | Steam rolled | | 25.6 | | 54 | | | 36 | 88.7 | | | |
| | | | Steam flaked | | 25.1 | | 76 | | | 23 | 97.9 | | | |
| Callison et *al.* (2001) | *In vivo* | Maize | Finely ground | 1200 | 18.4 | 4.92 | 70.1 | 65.2 | 19.9 | | 98 | 5.82 | 45.6 | 66.4 |
| | | | Medium ground | 2600 | 18.7 | 5.17 | 31.9 | 79.1 | 60.2 | | 92.2 | 5.95 | 51.5 | 66.5 |
| | | | Coarsely ground | 4800 | 18.8 | 5.44 | 35.2 | 66.4 | 47.7 | | 91.3 | 5.87 | 52.7 | 65.2 |
| | | | Steam rolled | | 18 | 5.21 | 52.2 | 70.3 | 36.9 | | 95 | 5.52 | 47.5 | 62.8 |
| San Emetorio et *al.* (2000) | *In vivo* | Maize | Finely ground | 1110 | 24.7 | 8.73 | | | | | 88.1 | 6.5455 | | 56.5 |
| | | | Coarsely ground | 3280 | 25.8 | 9.13 | | | | | 80.4 | 6.837 | | 52.8 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Yu et *al.* (1998) | *In vitro* | Maize | Finely ground | 1180 | 23.1 | | | | | | 95.8 | 7.161 | | 54.4 |
| | | | Coarsely ground | 2420 | 27.5 | | | | | | 87.4 | 9.6525 | | 62.8 |
| | | | Steam flaked at low density | 3840 | 27.8 | | | | | | 97.5 | 8.062 | | 41 |
| | | | Steam flaked at high density | 4700 | 27 | | | | | | 95.7 | 9.126 | | 57 |
| | | | Steam rolled | 5300 | 26.7 | | | | | | 91.3 | 9.2649 | | 69.3 |
| Theurer et *al.* (1999) | | Soghum | Dry rolled | | 6.761 | 4.002 | 66.8 | 85 | 28.5 | | 96.5 | | | |
| | | | Steam flaked at density 437 g/L | | 6.456 | 3.612 | 76.6 | 88.7 | 20.5 | | 97.7 | | | |
| | | | Steam flaked at density 360 g/L | | 6.895 | 3.76 | 81.5 | 93 | 17.5 | | 99.3 | | | |
| | | | Steam flaked at density 283 g/L | | 6700 | 3.867 | 89.4 | 91.5 | 9.8 | | 99.6 | | | |
| Poore et *al.* (1993) | | Sorghum and Alfafa hay | Dry-rolled Sorghum | 1000 | | 6.19 | 42.6 | 69.1 | | | 84.6 | 5.14 | 46.4 | 41.9 |
| | | | Steam flaked Sorghum | 4000 | | 6.13 | 71.1 | 92.1 | | | 97.8 | 5.35 | 39.8 | 40 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Joy et *al.* (1997) | *In vitro* | Maize | Dry-rolled | 20.94 | 2.9 | 34.33 | | | 43.66 | 77.99 | 7.72 | 62.72 | 66.02 |
| | | | Steam-flaked (0.39 kg/L) | 20.1 | 2.93 | 27.258 | | | 57.88 | 85.13 | 7 | 51.77 | 58.56 |
| | | | Steam-flaked (0.31 kg/L) | 21.4 | 3.2 | 44.81 | | | 49.59 | 94.4 | 7.04 | 51.23 | 58.83 |
| McNiven et *al.* (1995) | *In vivo* | Barley | Control | 14 | 3.56 | 85 | 80.8 | | | 97.3 | 4.44 | 59.7 | 62.8 |
| | | | Roasted | 13.6 | 3.3 | 86.9 | 80.6 | | | 98.1 | 4.19 | 54 | 58.5 |
| | | | Sodium Hydroxide | 13.1 | 2.74 | 66.9 | 20.3 | | | 85.3 | 4.02 | 69 | 69 |

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# Chapter 3

## Short communication: Effects of similar theoretical grinding size on particle size distributions of hard and soft maize

### 3.1 Abstract

Grains are an important constituent of ruminant diets and milling of grains is routinely used in the livestock industry to improve digestibility. Particle size influences grains digestibility, with finely milled grains having a higher extent and rate of starch digestion than coarsely milled grains regardless of starch content. However, there is evidence that different grains react differently to milling under similar conditions, thus potentially producing an inconsistent product. If different grains react differently to milling, resulting in different particle size distributions, and it is known that particle sizes interact with digestion, it is possible that some differences in digestibility could occur. Therefore, we hypothesised that there exists an interaction between a specific grain and the mill. The purpose of this study was to examine the particle size distribution and geometric mean particle size (GMPS) of different grains milled in a similar fashion. Two maize types were selected based on hardness, and milled using a Wiley mill fitted with either a 3mm screen or 4.5mm screen. The maize was then sieved and separated using the following screen sizes: 106µm, 125 µm, 150 µm, 180 µm, 250 µm, 500 µm, 850 µm, 1180 µm, 2000 µm, and 3350 µm with a sieve shaker at an amplitude of 100 for 20 minutes. Hard and soft maize produced a nominal geometric mean particle size (NGMPS) of 274.58 ± 0.87 and 470.91 ± 0.87 when milled at 3mm and 4mm respectively; and a NGMPS of 396.64 ± 0.87 and 576.66 ± 0.87 respectively. These results clearly indicate that an interaction exists between the mill and a specific grain.

## 3.2 Introduction

Grains are an important constituent of ruminant diets. Milling of grains is routinely used in the livestock industry to improve digestibility, as the hard outer shell, or pericarp, is very indigestible (Rowe et al., 1999).

Knowing not only the nutritional value, but also the digestibility of feed ingredients when formulating diets is essential. For instance coarsely milled grains and finely milled grains have similar starch content, but the latter have higher extent and rate of starch digestion, not only in the rumen but post-ruminally as well (Callison et al., 2001; Yang et al., 2001; Remond et al., 2004; Ewing et al., 1986). Accurate and precise information relative to digestion kinetics enables us to formulate rations that maximise production in a cost effective manner while preventing diet related diseases, especially in higher producing ruminants.

Digestibility of grains can vary considerably. These differences are often attributed to differences in nutritional value, genetics, variety, geographical locations, year, climatic conditions and agronomic practices (Huntington, 1997; Offner et al., 2003). The effects of the specific mill used is often ignored.

There are various examples of how similar feedstuffs are milled at the same theoretical size and yet produce different particle size distribution and geometric mean particle size (Crawford and Hoover, 1984; Ehle, 1984; Cherney et al., 1988; Emanuele and Staples, 1988).

In a study by Greffeuille e*t al.* (2006), the milling properties of two near iso-genic lines of wheat grains were tested, differing only in hardness. The results showed that harder wheats tended to break into coarser particles, whereas the softer wheats led to fine particles (Greffeuille et al., 2006). These two grains were near identical and yet responded differently to the mill and produced different particle size distributions.

In another experiment, Bitra e*t al.* (2009), measured various milling effects on particle size distribution of milled switchgrass, wheat straw and maize stover. It was found that, amongst other effects, even the speed of milling influenced particle size distribution, with higher speeds favouring smaller particle size (Bitra et al., 2009). It is therefore possible that one particular feed ingredient could produce different particle size distributions depending on the mill, regardless of what screen size is used.

When performing an experiment, it is routine practice to mill all feed ingredients at the same screen size. It is then assumed that any differences are due to the treatment effect. However, if different grains react differently to milling, resulting in different particle size distributions, and it is known that particle sizes interact with digestion, it is possible that some of the differences seen in digestibility within and amongst various studies or among dairy farms could in part be due to size differences and respective digestibility.

Therefore, we hypothesised that there exists an interaction between a specific grain and the mill. The purpose of this study was to examine the particle size distribution and geometric mean particle size (GMPS) of maize samples with different hardness milled in a similar fashion.

### 3.3 Materials and Methods

Two maize samples, provided by Agricol (Cape Town, South Africa) and marketed as hard and soft maize, were used in the experiment. A sub-sample of 100 g was milled using a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) fitted with a 3-mm screen or 4.5-mm screen. The maize was then sieved and separated using the following screen sizes: 106, 125, 150, 180, 250, 500, 850, 1180, 2000 and 3350 µm with a sieve shaker (Kingtest laboratory test sieve, Retsch GmbH, Series AS 200 basic, Germany), at an amplitude of 100 for 20 minutes.

The amount of 100 g was decided after preliminary tests designed to decide the amount of sample that would give the highest repeatability, accuracy and precision, using the mentioned sieve shaker and samples. Weights for each screen were recorded and used to determine geometric mean particle size. The process was then repeated to produce 3 runs.

**Statistical analyses**

Particle size distributions and nominal geometric mean particle size (NGMPS) were analysed as response variables by the GLM procedure of SAS (version 9.3; SAS Instiyute, Cary – NC, USA), using a factorial arrangement of maize type and screen for NGMPS and, maize type, screen and sieve for particle distribution. All the respective interactions and run as random factor were also included. Differences between means were declared significant at $P \leq 0.05$ using the least squares means and the Tukey adjustment. Statistical differences resulting in $0.05 < P \leq 0.10$ were considered tendencies. Treatments results are reported as least squares means unless specified.

### 3.4 Results and Discussion

The particle distribution and NGMPS for hard and soft maize ground at 3 mm and 4.5 mm can be found in Table 3.2. Hard and soft maize produced a NGMPS of 274.58 ± 0.87 and 470.91 ± 0.87 when milled at 3 mm, respectively; and a NGMPS of 396.64 ± 0.87 and 576.66 ± 0.87, respectively. The type of maize used (hard or soft) was highly significant ($P < 0.0001$) and the interaction between the type of maize and screen size was also significant ($P < 0.0086$).

**Table 3.1.** Particle size distribution hard and soft maize milled at 3 mm and 4.5mm

| | 3 mm | | 4,5 mm | |
|---|---|---|---|---|
| Sieve (microns) | Soft | Hard | Soft | Hard |
| 2000 | 0.38[a] | 0.96[ab] | 2.14[b] | 2.62[b] |
| 1180 | 2.66[a] | 5.70[b] | 9.86[c] | 13.97[d] |
| 850 | 6.10[a] | 15.15[b] | 12.44[b] | 20.16[c] |
| 500 | 12.22[a] | 28.88[d] | 16.73[b] | 25.31[c] |
| 250 | 33.90[c] | 30.09[b] | 25.75[a] | 24.93[a] |
| 180 | 9.76[b] | 7.32[ab] | 11.96[c] | 6.28[a] |
| 150 | 7.33[b] | 2.00[a] | 4.26[a] | 1.99[a] |
| 125 | 12.59[b] | 8.17[a] | 8.52[a] | 5.93[a] |
| 106 | 8.20[b] | 1.45[a] | 6.29[b] | 1.17[a] |
| Pan | 6.87[c] | 0.27[a] | 3.41[b] | 0.25[a] |
| NGMPS* | 274.58[a] | 470.91[b] | 396.64[c] | 576.66[d] |

[a-d] Means within a row not sharing a superscript differ ($P<0.05$).
Particle size distribution is given as the proportion (%) of total material recovered on top of each sieve.
*NGMPS (Nominal geometric mean particle size)

The particle size distributions show considerable differentiation not only between screen sizes, but also between maize types. At 3 mm the majority of particles for hard maize are retained on sieve size larger than 250 µm, with very little material on the remaining sieves. In comparison, soft maize retained the majority of particle below 500 µm with virtually none retained at 2000 µm and 1180 µm. At 4.5 mm screen size a similar pattern can be observed with hard maize favouring the production of particles larger than 250 µm and soft maize favouring the production of finer particles.

### 3.5 Conclusion

These results clearly indicate that an interaction exists between the mill and a specific grain. Milling of grains at similar screen sizes might produce a product that appears similar, however there are differences in the particle size distribution. In practice this undoubtedly leads to more variability than producers anticipate and ultimately an inconsistent product.

Consequently, rumen digestion, and probably passage, will be affected and therefore methods to better quantify how these differences will affect digestion are needed.

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# Chapter 4

## Effects of maize particle size and starch-to-fibre ratio on in-vitro starch and NDF degradability

### 4.1 Abstract

Milling of grains is considered to have a great impact on the rate and extent of starch digestion, however differences in milling conditions lead to variation in particle and often an inconsistent product. The purpose of this study was to investigate the effects that particles with specific sizes have on starch and fibre digestion *in vitro*. Ground maize was divided into five different fractions: very fine (<250 µm), fine (250-500 µm), medium (500-1180 µm), coarse (1180-2000 µm), and cracked (2000-3350 µm). Fractions were combined with either lucerne or oat hay to create combinations of either high or low starch-to-NDF ratios. The samples were analysed for *in vitro* starch and NDF digestibility. Starch digestion and rate were influenced by particle size, but not starch-to-NDF ratio, with larger values as particle size decreased. Starch digestibility for very fine, fine, medium, coarse, and cracked maize were 64.33, 62.28, 59.84, 47.58, and 42.15% respectively, and rate of starch digestion was 18.24, 13.48, 10.02, 7.16, and 3.77 %/h respectively. Fibre digestion was influenced by particle size, starch level and forage, resulting in the highest NDFd when combined with coarse or cracked maize, 43.15% and 44.15% respectively, and lowest with fine maize, 32.99%. The rate of NDFd for oat hay and lucerne was 3.11 %/h and 5.11 %/h respectively and it was influenced by particle size, with very fine maize reducing the rate. Better characterization of grains' particle sizes has the potential to better describe the specific feed's starch digestibility and to likely better satisfy requirements of animals in different physiological stages.

## 4.2 Introduction

Maize is a valuable and expensive resource in the dairy industry. It is routinely used in ruminants' diets as an energy concentrate to ensure that the high energy demands of high performance animals are met, especially during lactation. During 2016 maize prices reached a record high and due to droughts in 2015 and 2016 the milk to feed price ratio in South Africa is now at the lowest since 2007 (Bureau for Food and Agricultural Policy - BFAP, 2016). Compared to the beef industry, the beef to maize price ratio has been able to remain relatively stable due to increased exports. For the dairy industry, the importance of having a diet that is accurately formulated and fine-tuned to stage of lactation has never been more evident.

In South Africa, feed companies such as Meadow feeds (Roodepoort, South Africa) and Afgri (Centurion, South Africa) standardly mill grains at the theoretical size of either 2 or 4 mm (B. van Zyl and P. Henning, personal communications). There are various reports showing the effects of the milling used on forages on resulting mean particle size and distribution (Hooper and Welch, 1985b). Various factors can influence the resulting particle size distribution during milling, such as type of grain and endosperm type (hard or soft) (Greffeuille e*t al.*, 2006). Even the speed of milling has shown to influence particle distribution regardless of grain characteristics (Bitra e*t al.*, 2009). This may lead to a variable distribution in particle size and an inconsistent product.

Digestibility of grains can vary considerably. These differences are often attributed to differences in nutritional value, genetics, variety, geographical locations, year, climatic conditions and agronomic practices (Huntington, 1997; Offner e*t al.*, 2003). With regards to experimental procedure, when preparing samples to be analysed it is routine practice to mill all feed ingredients at the same theoretical size. It is then assumed that any difference in digestibility is due to treatment effect or intrinsic characteristics of the sample. However, there

exists considerable differences in the starch digestibility between whole, cracked, ground and finely ground maize. Thus, if different grains react differently to milling, resulting in different particle size distributions, and it is known that particle sizes interact with digestion, it is possible that differences seen in digestibility within and amongst various studies could in part be due to size differences and respective digestibility. The difference between two kinds of maize may therefore be augmented by milling, with higher quality maize resulting in finer and more digestible particles and vice-versa for lower quality maize, assuming soft maize being of higher quality than hard maize (Almeida-Dominguez e*t al.*, 1997).

The importance of maintaining an adequate forage to concentrate ratio has been well documented in the past (Miller and O'Dell, 1969; Weiss and Shockey, 1991; Mertens, 1997; Yang e*t al.*, 2001a). The mode of digestion of a feed is not only influenced by its chemical composition, as is measured by its neutral detergent fibre (NDF), starch, etc., but also by its physical characteristics, most importantly particle size (Mertens, 1997). This is a well-documented phenomenon in forages, and as a consequence various methods have been put forward to measure fibre such as the physically effective NDF (peNDF) system which takes particle size of forages into account. However, no attempt has ever been made to quantify the effect of concentrates in a similar fashion. We can safely assume that the whole diet, and not only the peNDF fraction, stimulates chewing for example. Furthermore, it is well known that particle size influences the rate and extent of starch digestion in the rumen (Ewing e*t al.*, 1986; Callison e*t al.*, 2001; Yang e*t al.*, 2001b; Remond e*t al.*, 2004). Incorporating particle size as a factor of starch content could enable us to better characterize feeds and thus fine tune diets.

We hypothesize that once ground maize is separated into specific ranges of particle sizes, each of these fractions will be unique with regards to extent and rate of starch digestion. There is evidence that starch decreases fibre digestion, however most *in vivo* results are

confounded by the effect of intake (Armentano and Pereira, 1997; Beckman and Weiss, 2005), and *in vitro* analysis do not typically include both the effect of starch-to-NDF ratio and the size of the starch source or a different NDF source. Therefore, our objective is to determine how the effect of each specific fraction on NDF digestibility varies, according to the starch-to-NDF ratio and to forage type.

Usually it is also assumed that starch digestion is not limited by other substrates. However, we wish to examine the effect of forage type and different ratios of starch-to-NDF will have on rate and extent of starch digestion.

## 4.3 Materials and Methods

### Substrates

All samples were milled using a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA). A single batch of yellow maize hybrid (Agricol, Cape Town, South Africa) was milled using a 1 or 2-mm screen. Milled maize was then sieved through a series of sieves with mesh size: 250 µm, 500 µm, 1180 µm, 2000 µm, and 3350 µm (Kingtest laboratory test sieve, Retsch GmbH, Series AS 200 basic, Germany), at an amplitude of 100 for 20 minutes. Five different fractions were obtained: very fine (<250 µm), fine (250-500 µm), medium (500-1180 µm), coarse (1180-2000 µm), and cracked (2000-3350 µm). Forages, lucerne and oat hay, were milled to pass through a 1-mm screen.

### Chemical analyses

All maize particle sizes' fractions were separately analysed for dry matter (DM) (AOAC, 1995, Method 930.15); organic matter (OM) (AOAC method 920.39); crude protein (CP) using a Nitrogen Gas Analyzer FP528 (LECO Africa Pty Ltd, Kempton Park) (AOAC, 2002); amylose-amylopectin using an Amylose/Amylopectin kit (Megazyme Ireland

International, Ltd., Bray, Ireland); amino acids using AccQ-Tag kit (Waters) and Waters Acquity Ultra Performance Liquid Chromatograph (UPLC) in accordance with manufacturer's instructions; crude fat (CF) using a Tecator Soxtec System HT 1043 Extraction Unit (AOAC, 2002; Method 920.39); Fatty Acids using Heptadecanoic acid (C17:0) as internal standard (catalogue number H3500, Sigma-Aldrich, Gauteng, South Africa) and a Thermo TRACE 1300 series gas-chromatograph (Thermo Electron Corporation, Milan, Italy) (Folch e*t al.*, 1957); starch (Hall, 2008) and NDF (Mertens, 2002).

Vitreousness of maize was determined by X-ray scanning of whole maize kernels (Figure 4.1) by X-ray micro-computed tomography scanning using a Phoenix V|Tome|X L240 (General Electric Sensing and Inspection Technologies, Wunstorff, Germany). The 2-D X-ray images were then rendered into 3-D volumes using the integrated Phoenix Datos acquisition and reconstruction software (General Electric Sensing and Inspection Technologies, Wunstorff, Germany).

Lucerne and oat hay were analysed for DM (AOAC, 1995, Method 930.15); OM (AOAC method 920.39); starch (Hall, 2008); crude protein (CP) using a Nitrogen Gas Analyzer FP528 (LECO Africa Pty Ltd, Kempton Park) (AOAC, 2002); crude fat (CF) using a Tecator Soxtec System HT 1043 Extraction Unit (AOAC, 2002; Method 920.39); acid detergent lignin (ADL; (Van Soest and McQueen, 1973); NDF (Mertens, 2002); and *in vitro* NDF digestibility (ivNDFd; (Raffrenato and Van Amburgh, 2010). Specifically, amylase, sodium sulfite and ashing at 550°C were applied to the NDF analyses. Table 4.1 shows composition of the samples used in the study.

***In vitro* fermentations**

The different maize fractions (very fine, fine, medium, coarse, and cracked) were combined with either lucerne or oat hay in order to create combinations with a high or low

starch-to-NDF ratio. Lucerne combined with maize had a starch-to-NDF ratio of 1.75 for high starch and 1.33 for low starch. Oat hay had instead a starch-to-NDF ratio of 2.20 for high starch and 1.67 for low starch. Because of initial analytical problems, the differences between the ratios were smaller than planned. Preliminary observations suggested to increase the starch proportions relative to what found in common dairy cows rations to challenge the buffering capacity of the *in vitro* medium used (Goering and Van Soest, 1970). The starch level was also higher for lucerne to compensate for the stronger lucerne buffering capacity (Jasaitis e*t al.*, 1987). Since particle size was one of the treatments, we could not isolate the starch from the rest of the maize kernel and therefore, both, forages and maize contributed to NDF and starch when the final starch-to-NDF ratios were calculated. Our objective was to measure both NDF and starch disappearance, therefore the fermentations were run in parallel to obtain residues for either starch or NDF measurements. All the combinations were thus analysed for *in vitro* starch (ivSd) and NDF digestibility (ivNDFd; (Goering and Van Soest, 1970; Hall, 2000; Raffrenato and Van Amburgh, 2010). The fermentation controls included either 100% maize, for all sizes, or 100% lucerne or oat hay, and they were analysed only for ivSd and ivNDFd, respectively.

Rumen fluid was collected from two lactating cows at Welgevallen experimental farm of Stellenbosch University. The cows were fed a total mixed ration with maize as the main starch source and NDF mainly from lucerne and wheat straw. All procedures carried out in this experiment were approved by the Research Ethics Committee for Animal Care and Use of Stellenbosch University (protocol number SU-ACUD14-00052). Rumen fluid was collected by hand and transferred into a pre-warmed insulated flask. The rumen fluid was then filtered through 4 layers of cheesecloth, glass wool, and a double layer of 200 µm porosity mesh into another pre-warmed Erlenmeyer flask. Carbon dioxide was pumped into the flask to purge any air. Samples were weighed into 125-ml Erlenmeyer flasks and 40 ml of *in vitro* medium

(adapted from Goering and Van Soest, 1970) was added to each flask. The prepared flasks were then placed in a water bath (39.5°C) and flushed with CO2 before adding rumen fluid. A pre-warmed syringe was used to inject rumen fluid into the flasks. Combinations and controls fermented for ivSd were incubated in duplicate for 3, 6, 9, 12, and 24 h. Longer incubations were not performed since preliminary observations resulted in the majority of starch being depleted at about 24 hours, and resistant starch was not present. For ivNDFd samples were incubated in duplicate for 6, 12, 24, 48, 120 and 240 h. Indigestible NDF (iNDF) was estimated using the undigested residue at 240 h (Raffrenato and Van Amburgh, 2010). All fermentations were completed across 3 runs.

**Statistical analyses**

Rates of NDF and starch digestion were computed using a first order decay model for both fractions according to the following equations:

Eq. 1: $NDF_{(t)} = pdNDF_{(0)} * e^{-kNDF(t-LNDF)} + iNDF$

Eq. 2: $S_{(t)} = S_{(0)} e^{-kS(t-LS)}$

Where $pdNDF_{(0)}$ and $S_{(0)}$ are the size at time 0 of the potentially digestible NDF and starch; $K_{NDF}$ and $K_S$ are the fractional rates of digestion of NDF and starch, respectively; $L_{NDF}$ and $L_S$ are the lags and iNDF is the indigestible NDF. Starch was assumed to be all digestible. Simultaneous estimations of the parameters pdNDF, $K_S$, $K_{NDF}$, iNDF and L were initially obtained using PROC NLIN of SAS (version 9.3; SAS Institute, Inc., Cary, NC) and the Marquardt algorithm. The Marquardt algorithm was selected to improve the efficiency of providing least-squares estimation for the non-linear curve fitting approach. Non-linear regression was chosen as the standard procedure because the method assumes equal error at each observation and by simultaneously fitting all parameters to the data, the result provides the smallest residual sums of squared deviations. The necessity of establishing initial

parameters values for the non-linear estimations was solved using a linear approach to seed the non-linear estimation as done by Grant and Mertens (1992). We used the log-linear approach of Van Soest e*t al.* (2005) to generate the initial values for each sample to parameterize the decay model, including an indigestible pool for the model using 240 h residual NDF to estimate the pdNDF. *In vitro* starch and NDF digestibility values and the rates estimated by nonlinear regression were analysed as response variables by the GLIMMIX procedure of SAS (version 9.3; SAS Institute, Inc., Cary, NC) using a factorial arrangement of maize size, forage, starch-to-NDF ratio, respective interactions and with fermentation run included as random effect. The highest order interaction (forage × time × ratio × size) was removed from the model because non-significant, when starch digestibility was the response variable. The control parameters for starch and NDF were the digestibility and rates of the maize fractions and forages, respectively, when fermented alone. Differences between means and the control were declared significant at $P \leq 0.05$ using the least squares means and the Tukey adjustment. Statistical differences resulting in $0.05 < P \leq 0.10$ were considered tendencies. Treatments results are reported as least squares means unless specified.

### 4.4 Results and discussion

The chemical composition of maize, lucerne and oat hay can be found in Table 4.1. Maize vitreousness was determined by X-ray scanning as this was deemed the most reliable mode of obtaining the results (Figure 4.1). The accuracy of methods, such as manual dissection, rely on the skill of the technician, while x-ray scanning provides a more unbiased result (Louis-Alexandre e*t al.*, 1991). The vitreousness was found to be 70.30% (Table 4.1), which is typical for a dent variety (Corona e*t al.*, 2006). Among the different maize particle sizes, variation existed in starch, NDF, ADL, amylose, CP and EE content. Smaller particles typically contained more starch, but lower NDF, ADL, amylose, CP and EE. These differences can

indicate that certain parts of the maize kernel tend to break into smaller particles while others into larger particles. In other words, the pericarp, germ, or endosperm might be more concentrated in certain particle sizes. For instance, floury endosperm has less protein, more starch and less amylose than vitreous endosperm (Galyean e*t al.*, 1981). Therefore, the chemical composition of very fine and fine maize could be due to the preferential separation of floury endosperm into these particle size. Vitreous endosperm and germ have the highest protein content, and the germ has the highest fat content, it could therefore be assumed that these are separated into particle sizes with higher protein and fat content, such as medium particle size. Most of the fibre in grain is located in the pericarp, coarse and cracked grain had the highest NDF and ADF content and therefore contained the majority of the pericarp. The NDF procedure (Mertens, 2002) includes milling at 1 mm, but to avoid loss of fine particles during milling we preferred to use the same particles' sizes. To increase accuracy and precision during the NDF procedure, we used extra filtering aid (Whatman 934-AH, Whatman International Ltd, Maidstone, England, 1.5 µm pore size) to reduce loss of material and smashed the larger particles using pestle and mortar during the refluxing to release the starch within the coarser particles.

**Table 4.1** Chemical composition of maize and forage samples in % of DM.

| | Component | | | | | | |
|---|---|---|---|---|---|---|---|
| Sample | OM | Starch | NDF | ADL | Amylose | CP | EE |
| Very fine | 99.26 | 77.92 | 5.65 | 0.83 | 19.36 | 7.29 | 2.17 |
| Fine | 99.26 | 74.88 | 11.89 | 0.72 | 22.89 | 9.28 | 2.54 |
| Medium | 99.26 | 69.59 | 15.01 | 1.21 | 25.34 | 9.74 | 3.47 |
| Coarse | 99.26 | 67.35 | 20.56 | 1.75 | 30.88 | 9.35 | 3.78 |
| Cracked | 99.26 | 54.82 | 25.24 | 2.92 | 31.24 | 8.78 | 3.37 |
| Lucerne | 91.59 | 3.97 | 41.19 | 2.70 | N.A. | 18.25 | 1.63 |
| Oat hay | 95.02 | 8.42 | 63.56 | 4.70 | N.A. | 8.95 | 2.17 |

N.A.: not analysed.

The amino acids and fatty acids composition of the different maize particle sizes can be found in Tables 4.2 and 4.3. The CP content was comparable to that found in other research (Philippeau e*t al.*, 2000; Larson and Hoffman, 2008). A high concentration of glutamine and proline were found, which are common constituents of prolamins (Shewry and Halford, 2002c), with only slight differences seen among particle sizes. Prolamins typically make up 7.5% ± 0.52 of maize DM, they are an important storage protein and are negatively related to starch digestibility in maize (Philippeau e*t al.*, 2000). Very little variation was observed between the particle sizes for fatty acids.

**Table 4.2.** Amino acids profiles for all maize fractions.

| Amino acids, mg/g | Very Fine | Fine | Medium | Coarse | Cracked |
|---|---|---|---|---|---|
| His | 2.82 | 2.57 | 2.39 | 2.87 | 2.52 |
| Ser | 4.72 | 4.20 | 3.93 | 4.65 | 4.33 |
| Arg | 5.84 | 4.96 | 5.33 | 5.50 | 4.93 |
| Gly | 3.92 | 3.11 | 3.44 | 3.59 | 3.12 |
| Asp | 4.97 | 5.02 | 4.30 | 4.85 | 4.53 |
| Glu | 14.35 | 14.64 | 12.56 | 15.50 | 14.52 |
| Thr | 2.84 | 3.00 | 2.62 | 3.01 | 2.76 |
| Ala | 5.20 | 5.30 | 4.88 | 5.80 | 5.36 |
| Pro | 7.79 | 6.82 | 7.09 | 7.81 | 7.38 |
| Cys | 0.23 | 0.28 | 0.25 | 0.32 | 0.28 |
| Lys | 1.31 | 1.69 | 1.56 | 1.58 | 1.48 |
| Tyr | 4.24 | 3.10 | 3.46 | 3.57 | 3.40 |
| Met | 1.11 | 1.00 | 1.23 | 1.27 | 0.75 |
| Val | 3.57 | 3.48 | 3.47 | 3.62 | 3.45 |
| Ile | 1.98 | 1.97 | 2.09 | 2.29 | 2.03 |
| Leu | 10.12 | 9.83 | 9.61 | 11.28 | 10.46 |
| Phe | 6.04 | 4.67 | 4.87 | 5.32 | 4.79 |
| Asn | 0.80 | 0.38 | 0.61 | 0.50 | 0.52 |
| Trp | 0.34 | 0.09 | 0.10 | 0.14 | 0.18 |

**Table 4.3.** Fatty acid composition for all maize fractions.

| Fatty acid, % of total | Very Fine | Fine | Medium | Coarse | Cracked |
|---|---|---|---|---|---|
| C16:0 | 14.07 | 14.81 | 15.05 | 13.96 | 14.81 |
| C18:0 | 2.88 | 2.33 | 2.68 | 2.11 | 2.24 |
| C18:1n9c | 27.45 | 26.16 | 24.56 | 26.76 | 26.27 |
| C18:2n6c | 50.64 | 52.19 | 52.20 | 52.73 | 52.03 |
| C18:3n3 | 1.65 | 1.63 | 1.89 | 1.36 | 1.54 |

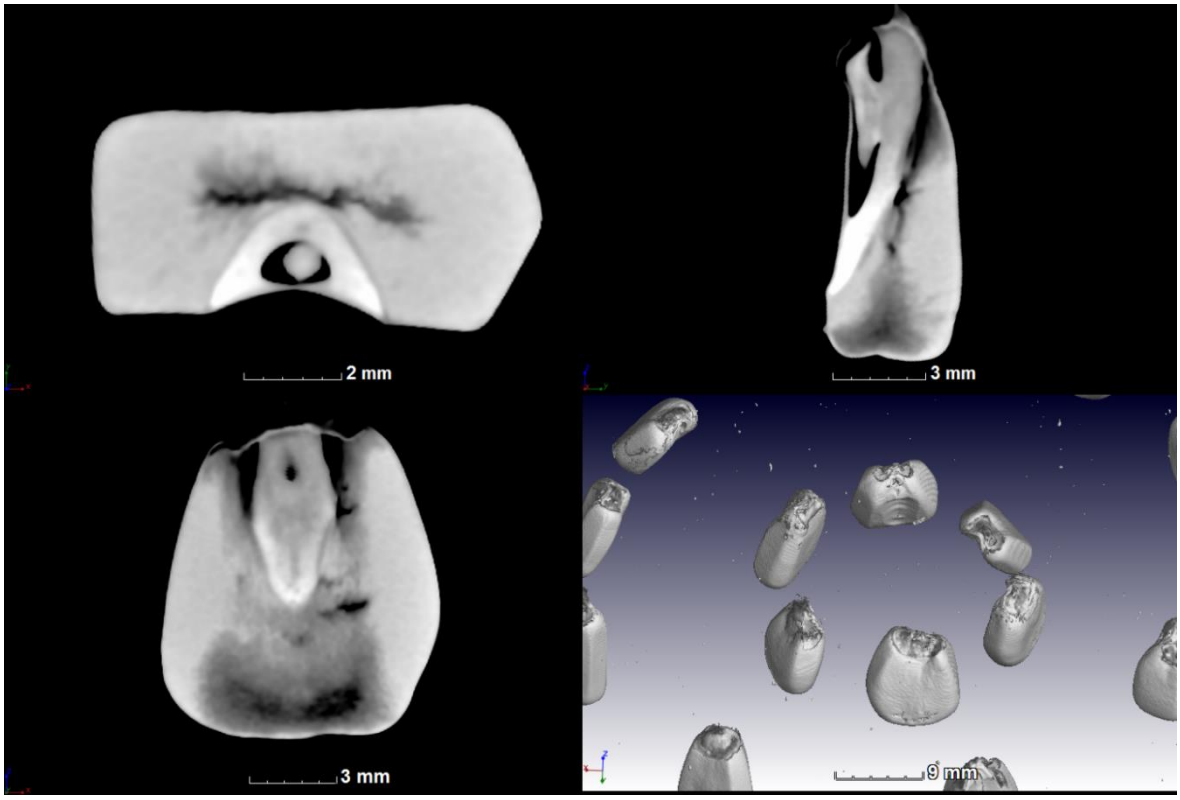


**Figure 4.1.** 2-D X-ray µCT slice image of whole maize kernel depicting external and internal (germ, floury endosperm, vitreous endosperm, and cavities) structures.

***Starch digestion***

For starch digestion, the effect of particle size was significant ($P < 0.05$), but starch level was not ($P = 0.1026$), indicating that particle size had a greater effect on starch digestion than starch-to-NDF ratio. The least squares means of starch digestibility are shown in Figure 4.2. As expected, starch digestibility increased as particle size decreased ($P < 0.05$).

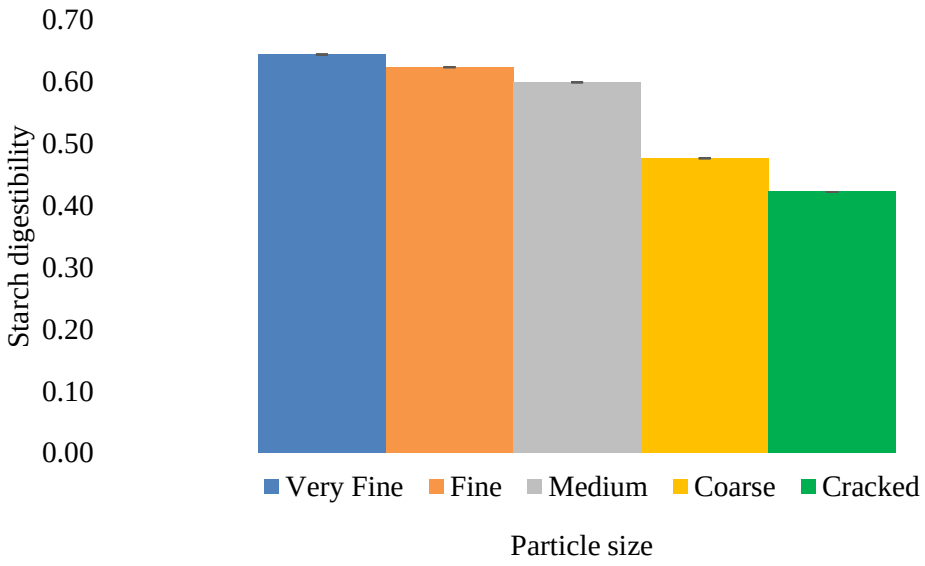


**Figure 4.2.** Least squares means of starch digestibility across maize particle sizes.

The least square means of starch digestibility over 24 hours for maize are shown in Table 4.4.

By 24 hours almost all starch had disappeared, with very fine starch having the highest disappearance (93%; $P < 0.05$). Starch digestibility decreased as particle size increased for each time point, though the difference was not always significant. The greater starch digestibility for finer particles is due to higher degree of access to starch. Starch within coarser particles is contained in granules within the floury and vitreous endosperm and is in places still protected by pericarp making it difficult for microbes to access (Huntington, 1997).

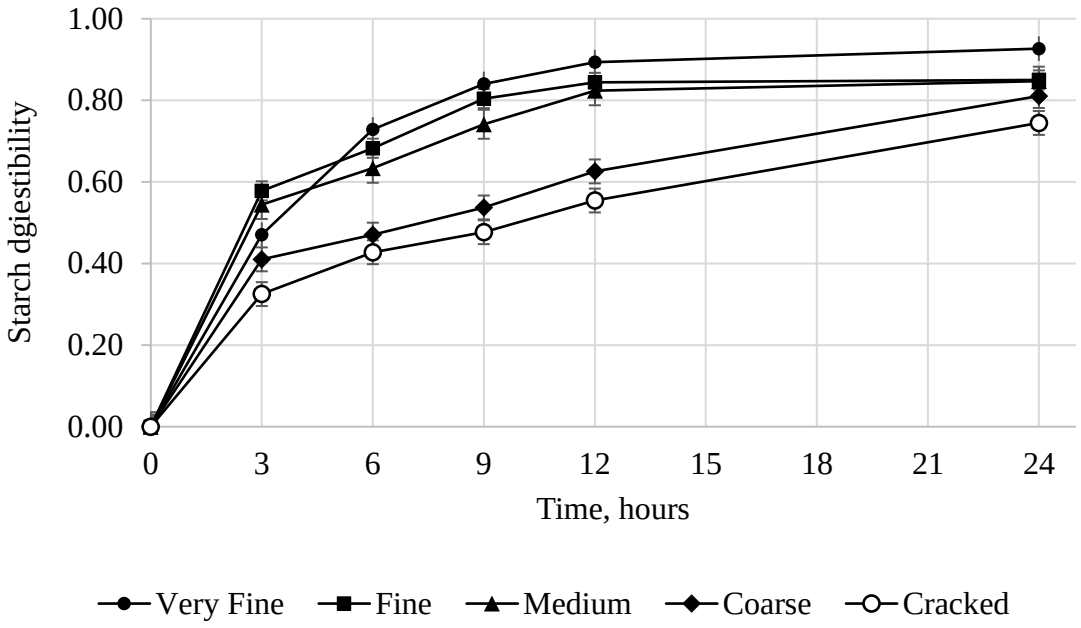


**Figure 4.3.** Least Square means of starch digestibility for the various particle sizes.

**Table 4.4.** Least squares means of starch digestibility across all maize fractions, when pooling forages.

| | Particle size | | | | | | |
|---|---|---|---|---|---|---|---|
| Time, h | Very Fine | Fine | Medium | Coarse | Cracked | SEM | *P*-value |
| 3 | 0.47$^{bc}$ | 0.58$^{a}$ | 0.55$^{ab}$ | 0.41$^{cd}$ | 0.33$^{d}$ | 0.039 | 0.0358 |
| 6 | 0.73$^{a}$ | 0.68$^{a}$ | 0.63$^{a}$ | 0.47$^{b}$ | 0.43$^{b}$ | 0.045 | 0.0009 |
| 9 | 0.84$^{ab}$ | 0.80$^{b}$ | 0.74$^{b}$ | 0.54$^{c}$ | 0.48$^{c}$ | 0.055 | 0.0004 |
| 12 | 0.89$^{ab}$ | 0.84$^{a}$ | 0.82$^{a}$ | 0.63$^{b}$ | 0.55$^{b}$ | 0.034 | 0.0034 |
| 24 | 0.93$^{a}$ | 0.85$^{abc}$ | 0.85$^{abc}$ | 0.81$^{bc}$ | 0.75$^{c}$ | 0.022 | 0.0022 |

$^{a-d}$ Means within a row not sharing a superscript differ ($P < 0.05$).

The effects of size and forage were significant ($P < 0.05$), while the effect of starch-to-NDF ratio was not ($P = 0.1026$). The interaction between particle size and forage was significant ($P < 0.05$) and the interaction particle size x forage x time was highly significant ($P < 0.0001$). The interaction between particle size and forages over time is shown in Table 4.5 and Figures 4.3 and 4.4. Typically, starch digestion was higher for finer particle sizes at all time points, however the differences were not always significant. Surprisingly, when fermented with lucerne, starch digestion of coarse maize was lower than for cracked maize at all time points, however the difference was not significant. The biggest differences are seen when very fine, fine, and medium are compared to coarse and cracked maize, with the finer maize typically having more starch digestion ($P < 0.05$). The finer particles' sizes typically reached maximum starch digestion before larger ones. Very fine, fine and medium maize reached maximum starch digestion between 9 and 12 hours. Coarse and cracked maize continued starch digestion beyond 12 hours. Very fine, fine, and medium maize had higher starch digestion in combination with oat hay. While coarse and cracked maize had higher starch digestion when combined with lucerne, possibly due to the larger amount of protein available, 18.25% CP for lucerne vs. 8.95% CP for oat hay (Table 4.1).

**Table 4.5.** Least squares means of starch digestibility across maize fractions.

| Lucerne | Particle size | | | | | | |
|---|---|---|---|---|---|---|---|
| Time, h | Very Fine | Fine | Medium | Coarse | Cracked | SEM | *P*-value |
| 3 | 0.56 | 0.69 | 0.67 | 0.45 | 0.57 | 0.052 | <0.0001 |
| 6 | 0.82[a] | 0.83[a] | 0.73[a] | 0.46[b] | 0.68[ab] | 0.049 | <0.0001 |
| 9 | 0.87[a] | 0.86[a] | 0.81[a] | 0.49[b] | 0.72[ab] | 0.042 | <0.0001 |
| 12 | 0.91[a] | 0.86[ab] | 0.84[ab] | 0.56[b] | 0.80[ab] | 0.049 | 0.0003 |
| 24 | 0.92 | 0.86 | 0.86 | 0.78 | 0.92 | 0.048 | 0.0012 |
| Oat hay | Particle size | | | | | | |
| Time, h | Very Fine | Fine | Medium | Coarse | Cracked | SEM | *P*-value |
| 3 | 0.50[a] | 0.65[a] | 0.71[a] | 0.52[a] | 0.23[b] | 0.056 | <0.0001 |
| 6 | 0.70[a] | 0.73[a] | 0.75[a] | 0.58[a] | 0.34[b] | 0.033 | <0.0001 |
| 9 | 0.87[a] | 0.83[a] | 0.84[a] | 0.63[b] | 0.41[c] | 0.045 | <0.0001 |
| 12 | 0.94[a] | 0.87[a] | 0.91[a] | 0.71[a] | 0.51[b] | 0.054 | <0.0001 |
| 24 | 0.99[a] | 0.89[ab] | 0.93[ab] | 0.87[ab] | 0.73[ab] | 0.022 | 0.0002 |

[a-c] Means within a row not sharing a superscript differ ($P < 0.05$).

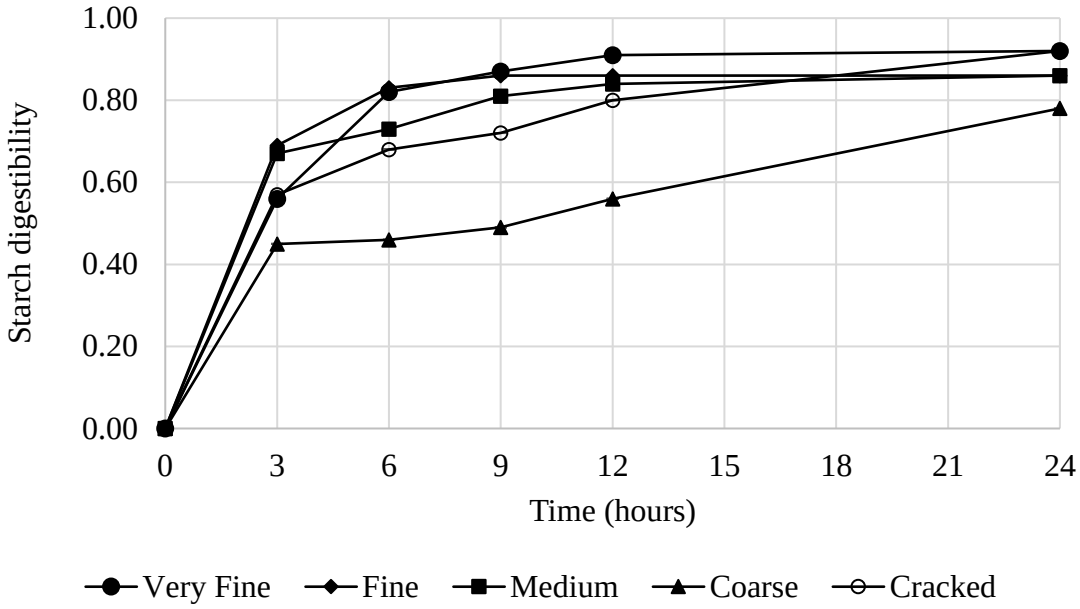


**Figure 4.4.** Least squares means of starch digestibility across all maize particle sizes for lucerne. Significant differences are shown in Table 4.5.

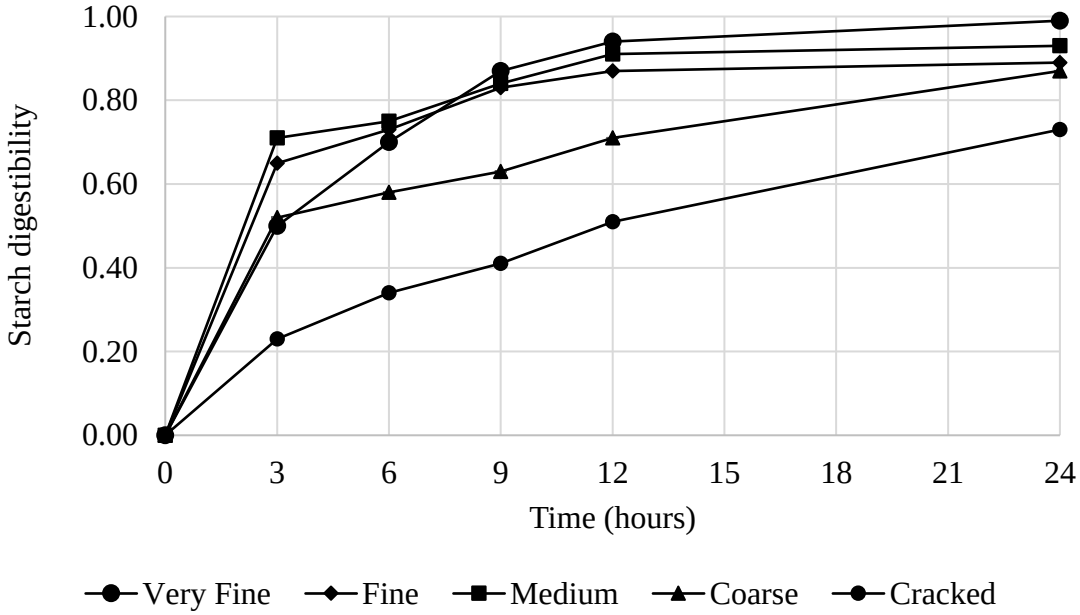


**Figure 4.5.** Least squares means of starch digestibility across all maize fractions for oat hay. Significant differences are shown in Table 4.5.

The effects of maize particle size, combined with either lucerne or oat hay at high or low level of starch on the rate of starch digestion are shown in Table 4.6. For the control, the

rate of starch digestion decreases as particle size increases ($P < 0.05$). This is in agreement with other research indicating that smaller particles are digested faster (Cone, 1991). Gallo e*t al.* (2016) determined the in vitro rate of starch digestibility of maize ground at 2 mm using both gas production and enzymatic methods. The rate of gas production was 8.8 %/h for ground maize when mean particle size was <750 µm; and 5.5 %/h when mean particle size was >750 µm. The *in vitro* rate of starch digestibility for ground maize was 7.76 %/h when mean particle size was <750 µm and 6.6 %/h when mean particle size was >750 µm using enzymatic methods to determine starch digestion (Gallo e*t al.*, 2016). Smaller particles were digested faster than larger particles because of greater degree of enzyme access to starch granules, greater surface area availability for microbial attachment, and lack of protection from pericarp as is seen in coarse and cracked maize (Huntington, 1997).

The addition of forages increased the rate of digestion for all particle sizes. As of yet we can only speculate as the cause of this phenomenon. It is unlikely that pH plays a role as the Van Soest buffer that was used has an excellent buffering capacity. Most likely though this is due to the amount of substrate used for each treatment. In order to create the control (only maize, no forage), and high and low starch-to-NDF combinations, different amounts of maize and forages were used. However, the amount of *in vitro* medium and rumen fluid remained constant for all treatments, resulting in different ratios medium-starch, and could possibly account for the difference in rate of starch digestion. Another explanation could possibly be given by a better micro-environment caused by the presence of more cell wall and fibrolytic bacteria.

According to the Nutritional Dynamic System (NDS, Ru.m.&N. Sas, Reggio Emilia, Italy) software's feed database, which is based on the Cornell Net Carbohydrate and Protein System (CNCPS, v.6.55) rate of starch digestibility is 5 %/h for whole, 10 %/h for cracked, 12

%/h for medium ground, and 15 %/h for finely ground maize. Most feed data set belonging to modern rationing software report rates of starch digestion and only a general description of the size (e.g.: fine, medium, coarse…). Therefore, it is not possible to compare our results to those values. However, our values would definitely be more representative of a grain milled with a specific screen whose particle size distribution is not known. Philippeau e*t al.* (1998) found the rate of *in situ* starch degradability of maize ground at 3 mm to be 8.08 %/h. Their results are similar to what we obtained for coarse maize (1180-2000 µm) 7.16 %/h. There is limited research concerning the rate of starch digestion for finely milled maize, however Sveinbjörnsson e*t al.* (2007) conducted an *in vitro* starch digestibility study, analysed in a similar method to our own, using cooked potato starch (97% starch). After 8 hours of fermentation the rate of starch digestion was determined as 19.7 %/h (Sveinbjörnsson e*t al.*, 2007), which is similar to the results we obtained for very fine maize 18.24 %/h after 24 hours. These results illustrate how difficult it is to compare digestibility across different studies, even when maize is processed similarly, differences in milling conditions, maize quality, and particle size distributions can produce different results.

**Table 4.6.** Least squares means of rate of starch digestion (%/h) for all combinations.

| Forage | Starch level | Particle size | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Very Fine | Fine | Medium | Coarse | Cracked | SEM | *P*-value |
| Lucerne | High | 28.50[a] | 25.73[a] | 19.73[ab] | 8.91[b] | 11.68[b] | 5.434 | 0.002 |
| | Low | 28.41[ab] | 34.30[a] | 23.35[abc] | 10.79[c] | 23.70[abd] | 6.298 | 0.021 |
| Oat hay | High | 24.81[a] | 24.11[a] | 21.87[a] | 14.45[a] | 7.00[b] | 6.875 | 0.001 |
| | Low | 21.31[a] | 22.22[a] | 24.42[a] | 15.51[a] | 6.97[b] | 5.774 | 0.006 |
| Control | | 18.24[a] | 13.48[ab] | 10.02[abc] | 7.16[bc] | 3.77[c] | 4.321 | 0.003 |

[a-d] Means within a row not sharing a superscript differ ($P < 0.05$).

**NDF *digestion***

For NDFd the effects of particle size, starch level, and forage were all significant ($P < 0.05$). The NDFd was the highest when forages were fermented in combination with coarse and cracked maize (Figure 4.6). As expected, very fine maize resulted in the lowest NDFd. However, unexpectedly, medium maize had a numerical lower NDFd than fine maize but the differences between NDFd of very fine, fine and medium particle size maize were not significant ($P = 0.26$). Digestibility of NDF increased as particle size increased, and starch digestibility decreased as particle size increased (Figures 4.2 and 4.6). This is in agreement with Firkins e*t al.* (2001) and Callison e*t al.* (2001), who found NDFd to be inversely related to starch digestibility. The NDFd of lucerne and oat hay are shown in Figure 4.6. Lucerne had higher NDFd at all the time points ($P < 0.05$).

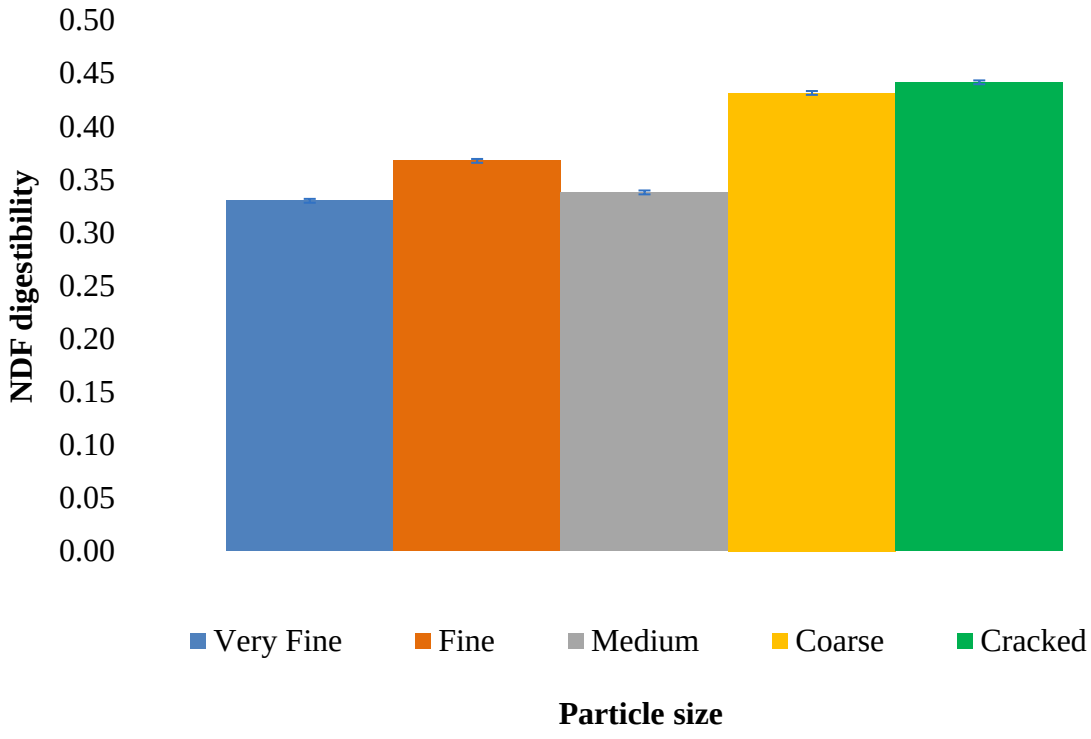


**Figure 4.6** Least squares means of NDF digestibility for pooled forages across maize particle size.

The least squares means of NDFd for pooled forages, alone or with maize, over time are shown in Table 4.7. Compared to the control, the addition of maize increased NDFd at all the time points, though the difference is not always statistically significant ($P > 0.05$). The NDFd values were calculated by estimating the total amount of NDF in the flasks being digested. The NDF digested during the *in vitro* fermentation of the controls (i.e. lucerne or oat hay alone) originates solely from forages. However, when maize and forages were combined and fermented in the same flasks, the NDF included cell wall components from both maize and forages. The different maize fractions contained between 5.65 and 25.24% NDF, from the very fine to cracked fractions, respectively. Interestingly, coarse and cracked maize fractions corresponded to the highest NDFd ($P < 0.05$). This makes it difficult to distinguish the direct effect that maize and starch from maize had on forage NDF digestion and can account for the improvement on NDFd with the addition of maize, as is further evidenced by the fact that NDFd increased as particle size increased and coarser maize had greater NDF content (Table 4.1).

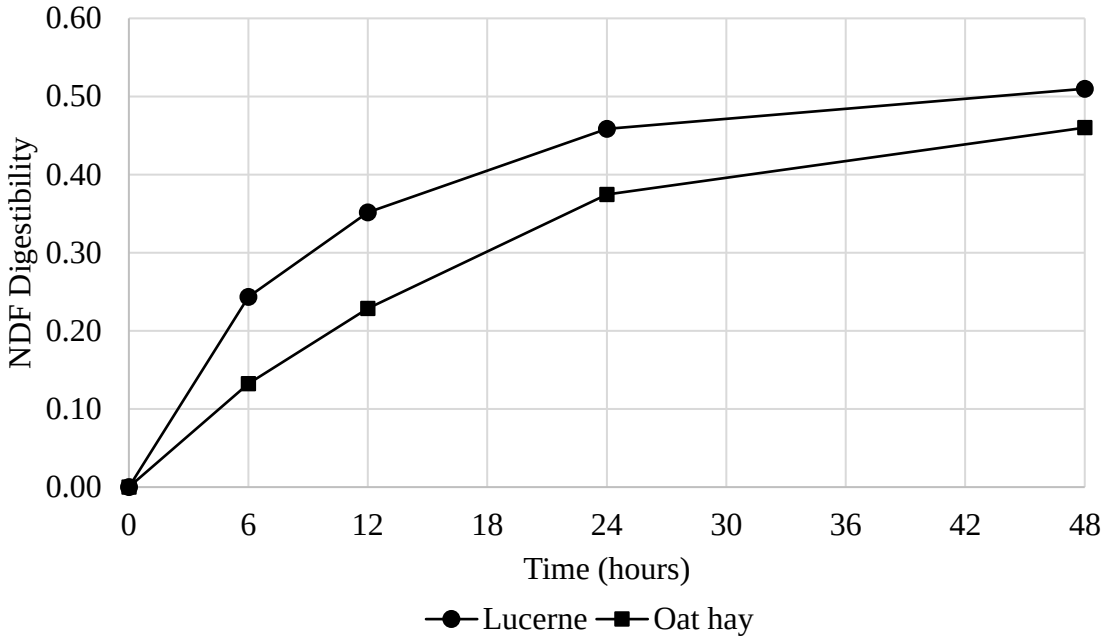


**Figure 4.7.** Least squares means of NDF digestibility for lucerne and oat hay, for pooled maize particle size.

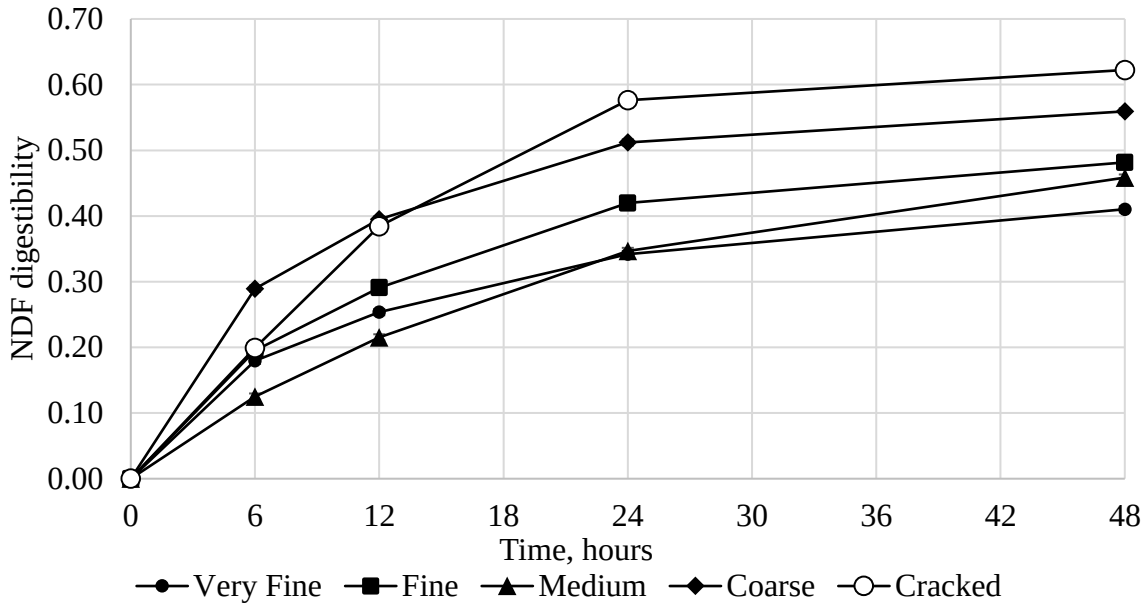


**Figure 4.8.** Least squares means of NDF digestibility for pooled forages across maize particle size used.

**Table 4.7.** Least squares means* of NDF digestibility, for pooled forages, across maize fractions.

| | Particle size | | | | | | SEM | *P*-value |
|---|---|---|---|---|---|---|---|---|
| Time, h | Very Fine | Fine | Medium | Coarse | Cracked | Control* | | |
| 6 | 0.18$^{c}$ | 0.20$^{bc}$ | 0.13$^{c}$ | 0.29$^{a}$ | 0.20$^{b}$ | 0.14$^{c}$ | 0.0083 | <0.0001 |
| 12 | 0.25$^{c}$ | 0.29$^{b}$ | 0.22$^{d}$ | 0.40$^{a}$ | 0.38$^{a}$ | 0.20$^{c}$ | 0.0095 | <0.0001 |
| 24 | 0.34$^{d}$ | 0.42$^{c}$ | 0.35$^{d}$ | 0.51$^{b}$ | 0.58$^{a}$ | 0.30$^{e}$ | 0.0112 | <0.0001 |
| 48 | 0.41$^{e}$ | 0.48$^{c}$ | 0.46$^{d}$ | 0.56$^{b}$ | 0.62$^{a}$ | 0.38$^{f}$ | 0.0099 | <0.0001 |

$^{a-f}$ Means within a row not sharing a superscript differ ($P < 0.05$).
*Control values represent the least squares means for pooled forages in absence of maize

The least squares means of NDFd for lucerne, alone or combined with maize, can be found in Table 4.8. As in Table 4.7, the addition of maize increased NDFd compared to the control across all particle sizes and for most time points ($P < 0.05$). Larger particle sizes corresponded to higher NDFd digestibility and the differences become more pronounced at later time points ($P < 0.05$).

The least squares means of NDFd for oat hay, alone or combined with maize, can be found in Table 4.8. Unlike lucerne, the addition of maize only improved NDFd of oat hay when in combination with larger particle size ($P < 0.05$). We speculate that lucerne has a greater buffering capacity than oat hay, this could possibly explain why NDFd did not improve for oat hay in combination with smaller particles. Furthermore, lucerne had greater NDFd than oat hay across all treatments ($P < 0.05$). For all forages the addition of a slowly digesting starch source, such as cracked or coarse maize, increased the NDFd considerably. Opatpatanakit e*t al.* (1995) observed a similar effect when sorghum was incubated with lucerne. They hypothesised that the slow rate at which sorghum is fermented provided energy to cellulolytic bacteria, which increased their fermentation rate, without there being substrate competition or changes in pH (Opatpatanakit e*t al.*, 1995). During our experiments we could not measure the pH within the flasks for logistics reasons, since we should have had extra flasks within the same run. Measuring the pH would have then prevented us to run all the time points within the same fermentation and thus biasing the results.

The rate of NDFd for all combinations can be found in Table 4.9. The rate of NDFd for lucerne was 5.11 %/h, and 3.36 %/h for oat hay, which are similar to results found in the literature. *In vitro* gas production studies determined fractional NDF digestibility rate of isolated NDF to be 5.30 %/h for immature and 6.30 %/h for mature lucerne, and lag was 0.4 and 4.2 h for mature and immature lucerne, respectively, which probably affected the rates

results for that study (Doane e*t al.*, 1997). In our study, lag was estimated by the non-linear regressions but it is not reported, as it did not differ across treatments ($P$ = 0.662). The $K_{NDF}$ for oat grass *in situ* was determined to be 3.69 %/h (Khan e*t al.*, 2006). *In vitro* gas production studies determined the rate of digestion of oat hay NDF to be 2.20 %/h, and 3.70 %/h for the whole forage (Calabro e*t al.*, 2005).

Compared to the control, the addition of very fine maize reduced the rate of NDFd. For all other particle sizes, the addition of maize increased the rate of NDFd ($P < 0.05$), except for medium maize and lucerne at low starch level which has unexpectedly low rate of NDFd. This is in contrast with previous research where the addition of starch negatively affects the $K_{NDF}$ of forages (Grant and Mertens, 1992; Oba and Allen, 2003). In a study by Grant (1994) the $K_{NDF}$ of lucerne decreased from 7.12 %/h to 5.90 %/h when ground maize with a particle size of <250µm was added, and 4.10 %/h when pure corn starch was added. In contrast to this Beckmann and Weiss (2005) found that when the confounding effects of DMI was removed starch had no effect on NDF digestibility *in situ.* However, as mentioned previously the residues obtained during NDF analysis originate not only from forages but also from maize. Even though the proportion of NDF coming from maize is small it is highly digestible and can explain the increase in $K_{NDF}$ of the treatments compared to the control. In the study by Grant (1994), maize was milled and passed through a 250-µm sieve in order to remove most of the NDF (Grant, 1994). The results from Grant (1994) agree with our hypothesis that some of the NDF originated from maize. In fact, pure starch decreased rate of NDF digestion more (4.10 %/h) than when maize ground at <250 µm was used (5.90 %/h).

**Table 4.8.** Least squares means of NDF digestibility across particle size for forages

| Lucerne | Particle size | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Time. h | Very Fine | Fine | Medium | Coarse | Cracked | Control | SEM | *P*-value |
| 6 | 0.28$^{b}$ | 0.25$^{c}$ | 0.18$^{d}$ | 0.32$^{a}$ | 0.23$^{c}$ | 0.20$^{d}$ | 0.0086 | <0.0001 |
| 12 | 0.36$^{b}$ | 0.37$^{b}$ | 0.28$^{c}$ | 0.41$^{a}$ | 0.44$^{a}$ | 0.25$^{d}$ | 0.0077 | <0.0001 |
| 24 | 0.41$^{d}$ | 0.47$^{c}$ | 0.37$^{e}$ | 0.53$^{b}$ | 0.64$^{a}$ | 0.34$^{f}$ | 0.0085 | <0.0001 |
| 48 | 0.42$^{e}$ | 0.52$^{c}$ | 0.45$^{d}$ | 0.60$^{b}$ | 0.67$^{a}$ | 0.40$^{e}$ | 0.0056 | <0.0001 |
| Oat hay | | | | | | | | |
| Time. h | Very Fine | Fine | Medium | Coarse | Cracked | Control | SEM | *P*-value |
| 6 | 0.08$^{d}$ | 0.14$^{c}$ | 0.07$^{e}$ | 0.26$^{a}$ | 0.17$^{b}$ | 0.08$^{d}$ | 0.0074 | <0.0001 |
| 12 | 0.14$^{d}$ | 0.21$^{c}$ | 0.16$^{d}$ | 0.38$^{a}$ | 0.33$^{b}$ | 0.15$^{d}$ | 0.0066 | <0.0001 |
| 24 | 0.28$^{d}$ | 0.38$^{b}$ | 0.33$^{c}$ | 0.49$^{a}$ | 0.51$^{a}$ | 0.27$^{d}$ | 0.0045 | <0.0001 |
| 48 | 0.40$^{d}$ | 0.44$^{c}$ | 0.46$^{c}$ | 0.52$^{b}$ | 0.58$^{a}$ | 0.35$^{e}$ | 0.0053 | <0.0001 |

$^{a-f}$ Means within a row not sharing a superscript differ ($P < 0.05$).

**Table 4.9.** Least squares means of rate of NDF digestion for all combinations.

| Forage | Starch level | Particle size | | | | | | SEM | *P*-value |
|---|---|---|---|---|---|---|---|---|---|
| | | Very Fine | Fine | Medium | Coarse | Cracked | Control | | |
| Lucerne | High | 4.73$^{d}$ | 8.03$^{ab}$ | 6.17$^{c}$ | 7.29$^{b}$ | 9.07$^{a}$ | 5.11$^{cd}$ | 0.588 | <0.0001 |
| | Low | 5.79$^{bc}$ | 6.82$^{b}$ | 1.81$^{d}$ | 6.34$^{b}$ | 9.44$^{a}$ | 5.11$^{c}$ | 0.466 | <0.0001 |
| Oat hay | High | 3.21$^{c}$ | 4.08$^{c}$ | 3.97$^{c}$ | 6.78$^{b}$ | 8.90$^{a}$ | 3.36$^{c}$ | 0.623 | <0.0001 |
| | Low | 3.33$^{b}$ | 3.32$^{b}$ | 3.20$^{a}$ | 5.13$^{a}$ | 5.89$^{a}$ | 3.36$^{b}$ | 0.334 | <0.0001 |

$^{a\text{-}d}$ Means within a row not sharing a superscript differ ($P < 0.05$).

## 4.6 Conclusion

Studies similar to ours exist in the literature. However, grains were usually milled with two or more screens of different size and all the resulting products were tested in the specific study. The only difference in those studies was therefore particle distribution and the products obtained were chemically identical. Our aim was however to separate the fractions more accurately of the same ground maize using narrow ranges of sieves, and assuming heterogeneous chemical fractions. The chemical composition of the individual fractions concurs with our hypothesis, especially relative to NDF, starch, lignin and amylose amounts. All these chemical constituents, together with the particle size have likely affected our results. The results clearly show that maize particle size has an important influence on both starch and fibre digestion. As mentioned, different maize particle sizes differed not only in starch content but also greatly in NDF. Particle size influenced both rate and extent of starch digestion of maize, with smaller particles having the greatest digestion. We clearly demonstrated that rate estimation of maize ground at 2 or 4 mm cannot be extremely accurate and precise unless particle size distributions are measured and rates of starch digestion are estimated in more than one particle size range. Furthermore, there exists a possibility of fine tuning diets for starch digestion by incorporating particle size as a factor to better characterize starch. This study focused only on maize. Results for other grains can however differ. Given the large ranges found for rates of NDF and starch digestions in our study, a more accurate knowledge of the specific particles sizes fed of grains would likely result in a more accurate prediction of the starch digested in the rumen. Also, by feeding a narrow and known range of particles size, we would be able to better formulate diets for specific physiological stages.

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# Chapter 5

## Effects of amylose and amylopectin starch and starch-to-fibre ratio on *in vitro* starch and NDF degradability.

### 5.1 Abstract

Proportions of starch type (amylose/amylopectin) impact fermentability of grains in the rumen. The purpose of this study was to isolate and determine the specific effects of amylose and amylopectin on rate and extent of starch digestion, when combined with two forages and at different levels. Two different starch sources were used with different amounts of amylose, Hylon VII (74% amylose starch) and Amioca (98% amylopectin starch). Both samples were combined with either lucerne or oat hay in order to create combinations of either high or low starch-to-NDF ratios. The samples were analysed for *in vitro* starch and NDF digestibility. Amioca had the greatest starch digestibility and the addition of forages increased starch digestion. Rate of starch digestion was 12.55 %/h and 6.13 %/h for Amioca and Hylon respectively and it was influenced by starch, and forage type, but not by starch level. Neutral detergent fibre digestion was influenced by forage and starch type. Lucerne had the greatest NDFd and the addition of starch reduced NDFd of forages. Forages in combination with Amioca had the lowest NDFd. The $K_{NDF}$ was 7.35%/h for Lucerne and 3.87%/h for Oat hay. The rate of NDFd for oat hay was not influenced by starch or starch type, 3.15 %/h for Amioca and 3.30 %/h for Hylon. Rate of NDF digestion for lucerne was significantly reduced by the addition of starch, 7.07 %/h for Amioca and 5.88 %/h for Hylon. By knowing the exact proportions of each starch type in grains we can better characterise the starch fraction digestion characteristics. When other factors affecting starch digestion are known, a well-defined amylose-amylopectin ratio can be obtained to better quantify, and

adjust if needed, the speed of starch digestion in the rumen, which can easily affect rumen health in dairy cows.

## 5.2 Introduction

In diets of high performing ruminants', starch functions as a source of energy and is commonly provided by cereals such as maize, wheat, barley or sorghum. Among the various factors affecting the digestibility of grains is the type of starch (i.e. amylose or amylopectin) contained within the starch granules of the endosperm (Huntington e*t al.*, 2006).

Starch is a polysaccharide molecule comprised of α–D-glucose units (Tester et al., 2004). Amylose is a linear molecule consisting of (1-4) linked α-D-glucopyranosyl units (Buléon et al., 1998a). Amylopectin is a highly branched molecule and is formed through chains of α-D-copyranosyl residues linked by (1-4) linkages and (1-6) linkages (Buléon et al., 1998a). Amylose, as a percentage of total starch, was found to be 3-46% in barley (Åkerberg et al., 1998), 0-70% in maize (Morrison et al., 1984b), 3-31% in wheat, and 0-30% in sorghum (Beta et al., 2001; Sang et al., 2008).

Starch occurs naturally in highly organised water insoluble granules contained within the endosperm of grain kernels (Pflugfelder, 1986; Huntington, 1997). Starch granules are created by depositing starch in layers containing various amounts of amylose and amylopectin held together by hydrogen bonds, the layers alternate between semi-crystalline and amorphous in nature (Buléon et al., 1998b). The crystalline regions are quite impervious to water, while the amorphous region allow free movement of water (Pflugfelder, 1986; Nocek and Tamminga, 1991a).

The ratio of amylose to amylopectin has proved to influence the digestibility of grains (Sajilata et al., 2006). Stevnebø e*t al.* (2006) investigated the effect of amylose level of barley starch on *in vitro* rumen digestibility. They found that cultivars with low amylose levels had

higher starch digestion than normal or high amylose cultivars, for both isolated starch and ground samples (Stevnebø et al., 2006). The reason that amylopectin is more readily digested then amylose is because amylose has tighter intermolecular bonding between starch molecules (Buléon et al., 1998a). This leads to a more compacted structure of the starch granules in the endosperm. Therefore, grains with greater proportions of amylopectin have greater rumen starch and total tract starch digestion.

However, various other factors, such as nutritional value, genetics, variety, geographical locations, year, climatic conditions and agronomic practices, can influence the digestibility of grains (Huntington, 1997; Offner et al., 2003). Therefore, the results of previous studies may be confounded by these factors and their interactions. The direct effect of amylose and amylopectin on starch digestion thus needs clarification.

The aim of this study was to isolate and determine the specific effects of amylose and amylopectin on rate and extent of starch digestion. Furthermore, it is known that starch digestion negatively affects fibre digestion (Grant and Mertens, 1992; Oba and Allen, 2003). However, the majority of research does not distinguish between amylose and amylopectin starch on fibre digestion. Therefore, our objective was to determine how amylose and amylopectin starch affects NDF digestibility *in vitro*, according to the starch-to-NDF ratio and to forage type.

Usually it is also assumed that starch digestion is not limited by other substrates. However, we wish to examine the effect of forage type and different ratios of starch-to-NDF will have on rate and extent of starch digestion.

## 5.3 Materials and Method

### Substrates

Starch samples included Hylon VII Powder (74% amylose starch) and Amioca powder (98% amylopectin starch). Samples were provided by Ingredion (Ingredion Germany, GmbH – Hamburg, Germany). Forages, lucerne and oat hay, were milled using a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) to pass through a 1-mm screen.

### Chemical Analyses

Starch samples were analysed for dry matter (DM) (AOAC, 1995, Method 930.15); organic matter (OM) (AOAC method 920.39); crude protein (CP) using a Nitrogen Gas Analyzer FP528 (LECO Africa Pty Ltd, Kempton Park) (AOAC, 2002); crude fat (CF) using a Tecator Soxtec System HT 1043 Extraction Unit (AOAC, 2002; Method 920.39); starch (Hall, 2008) and neutral detergent fibre (NDF) (Mertens, 2002).

Lucerne and oat hay were analysed for DM (AOAC, 1995, Method 930.15), OM (AOAC method 920.39), starch (Hall, 2008), acid detergent lignin (ADL; (Raffrenato and Van Amburgh, 2011) and NDF (Mertens, 2002). Specifically, amylase, sodium sulfite and ashing at 550°C were applied to the NDF analyses. Table 5.1 shows composition of the samples used in the study.

**Table 5.1** Chemical composition of starch and forage samples used on dry matter (DM) basis.

| Material | OM% | Starch% | NDF% | ADL% | Amylose% | CP% | EE% |
|---|---|---|---|---|---|---|---|
| Hylon | 99.56 | 78.22 | 0.84 | N.A. | 74.00 | 0.93 | 0.11 |
| Amioca | 99.63 | 87.03 | 0.73 | N.A. | 2.00 | 0.78 | 0.27 |
| Lucerne | 91.59 | 3.97 | 41.19 | 2.7 | N.A. | 18.25 | 1.63 |
| Oat hay | 95.02 | 8.42 | 63.56 | 4.7 | N.A. | 8.95 | 2.17 |

N.A.: not analysed

***In vitro* fermentation**

Hylon and Amioca were combined with forages (lucerne and oat hay) in order to create either a high starch (60% starch - 40% NDF) or a low starch (50% starch - 50% NDF) to NDF ratio. Starch content, relative to NDF, was planned to be higher than real *in vivo* situation to attempt to challenge the well buffered *in vitro* system. Combinations with lucerne had a starch-to-NDF ratio of 1.30 for high starch and 1.88 for low starch. Combinations with oat hay had a starch-to-NDF ratio of 1.67 for high starch and 3.06 for low starch. Because of initial analytical problems, the differences between the ratios were different than planned.

Since our objective was to measure both NDF and starch disappearance, the fermentations were run in parallel to obtain flasks for either starch or NDF measurements. All the combinations were therefore analysed for *in vitro* starch digestibility (ivSd) and *in vitro* NDF digestibility (ivNDFd) (Goering and Van Soest, 1970; Hall, 2000; Raffrenato and Van Amburgh, 2010). The fermentation controls included either 100% Hylon or Amioca; or 100% lucerne or oat hay, and they were analysed for ivSd and ivNDFd, respectively.

Rumen fluid was collected and mixed from two lactating cows at Welgevallen experimental farm of Stellenbosch University. The cows were fed a total mixed ration (TMR) with about 26% starch on dry matter basis and with maize being the main starch source and

containing both amylose and amylopectin. All procedures carried out in this experiment were approved by the Research Ethics Committee: Animal Care and Use (REC: ACU) at Stellenbosch University (protocol number SU-ACUD14-00052). Rumen fluid was collected by hand and transferred into a pre-warmed insulated flask. The rumen fluid was then filtered through 4 layers of cheesecloth, glass wool, and a double layer of 200 µm porosity mesh into another pre-warmed Erlenmeyer flask. Carbon dioxide was pumped into the flask to purge any air. Samples were weighed into 125-ml Erlenmeyer flasks and 40 ml of *in vitro* medium (adapted from Goering and Van Soest, 1970) was added to each flask. The prepared flasks were then placed in a water bath (39.5°C) and flushed with CO2 before adding rumen fluid. A pre-warmed syringe was used to inject rumen fluid into the flasks. Combinations and controls fermented for ivSd were incubated in duplicate for 3, 6, 9, 12, and 24 h. Longer incubations were not performed as the majority of starch is depleted at 24 hours. For ivNDFd samples were incubated in duplicate for 0, 6, 12, 24, 48, 120 and 240 h and residual ash free NDF of the fermented samples were obtained (Mertens, 2002; Goering and Van Soest, 1970). Digestibility values obtained at 240 h were used as estimated indigestible NDF (iNDF) as suggested by Raffrenato and Van Amburgh (2010). All fermentations were completed across 3 runs.

**Statistical Analyses.**

Rates of NDF and starch digestion were computed using a first order decay model for both fractions according to equations 1 and 2 below, respectively:

Eq. 1: $NDF_{(t)} = pdNDF_{(0)} * e^{-kNDF(t-LNDF)} + iNDF$

Eq. 2: $S_{(t)} = S_{(0)}\, e^{-kS(t-LS)}$

Where $pdNDF_{(0)}$ and $S_{(0)}$ are the size at time 0 of the potentially digestible NDF and starch; $K_{NDF}$ and $K_S$ are the fractional rates of digestion of NDF and starch, respectively; $L_{NDF}$ and $L_S$ are the lags and iNDF is the indigestible NDF. Starch was assumed to be all digestible.

Simultaneous estimations of the parameters pdNDF, $K_S$, $K_{NDF}$, $L_{NDF}$, $L_S$ and iNDF were initially obtained using PROC NLIN of SAS (SAS Institute, Inc., Cary, NC) and the Marquardt algorithm. The Marquardt algorithm was selected to improve the efficiency of providing least-squares estimation for the non-linear curve fitting approach. Non-linear regression was chosen as the standard procedure because the method assumes equal error at each observation and by simultaneously fitting all parameters to the data, the results provide the smallest residual sums of squared deviations. The necessity of establishing initial parameters values for the non-linear estimations was solved using a linear approach to seed the non-linear estimation as done by Grant and Mertens (1992). We used the log-linear approach of Van Soest e*t al.* (2005) to generate the initial values for each sample to parameterize the decay model, including an indigestible pool for the model using 240 h residual NDF to estimate the pdNDF. *In vitro* starch and NDF digestibility values and rates estimated by nonlinear regressions were analysed as response variables by the GLIMMIX procedure of SAS using a factorial arrangement of Starch type, forage, starch:NDF ratio, all resulting interactions and fermentation run as a random effect. The highest order interactions (forage × time × ratio × starch type for Sd and NDFd; forage × ratio × starch type for $K_S$ and $K_{NDF}$) were removed because non-significant. The 2$^{nd}$ order interactions were also removed when NDFd was the response variable because non-significant. The control parameters for starch and NDF were the digestibility and rates of the starch types and forages, respectively, when fermented alone. Differences between means and the control were declared significant at $P \leq 0.05$ using the least squares means and the Tukey adjustment. Statistical differences resulting in $0.05 < P \leq 0.10$ were considered tendencies. Treatments results are reported as least squares means unless specified.

## 5.4 Results and discussion

As shown above (Table 5.1), Hylon and Amioca had a starch content of 78.22% and 87.03%, respectively. Both NDF and ADL content in Hylon and Amioca were too small to be detected and therefore are not reported. Crude protein, fat and fibre content for both starch types were so small as to have little effect on subsequent digestibility. Thus Hylon and Amioca are very similar in every respect, except starch type. Hylon and Amioca contained 74% and 2% amylose, respectively. Differences in digestibility between the two can therefore be attributed to interaction between amylose and amylopectin and digestion.

### *Starch digestion*

The starch digestibility of Hylon and Amioca (Table 5.2) differed significantly ($P < 0.05$) at every time point, with Amioca having greater starch digestibility at every time point except 24 hours where Hylon had greater starch digestibility. This is in agreement with the literature, confirming that amylose has lower starch digestibility than amylopectin (Li e*t al.*, 2004; Stevnebø e*t al.*, 2009). The lower starch digestibility of amylose has been attributed to tighter intermolecular bonding between starch molecules (Corona e*t al.*, 2006). It can be assumed that once these intermolecular bonds have been broken starch is rapidly digested, which is a possible explanation for the large increase in ivSd between hours 12 and 24 for Hylon.

**Table 5.2** Least squares means* of starch digestibility of Hylon and Amioca, when pooling forages.

| | Time | | | | | | |
|---|---|---|---|---|---|---|---|
| | 3 | 6 | 9 | 12 | 24 | SEM | *P*-value |
| Amioca | 0.53[d] | 0.73[c] | 0.81[b] | 0.84[a] | 0.85[a] | 0.012 | <0.0001 |
| Hylon | 0.43[e] | 0.52[d] | 0.63[c] | 0.79[b] | 0.90[a] | 0.018 | <0.0001 |

[a-d] Means within a row not sharing a superscript differ ($P < 0.05$).
*Values represent the least squares means across oat hay and lucerne in combination with each starch type

The interaction starch type x forage x time was highly significant ($P < 0.0001$) (Table 5.3). When Hylon and Amioca were combined with forages starch digestibility improved ($P < 0.05$). As of yet we can only speculate as to the cause of this phenomenon. It could indicate that amylolytic bacteria thrives or starch digestion is improved by the presence of a fibre source *in vitro*. It is unlikely that pH plays a role as the Goering and Van Soest (1970) buffer that was used has an excellent buffering capacity. Most likely though this is due to the amount of substrate used for each treatment. In order to create the control (only Hylon or Amioca, no forage) different amounts of starch and forages were used, to have a relatively constant amount of sample within each flask. However, the amount of *in vitro* medium and rumen fluid remained constant for all treatments and could possibly account for the difference in starch digestion. Another explanation could possibly be given by a better micro-environment caused by the presence of more cell wall and fibrolytic bacteria. Starch digestibility of Amioca was not influenced by type of forage, Amioca in combination with lucerne did not differ significantly from Amioca with oat hay ($P > 0.05$). Hylon, however, was influenced by forage type, with the combination of Hylon and oat hay having higher starch digestion ($P < 0.05$).

**Table 5.3** Starch digestibility of Hylon and Amioca

| | Amioca | | | Hylon | | | | |
|---|---|---|---|---|---|---|---|---|
| Time (hours) | Control* | Lucerne | Oat hay | Control* | Lucerne | Oat hay | SEM | *P*-value |
| 3 | 0.22$^{d}$ | 0.69$^{a}$ | 0.68$^{a}$ | 0.20$^{d}$ | 0.50$^{b}$ | 0.58$^{c}$ | 0.023 | <0.0001 |
| 6 | 0.54$^{d}$ | 0.85$^{a}$ | 0.79$^{b}$ | 0.28$^{c}$ | 0.60$^{d}$ | 0.67$^{e}$ | 0.027 | <0.0001 |
| 9 | 0.67$^{b}$ | 0.89$^{a}$ | 0.88$^{a}$ | 0.43$^{c}$ | 0.68$^{b}$ | 0.77$^{d}$ | 0.022 | <0.0001 |
| 12 | 0.70$^{c}$ | 0.91$^{a}$ | 0.90$^{a}$ | 0.64$^{b}$ | 0.85$^{b}$ | 0.90$^{a}$ | 0.032 | <0.0001 |
| 24 | 0.73$^{c}$ | 0.91$^{b}$ | 0.91$^{b}$ | 0.77$^{c}$ | 0.98$^{a}$ | 0.94$^{ab}$ | 0.030 | <0.0001 |

$^{a-e}$ Means within a row not sharing a superscript differ ($P < 0.05$).
*Control values represent the least squares means of Hylon and Amioca in absence of oat hay and lucerne

The fractional rate of starch digestion ($K_S$) for Hylon and Amioca differed significantly ($P < 0.05$) with Amioca having a higher $K_S$ (Table 5.4). The rate of starch digestion was significantly influenced by starch ($P < 0.0001$) and forage ($P < 0.0001$). However, starch level ($P < 0.4291$) and the interaction between starch level and type of starch ($P = 0.8414$) was not significant. The fixed effect of forage was highly significant ($P < 0.0001$) and the interaction between starch type and forage was significanct ($P = 0.0052$). As can be seen in Table 5.4 the addition of forages greatly increased the $K_S$ for both Hylon and Amioca ($P < 0.05$) and, as previously mentioned, this could be due to the amount of substrate in relation to *in vitro* medium and rumen fluid. Amioca had a higher $K_S$, regardless of forage type. Hibberd e*t al.* (1982) found the 6 hour *in vitro* gas production of isolated starch from maize and sorghum with lower amylose content to have more starch digestion. Phillipeau e*t al.* (1998) measured the *in situ* rate of starch digestion of ground maize as 22.9, 6.7, and 5.8%/h with amylose content of 8.1, 22.6, and 46.8% amylose as a percentage of starch, respectively. Research comparing the $K_S$ of isolated starches differing in amylose content are limited.

**Table 5.4** Least squares means of fractional rates (%/h) of starch digestion for Hylon and Amioca in combination with forages and when fermented alone (controls), pooled for different starch levels.

| | Amioca | Hylon | SEM | *P*-value |
|---|---|---|---|---|
| Lucerne | 30.57[a] | 17.10[b] | 1.44 | <0.0001 |
| Oat hay | 25.81[a] | 18.24[b] | 1.24 | <0.0001 |
| Control* | 12.55[a] | 6.13[b] | 2.13 | <0.0001 |

[a-b] Means within a row not sharing a superscript differ ($P < 0.05$).
*Control values represent the least squares means of Hylon and Amioca in absence of oat hay and Lucerne

### *NDF* digestion

The fixed effect of forages, as well as the interaction between forage and time were highly significant ($P < 0.0001$). The NDFd of lucerne and oat hay, for pooled starch types, are shown in Figure 5.1. Lucerne had higher NDFd at all time points ($P < 0.05$).

The fixed effect of starch and the interaction starch x forage was significant ($P < 0.05$). The addition of Hylon and Amioca reduced NDFd for both forages, though not significantly (Table 5.5) ($P > 0.05$). This is in agreement with literature stating NDFd to be inversely related to the starch digestibility (Callison et *al.*, 2001; Firkins et *al.*, 2001). Amioca had greater starch digestibility and consequently had a greater negative effect on NDFd than Hylon ($P < 0.05$). Thus amylopectin reduced NDFd of forages to a greater extent than amylose ($P < 0.05$).

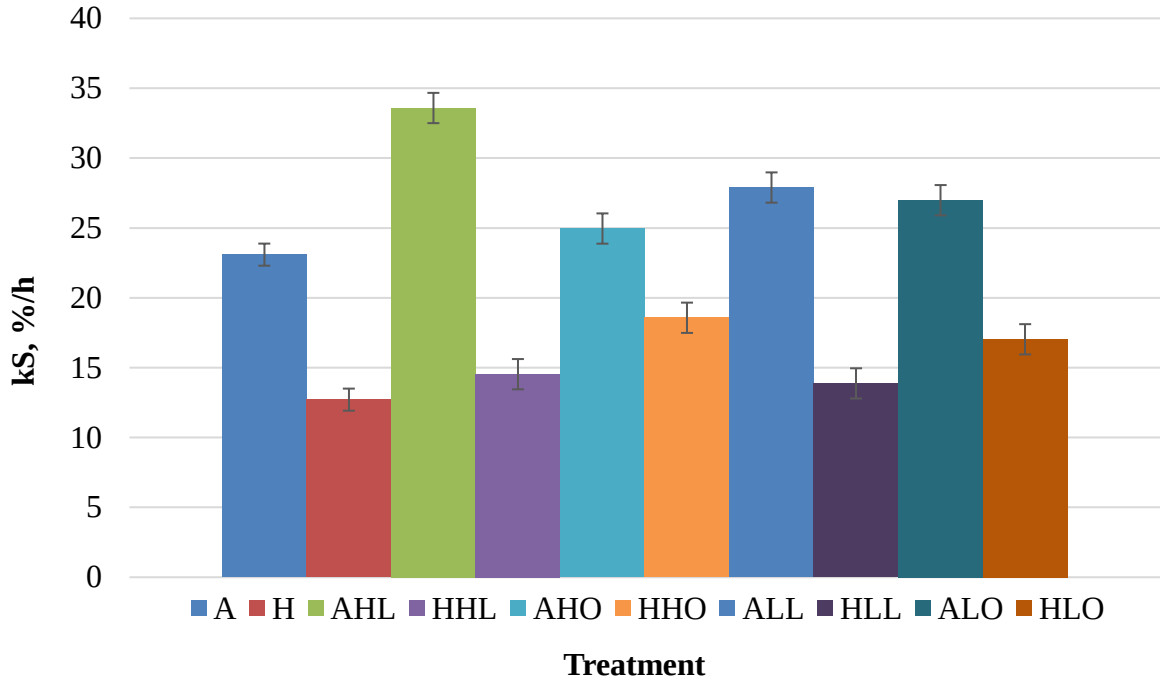


**Figure 5.1.** Least squares means of rates of starch digestion of Amioca and Hylon and forages at different starch levels.
A= Amioca with no forages; H= Hylon with no forages; AHL= Amioca with Lucerne at high starch level; ALL= Amioca with Lucerne at low starch level; HHL= Hylon with Lucerne at high starch level; HLL= Hylon with Lucerne at low starch level; AHO= Amioca with Oat hay at high starch level; ALO= Amioca with Oat hay at low starch level; HHO= Hylon with Oat hay at high starch level; HLO= Hylon with Oat hay at low starch level

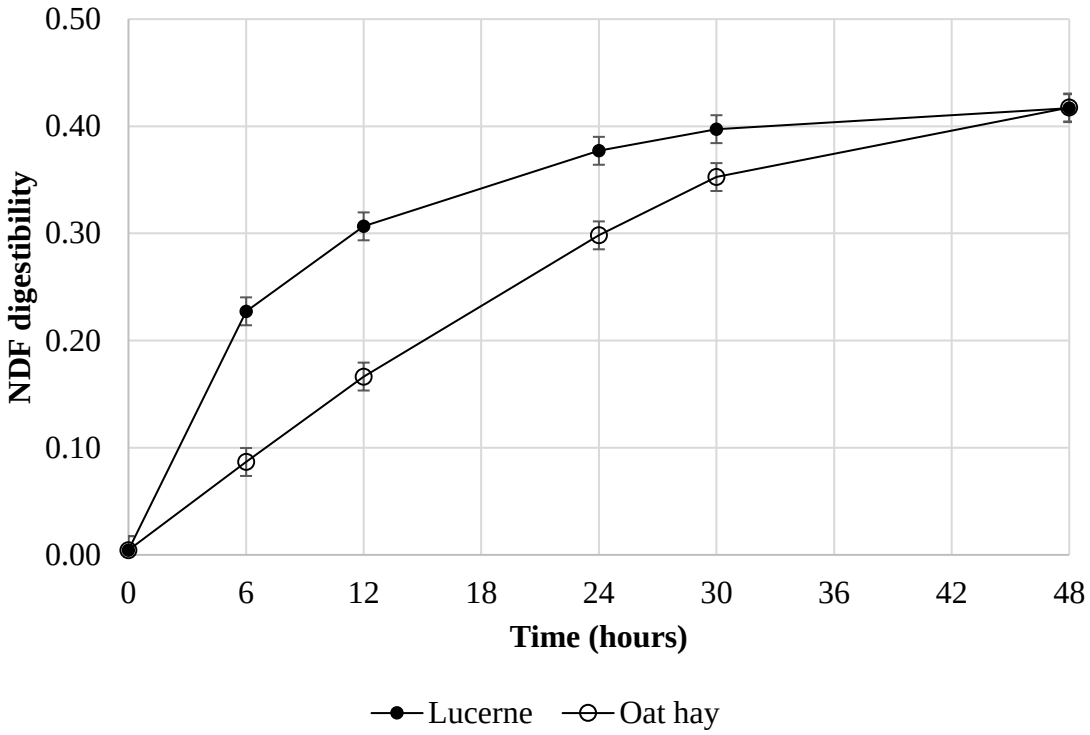


**Figure 5.2.** Least squares means of NDF digestibility for lucerne and oat hay, for pooled starch types, across time.

**Table 5.5.** Least squares means of NDF digestibility for forages fermented with either Hylon, Amioca or individually (controls).

| | Amoica | Hylon | Control | SEM | *P*-value |
|---|---|---|---|---|---|
| Lucerne | $0.30^{b}$ | $0.35^{a}$ | $0.35^{ab}$ | 0.010 | 0.0037 |
| Oat hay | $0.21^{b}$ | $0.24^{a}$ | $0.28^{ab}$ | 0.021 | 0.0098 |

Means not sharing a superscript differ ($P < 0.05$).
*Control values represent the least squares means of oat hay and lucerne in absence of Hylon and Amioca

The rate of NDF digestion of forages (pooled starch type and level) found the $K_{NDF}$ for oat hay (3.44 %/h ± 0.21) to be much lower than for lucerne (5.76%/h ± 0.21) ($P < 0.05$) which is similar to results found in the literature. According to the Nutritional Dynamic System (NDS, Ru.m.&N. Sas, Reggio Emilia, Italy) software's feed database, which is based on the Cornell Net Carbohydrate and Protein System (CNCPS, v.6.55) the rate of NDFd is 4.46%/h for oat hay and 6.72%/h for lucerne. *In vitro* gas production studies determined fractional NDF

digestibility rate of isolated NDF to be 5.30 %/h for immature and 6.3 %/h for mature lucerne (Doane et *al.*, 1997). The $K_{NDF}$ for oat grass *in situ* was determined to be 3.69 %/h (Khan et *al.*, 2006). *In vitro* gas production studies determined the rate of digestion of oat hay NDF to be 2.2 %/h, and 3.7%/h for the whole forage (Calabro et *al.*, 2005).

The rate of NDFd for the interaction between starch type and forage can be found in Table 5.6. The $K_{NDF}$ for oat hay was not influenced by the presence of starch ($P$ = 0.14 for control vs. Amioca, and $P$ = 0.26 for control vs Hylon) or by starch type ($P$ = 0.65). Rates of NDF digestion for lucerne wasreduced by the addition of starch ($P<0.005$), and Amioca had the greater negative effect on NDFd than Hylon ($P < 0.05$).

**Table 5.6.** Least squares means of rates of NDF digestion for forages fermented *in vitro* with either Hylon or Amioca, and control.

| Forages | Amioca | Hylon | Control* | SEM | *P*-value |
|---|---|---|---|---|---|
| Lucerne | 4.07[a] | 5.88[b] | 7.35[c] | 0.3321 | <0.0001 |
| Oat hay | 3.15 | 3.30 | 3.87 | 0.3021 | 0.1721 |

[a-c] Means within a row not sharing a superscript differ ($P < 0.05$).
*Control values represent the least squares means of oat hay and lucerne in absence of Hylon and Amioca

## 5.5 Conclusion

Studies examining the effects of amylose and amylopectin usually use grain varieties with different amounts of amylose and amylopectin content, and it is therefore difficult to infer only on the specific starch type. Our aim was to test the direct effect that amylose or amylopectin have on starch and fibre digestion by making use of isolated starch types. By making use of isolated starches we removed the effect that other grain components, such as vitreousness, granules composition, prolamin content, protein matrix, particle size and NDF content, would have on starch and fibre digestion. This study clearly shows that starch digestion

is directly influenced by the specific type. Amylopectin (Amioca) digested faster than amylose starch (Hylon), however after 24 hours they were both almost completely digested. As expected NDF digestion was inversely related to starch digestion and both starch types decreased NDF digestion of forages. However, amylose had the least negative effect on NDF digestion. High amylose grains have therefore the potential to provide an energy source to ruminants that does not negatively affect NDF digestion to the same extent as high amylopectin ones. Because amylose digests at a slower rate than amylopectin it could be of added benefit to ruminants as a slowly digesting starch would prevent any sudden changes in rumen pH.

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# Chapter 6

## Conclusions

The focus of this study was to better quantify some of the chemical and physical factors influencing starch digestion *in vitro* and the consequent interactions with fibre digestion. Previous studies have been conducted that analyse the effect of particle size or amylose-to-amylopectin ratio and have concluded that both these factors play an important role in the digestion of grains. However, these studies have predominantly been done on ground grains and thus various other factors could have contributed to the results. Therefore, this study aimed to reveal the specific effect of each particle size fraction, as well as the specific effect that amylose and amylopectin starch has on digestion.

Once maize was divided by particle size, the individual fractions were unique with regards to NDF, starch, lignin and amylose amounts and we theorize that all these chemical constituents, together with the particle size have likely affected our results. Consequently, the results clearly show that maize particle size has an important influence on both starch and fibre digestion. It is thus clear that rate estimation of ground maize cannot be accurate unless particle size distributions are measured and rates of starch digestion are estimated in more than one particle size range.

In the second study, by making use of isolated starches, we removed the effect that other grain components, such as vitreousness, granules composition, prolamin content, protein matrix, particle size and NDF content, would have on starch and fibre digestion. This study confirmed that starch and NDF digestion are directly influenced by the starch type. Even though we have come to a similar conclusion than previous studies, that amylose starch digests slower than amylopectin, this study has illuminated the exact extent of the relationship between

amylose and amylopectin and its effect on starch and NDF digestion. Furthermore, our study illustrates that starch digestion is affected not only by the presence of forages but also the type of forage