Degradation of Crude Protein and Starch of Corn and Wheat Grains in the Rumen

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DEGRADATION OF CRUDE PROTEIN AND STARCH OF CORN AND WHEAT GRAINS IN THE RUMEN

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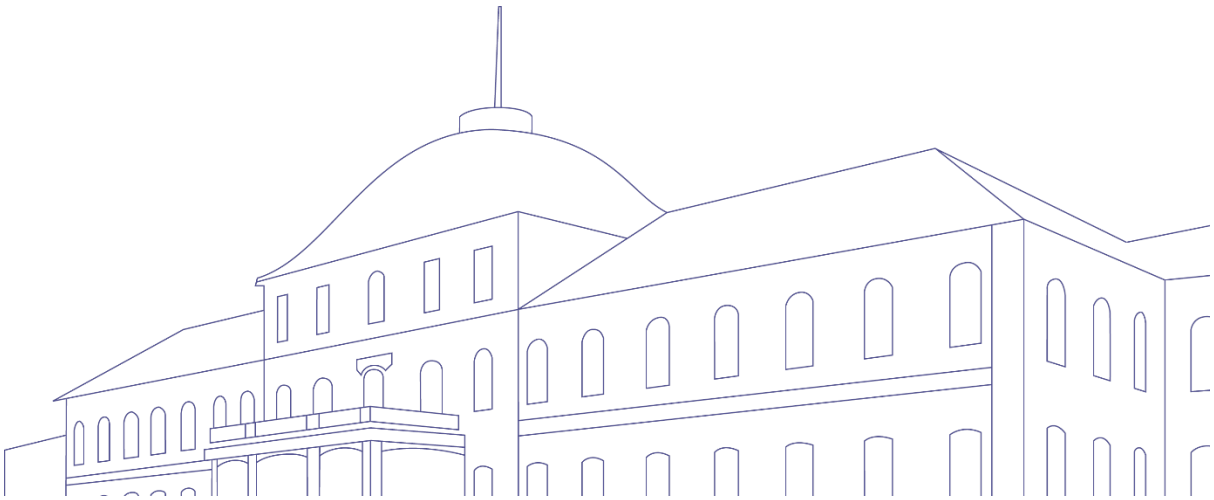

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LIST OF ABBREVIATIONS

With the exception of abbreviations only used in Manuscript 1–3

a	Rapidly disappearing fraction
AA	Amino acids
ADFom	Acid detergent fiber expressed exclusive of residual ash
adj r^2	Adjusted r^2
ADL	Acid detergent lignin
aNDFom	Neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash
AS	Aminosäuren
ATP	Adenosin triphosphate
b	Potential degradable fraction
c	Degradation rate
CP	Crude protein
Deg	Degradation after *t* hours
DM	Dry matter
ED	Effective degradation/Effektiver Abbau
EDCP	Effective degradation of crude protein
EDDM	Effective degradation of dry matter
EDST	Effective degradation of starch
FN	Falling number
GB	Gasbildung
GP	Gas production
GP_b	Potential gas production
GP_c	Gas production rate
k	Passage rate out of the rumen/Passagerate
KD	Kernel density
ME	Metabolizable energy
N	Nitrogen
NPN	Non-protein nitrogen
NSC	Non-starch carbohydrates
NSP	Non-starch polysaccharides
Pin	Puroindoline
rDM	Residual dry matter

RMSE	Root mean square error
RUSITEC	Rumen simulation technique/Pansensimulationssystem
SCFA	Short chain fatty acids
SD	Standard deviation/Standardabweichung
t	Time
TM	Trockenmasse
TSW	Thousand seed weight
TW	Test weight
UDP	Undegradable crude protein
XP	Rohprotein

AMINO ACIDS

Ala	Alanine
Arg	Arginine
Asp	Aspartic acid-asparagine
Cys	Cysteine
Glu	Glutamic acid-glutamine
Gly	Glycine
His	Histidine
Ile	Isoleucine
Leu	Leucine
Lys	Lysine
Met	Methionine
Phe	Phenylalanine
Pro	Proline
Ser	Serine
Thr	Threonine
Trp	Tryptophan
Val	Valine

MINERALS

Ca	Calcium
Cu	Copper
Fe	Iron
K	Potassium
Mg	Magnesium

Mn	Manganese
Na	Sodium
P	Phosphorus
Zn	Zinc

CHAPTER I

EXTENDED INTRODUCTION

1 EXTENDED INTRODUCTION

After many generations of genetic selection for productivity the milk production potential of the dairy cow has been steadily enhanced and is still further increasing. The high milk production potential especially in early lactation is not accompanied by a proportionally increased physical capacity for the consumption of dry matter (**DM**) to compensate for the high milk energy output. Meeting the nutritional needs of energy and protein is in most cases the limiting factor in dairy feeding management, and the composition of the diet is the major aspect influencing energy and protein uptake. The energy density of a dairy ration is primarily determined by varying proportions of fibrous and non-fibrous carbohydrates and is typically increased through the inclusion of cereal grains with starch being their main component ranging between 40 and 75% of DM. Compared to starch the crude protein (**CP**) contents of cereal grains are low with values ranging from 8 to 16% of DM but can contribute significantly to the protein supply of the animal when high proportions of cereal grains are included in dairy cow diets. However, the inclusion of cereal grains into dairy rations is limited due to the occurrence of rumen disorders like acidosis that are associated with an oversupply of starch. It is therefore highly relevant to have reliable information about the ruminal degradation characteristics of the main nutrients (starch and CP) to optimize the supplementation of cereal grains to forages without negative effects on rumen function and milk production.

The digestion and utilization of nutrients is determined by several factors and besides feeding regime (diet composition, frequency of feeding, etc.) and animal factors (feed intake, passage rate (***k***), rumination time, etc.), the intrinsic properties (nutrient concentration and physical characteristics) of the provided feedstuffs are very important. The chemical and physical characteristics of cereal grains are highly variable and depend on several factors like grain type, genotype, field conditions, climatic factors, cultural practices, and postharvest handling (Hornick, 1992). Therefore all of these factors can have an effect on the nutrient utilization by ruminants.

The main site of digestion is the rumen where starch and CP are fermented by rumen microbes. The degradation rates of CP and starch determine the effective degradation (**ED**) of those nutrients in the rumen and therefore the amount of nitrogen (**N**) and energy for the synthesis of microbial protein and the amount of undegradable crude protein (**UDP**) and bypass-starch. In general, the CP and starch of cereal grains are nearly completely degradable in the rumen, but soft cereal grains like wheat are more rapidly degraded in the rumen than hard cereal grains like corn (McAllister et al., 1993). Because of the fast fermentation of wheat grains, the ED of starch

(**EDST**) ranges between 90 and 95%, whereas for ground corn it is much lower ranging between 55 to 75% of ingested starch (Nocek and Tamminga, 1991; Offner et al., 2003). Differences can also be seen for CP degradation with ED values between 80 to 90% for ground wheat and only 35 to 53% for ground corn (Arroyo et al., 2009; Ramos et al., 2009).

Both, fast and slow fermentation of starch and CP in the rumen are associated with positive and negative effects on the physiological status of the animal. Thus, balancing diets with cereal grains to facilitate a high microbial protein synthesis in the rumen and to supply the optimal level of UDP and bypass-starch to the duodenum is the main goal in feeding high yielding dairy cows. The problems of acidosis, milk fat depression, and depressed fiber digestion that occur with an oversupply of fast fermentable starch to the rumen are well known (Beauchemin and Rode, 1997; Huntington, 1997; Jurjanz et al., 1998; Owens et al., 1998). Nevertheless, a moderate amount of fast degradable starch is needed for optimal microbial protein synthesis and ammonia utilization in combination with fast fermentable CP in the diet (Van Vuuren et al., 1990; Huntington, 1997). Conversely, a slow starch release can stabilize rumen pH and supplies energy for microbial synthesis over longer time periods (Axe et al., 1987; Philippeau et al., 1999b). Furthermore, higher amounts of UDP can improve animal performance through higher quantities of protein and therefore amino acids (**AA**) reaching the small intestine for absorption (Baker et al., 1996). In addition, higher amounts of bypass-starch influence glucose availability, and it is assumed that starch degradation in the small intestine is more energy efficient than starch degradation to short chain fatty acids (**SCFA**) and gluconeogenesis in the liver (Owens et al., 1986; Reynolds, 2006). Besides covering the nutrient requirements and ensuring a healthy rumen environment, the synchronization of N and energy in animal feeding is important to minimize N excretion. Nitrogen from animal manure is lost to the air as ammonia or nitrous oxide and can run-off into surface and groundwater as nitrate (Rotz, 2004). Eutrophication, acidification, and global warming are consequences of a permanent surplus of released N into the environment (Follett and Hatfield, 2001). Balancing farm level N flow is also of major concern in animal production systems to meet the legislative regulations in many countries worldwide. For adequate diet formulation in ruminant nutrition it is therefore necessary to have profound information about the ruminal degradation of the feedstuffs selected for inclusion.

The degradation of feedstuffs in the rumen depends on two competing processes: the degradation rate in combination with the degradability of the feedstuff, and the rate of passage from the rumen which determines the retention time for digestive action. It is therefore necessary to measure the dynamic and time-dependent degradation of the feedstuff and to

distinguish between soluble and rapidly degradable, potential but slowly degradable, and undegradable components (Mertens, 2005). The degradation of feeds in the rumen can be measured with *in vivo* techniques using double fistulated animals with surgically implanted cannulas in the rumen, abomasum or duodenum, and digestibility markers (Stern et al., 1997; Kitessa et al., 1999). However, the application of invasive animal experiments for research has become very difficult to justify, because of the decreasing public acceptance initiated through the animal-rights movement (Stern et al., 1997). Furthermore, *in vivo* measurements are very demanding, labor-intensive, expensive, time-consuming, and also not free of errors due to more or less difficulties with digesta flow marker technique and sampling site depending on the evaluated feedstuff (Huhtanen and Sveinbjörnsson, 2006). Alternative *in situ* and *in vitro* procedures which use time-series sampling to measure kinetics of degradation in the rumen are dependent on the research objectives. All of these methods have different advantages and disadvantages concerning accuracy, precision, simplicity, speed, and economic criteria. It is therefore necessary to choose the appropriate method based on the intention of the experiment.

For a comprehensive evaluation and description of the ruminal degradation kinetics of DM, starch, and CP from different grain types and genotypes within grain types it is necessary to measure a high number of different samples in a given time and to use techniques which are precise enough to detect differences in fermentation kinetics between and within grain species. Available methods fulfilling these requirements as its best are the *in situ* bag technique and the *in vitro* gas production (**GP**) technique. With the *in situ* bag technique the disappearance of a feedstuff from indigestible bags with a defined pore size after incubation for different time spans in the rumen of a fistulated animal is measured. It is then assumed that all the disappearance of the substrate is due to microbial degradation processes (Ørskov and McDonald, 1979). However, this assumption cannot be sustained because particles can be lost from the bags without degradation once their size is smaller than the pore size (López, 2005). The most recommended and used pore size in *in situ* degradation studies is 50 µm. Particle loss from the bag is mainly influenced by the interaction between bag pore size and sample characteristic (López, 2005). A pore size of 50 µm might be suitable when evaluating fiber and CP degradation of forages where particle losses during incubation are very likely to be negligible. On the other hand a pore size of 50 µm can be too large when evaluating the starch degradation of cereal grains in the rumen. Starch in cereal grains occurs in granular form with starch granules ranging in size between 2 and 36 µm depending on the cereal species (Jane, 1995). The granules are embedded in a continuous protein matrix in the endosperm of the grain. Whole grains are not degradable in the rumen and grinding is necessary prior to rumen

incubation to open the endosperm for rumen microbes (Huntington, 1997). During degradation process starch granules can then be released from the surrounding protein and structural carbohydrates. So once granules are free and further decreasing in size, they can pass the pores of the *in situ* bags easily without degradation resulting in the so called secondary starch particle losses (Huhtanen and Sveinbjörnsson, 2006). Secondary starch particle losses contribute to the degradation rate and may therefore result in an overestimation of the ED of DM (**EDDM**) and EDST and in an underestimation of the amount of bypass-starch leaving the rumen. McAllister et al. (1990) showed that the protein in the horny endosperm of corn grains is very resistant to rumen microbes. After 48 h of ruminal incubation, the microbial colonization of the protein matrix was very sparse and starch granules where degraded embedded in the protein structure. McAllister et al. (1990) further investigated that the protein matrix of wheat and barley, in contrast, was completely overgrown by a microbial biofilm and so rapidly degraded that starch granules can be released from the structural endosperm of the grain. Therefore, secondary starch particle losses might be a problem when incubating the soft *Triticeae*, like wheat and barley, but not the harder *Panicoideae*, like corn grains. However, irrespective of this widely mentioned hypothesis no experimental study has proven this statement, and a lot of studies evaluated the starch degradation of wheat and barley with the *in situ* method using bags with a pore size of 50 µm (Herrera-Saldana et al., 1990; Arieli et al., 1995; Garnsworthy and Wiseman, 2000).

As an alternative to the *in situ* technique, the fermentation profile and kinetic parameters can be modeled from the *in vitro* GP of a feedstuff incubated with buffered rumen liquor after different time spans. This technique is a good choice when a lot of samples have to be compared rapidly. It offers the opportunity to rank feeds according to their GP characteristics and to identify varieties which might be, dependent on a specific demand, superior to one another. When evaluating feedstuffs with high proportions of small particles (e.g. starch) the main advantage of the *in vitro* technique is that these particles can be considered in the GP measurements, whereas when using the *in situ* technique they can be washed out from the bags without degradation. That might be one reason why *in situ* data and GP data of feedstuffs with high amounts of non-fibrous carbohydrates, especially starch, often disagree (Valentin et al., 1999; Cone et al., 2002). In the case of cereal grains only few studies have been conducted to compare *in situ* degradation kinetics with their GP profiles obtained *in vitro* (Umucalilar et al., 2002; Hindle et al., 2005). To understand and interpret fermentation kinetics of cereal grains obtained with both methodical approaches it is necessary to evaluate a proper number of the same biological material under standardized experimental conditions. Then it would be possible to examine the commonalities and differences of fermentation characteristics obtained with

both methods and to detect the strength and weaknesses of the *in vitro* and *in situ* technique when evaluating cereal grains.

Both, the *in situ* and *in vitro* technique require rumen fistulated animals, and are too laborious for the use in routine analysis of the plant breeding and feed industry. Characterization of the ruminal degradation by easy measurable chemical and physical characteristics would be preferable. It is therefore necessary to determine the *in situ* degradation characteristics of a sufficient number of samples from one grain type for the development of equations to predict the ED from chemical and physical characteristics. These equations are necessary to implement nutritional goals in plant breeding activities and to adjust the ruminal degradation characteristics of different cereal grains and genotypes to the variable demands of ruminant nutrition.

CHAPTER II

OVERVIEW AND RESEARCH QUESTIONS OF THE INCLUDED MANUSCRIPTS

2 OVERVIEW AND RESEARCH QUESTIONS OF THE INCLUDED MANUSCRIPTS

The overall aim of the present thesis was to evaluate whether there are variations in CP and starch degradation of different corn and wheat grain genotypes in the rumen. To use the *in situ* technique, as the standard method in ruminal degradation studies, it was necessary to clarify methodical details related to the measurements of *in situ* starch degradation from cereal grains. To reduce the need of *in situ* evaluations in the future, it was further investigated, whether differences in ruminal degradation kinetics can be obtained by evaluation of the dynamics of *in vitro* GP, and if the ED of CP (**EDCP**) and EDST can be predicted from regression equations based on physical characteristics and chemical composition of the grains.

The studies of the present thesis were part of a collaborative research project referred to as GrainUp. The objectives of this project were the characterization of the feeding value of different genotypes of cereal grains for farm animals by innovative techniques and the development of methods for rapid estimates of grain quality. Cultivation, harvest, and storage, as well as chemical and physical analyses and characteristics of the 20 genotypes of wheat and corn grains used in the present thesis are described by Rodehutscord et al. (2016) and are therefore not separately listed in the present thesis.

Chapter 4 contains the abstracts and references to the three manuscripts in which the experimental studies of the present thesis are presented and evaluated. The objectives of each experimental trial can be characterized as follows.

MANUSCRIPT 1: *In vitro* and *in situ* evaluation of secondary starch particle losses from nylon bags during the incubation of different cereal grains

Secondary starch particle losses during ruminal *in situ* incubations of grains are often used as an explanation for the fast *in situ* starch degradation rates measured with soft cereal grains that lead to higher EDST compared to *in vivo* and *in vitro* measurements. However, the occurrence and extent of secondary starch particle losses has up to now not been evaluated. Therefore, the first objective was to test the hypothesis of the occurrence of secondary starch particle losses from bags used for *in situ* evaluation during incubation of cereal grains in degradation studies. The pore size of the bags used for *in situ* evaluation is not well standardized. In most cases a size of 50 µm is used, although the majority of the starch granules of different cereal grains are smaller. Thus, the second objective was to investigate the effect of bag pore size on secondary starch particle losses from different cereal grain types in an *in vitro* system. Furthermore, the

influence of bag pore size on the calculation of DM and starch degradation characteristics for different cereal grains was tested in a ruminal degradation study *in situ*.

MANUSCRIPT 2: Variation in *in situ* degradation of crude protein and starch from corn grains compared to *in vitro* gas production kinetics and physical and chemical characteristics

Corn grains are a major component of bovine animal diets in intensive production systems. Their feeding value for ruminants is mainly determined by starch content and degradation of starch in the rumen. Due to the high proportion in many diets, corn can also contribute significantly to the protein supply of the animal, although total CP content in corn is relatively low. As a result of many different corn breeding programs the variation of corn genotypes for livestock nutrition is steadily increasing. It is therefore necessary to investigate the variation of CP and starch degradation of a wide range of corn genotypes in the rumen, and to develop simple, rapid, and accurate methods to predict those variations for the use in plant breeding and livestock industry. The objectives of Manuscript 2 were therefore to investigate ruminal DM, CP, and starch degradation characteristics as well as GP kinetics from a set of grains of 20 different corn genotypes. Another objective was to predict the EDCP and EDST from EDDM or chemical and physical characteristics alone or in combination with *in vitro* GP measurements.

MANUSCRIPT 3: *In situ* starch and crude protein degradation in the rumen and *in vitro* gas production kinetics of wheat genotypes

In 2014, 20 new winter wheat varieties were officially registered in Germany and wheat producers can therefore choose from a total number of 133 varieties. These numbers illustrate the considerable effort of the wheat breeding industry to modify the genetic information of wheat grains to satisfy the multifarious requirements of agricultural practices. In general, all quality classes of wheat grains differing considerably in their chemical composition and physical characteristics are available for animal feeding. The high genetic variation might be reflected in the feeding value due to differences in the digestion of nutrients like starch and protein. Wheat can be an excellent source of fermentable carbohydrates for ruminants and also contribute to the protein supply of the animal. But, in contrast to barley, a soft cereal grain very well described in the literature, information about ruminal degradation of starch and CP from different genotypes of wheat grains is scarce. The first aim of Manuscript 3 was therefore to determine the variation of *in situ* ruminal degradation parameters of DM, CP, and starch and the ED of these nutrients based on a wide range of wheat grain genotypes. Because of

methodical deficiencies when evaluating wheat grains with the *in situ* technique, the second objective was to evaluate the relationship between the *in situ* degradation kinetics and the *in vitro* GP kinetics. Additionally, the influence of physical and chemical characteristics on ruminal fermentation characteristics were determined by correlation analysis and multiple regression approach.

CHAPTER III

GENERAL DISCUSSION

3 GENERAL DISCUSSION

One of the major challenges for the feeding management of high producing dairy cows in early lactation is the maximization of energy intake by taking into account the ruminants' demand for a minimum amount of fibrous feedstuff to avoid possible disturbances of rumen fermentation. It is therefore a walk on the tightrope between adequate structural fiber supply and providing an adequate amount of energy from concentrates predominantly based on cereal starches. Thus, it is of increasing importance to have reliable and differentiating information about the amount, rate, and extent of starch fermentation in the rumen. Although cereal grains are primary used as an energy source with protein being of secondary importance, studies on CP degradation characteristics are necessary to characterize the overall feeding value of the grains (Van Barneveld, 1999). In general, ruminal fermentation of starch and CP is faster in soft cereal grains (e.g. wheat or barley) than in hard cereal grains (e.g. corn or sorghum), and these differences are primarily attributed to the structure of the starch-protein matrix in the endosperm of the grains (McAllister et al., 1993). Thus, CP degradation is linked with the starch degradation of grains, and it is important to characterize the degradation of both fractions in the rumen.

Endosperm characteristics vary considerably between and within grain species. For overall evaluation of the nutritional quality a broad spectrum of different genotypes within each grain type must be evaluated regarding the degradation of starch and CP in the rumen. Therefore, the research described in the present thesis focused on the ruminal CP and starch degradation characteristics of different genotypes of wheat and corn representing grain types with different endosperm characteristics. To evaluate and understand the differences of ruminal fermentation between wheat and corn the following section gives an overview on their chemical composition and physical structure. Furthermore, variations of physico-chemical characteristics of different genotypes within grain type with possible influence on ruminal degradation characteristics are discussed. For the results obtained with the *in situ* and *in vitro* technique some methodical aspects are important to be considered. The last part will deal with the possibilities to estimate CP and starch degradation of wheat and corn in the rumen for the use in plant breeding and feed industries.

3.1 CHEMICAL AND PHYSICAL CHARACTERISTICS OF CORN AND WHEAT

Botanically all cereal grains are grasses and share many anatomical and chemical properties (Evers and Millar, 2002). Cereal grains are produced in the form of a caryopsis in which the

fruit coat (pericarp) is strongly fused together with the seed coat (testa). The anatomy of cereal grains is rather uniform. The fruit tissue consists of the epidermis and several thin inner layers (mesocarp, cross cells, and tube cells). Together with the testa it encloses the germ and the aleurone layer with the starchy endosperm (Koehler and Wieser, 2013). The major constituents of cereal grains are starch followed by proteins and cell wall polysaccharides which together account for about 90% of the dry weight. The remaining constituents are mainly lipids, sugars, minerals, and vitamins. The pericarp as the outer layer covers the grain and contains most of the fiber. The endosperm consists of the aleurone and the starchy endosperm with its main constituent being starch and also a significant amount of protein. The germ encloses the scutellum and embryo and contains mainly proteins, lipids, and a significant amount of sugars and vitamins (Evers and Millar, 2002; Koehler and Wieser, 2013). Although different cereal grain species share more chemical and structural similarities than differences they can vary substantially in their ruminal degradation characteristics (Nocek and Tamminga, 1991; Offner et al., 2003), and it has been shown that this is primarily due to variation in chemical and physical properties (Herrera-Saldana et al., 1990; Cerneau and Michalet-Doreau, 1991). Also within each grain type variation in degradation characteristics exist and can be associated to specific differences in grain structure and composition (Philippeau et al., 1998; Swan et al., 2006). These findings could be confirmed by the results of the present work. The 20 genotypes of wheat and corn grains used for *in situ* and *in vitro* evaluation in the present thesis cover a wide range of available qualities produced by breeding with differences in chemical and physical characteristics (Rodehutscord et al. 2016). Correlation analysis showed significant relationships of degradation characteristics and ED with several chemical and physical characteristics for wheat (Manuscript 3; Annex 1–3) and corn grains (Manuscript 2; Annex 1–3). It is therefore necessary to have a closer look at the different grain characteristics and to discuss their possible influences on ruminal degradation of both grain types.

3.1.1 STARCH

In the present thesis starch content of wheat and corn grains showed no significant correlation with the degradation characteristics and ED (k = 8%/h) of CP and starch ($P > 0.05$) within each grain type (Manuscript 2; Manuscript 3; Annex 2–3). However, pairwise comparison with separation by t test (n = 20 genotypes for each grain type) showed that the average starch degradation rate of corn (6.8%/h; Manuscript 2) was lower ($P < 0.05$) than that of wheat (65%/h; Manuscript 3). Correspondingly, the average EDST (k = 8%/h) of corn (55%; Manuscript 2) was lower ($P < 0.05$) than that of wheat (91%; Manuscript 3). Reasons for this

might be found in granular or molecular differences between wheat and corn starch and these issues are therefore examined more closely in the following section.

Starch is the main storage carbohydrate of all cereal grains. It is synthesized in the amyloplasts of the endosperm during ripening. Information on the structure, composition, and chemical and physical properties of cereal starches were reviewed extensively over the years for example by Swinkels (1985), Zobel (1988), Buléon et al. (1998), Singh et al. (2003), Lindeboom et al. (2004), Tester et al. (2004), Appelqvist and Debet (2009), Copeland et al. (2009), Pérez and Bertoft (2010), Vamadevan and Bertoft (2015), and many others. Summarized briefly for wheat and corn, starch in both grain types occurs as individual starch granules, meaning each amyloplast contains one granule. Wheat contains two types of starch granules, the relatively thick lenticular shaped granules with sizes between 15 and 36 µm and small spherical granules with diameters of only 2 to 10 µm (Jane et al., 1994; Tester et al., 2004). Corn starch granules are spherical or polyhedral in shape and all sizes between 2 and 30 µm can be found (Wang et al., 1993; Tester et al., 2004). On molecular level starch granules are composed of two polymers: amylose, a linear polymer of α-(1-4) linked glucose units with very few α-(1-6) linkages, and amylopectin, a highly branched polyglucan of α-(1-4) and about 5–6% of α-(1-6) linkages. In normal endosperm types, starch granules contain about 18 to 33% of amylose, with the remaining fraction composed of amylopectin and a number of minor constituents (1–2%) such as lipids, proteins, and low levels of minerals, mainly phosphorus, which can have significant impact on their functional properties and digestibility (Vamadevan and Bertoft, 2015). However, some mutants exist and in the so called "waxy" starches amylose content is lower than 15% and in high-amylose starches amylose content exceeds 40% (Buléon et al., 1998; Tester et al., 2004). Both, amylose and the exterior chains of amylopectin show double-helical structure, and the latter one is responsible for the deposition of individual starch granules in several "growth rings" that consist of alternating amorphous and semi-crystalline structures (Tester et al., 2004). The crystalline regions contain the double-helices of amylopectin and the amorphous growth rings consist of amylose and the amylopectin branch points. The degree of crystallinity varies between 20 and 40% for wheat and corn starches of different origins (Waterschoot et al., 2015).

Results from *in vitro* studies of hydrolysis of native starch granules by starch degrading enzymes are contradictory, and a number of factors are involved in the rate and amount of starch lysis. These factors include botanical origin, starch granule size, porosity of the starch granule, degree of crystallinity, amylose/amylopectin ratio, average molecular weight, and the amount of minor components like proteins, lipids, and phosphorus, and the type of starch degrading

enzymes used in experimental trials (Tester et al., 2006). It is therefore difficult to compare *in vitro* enzymatic degradation of wheat and corn starch, and studies evaluating both grain types exhibit different results. Planchot et al. (1995) and Blazek and Gilbert (2010) showed that there are only marginal differences between the extent of enzymatic digestion of isolated wheat and corn starch. However, Smith and Lineback (1976) and Sarikaya et al. (2000) showed that isolated corn starch was hydrolyzed more extensively than wheat starch, whereas Naguleswaran et al. (2012) demonstrated that isolated wheat starch granules were hydrolyzed significantly faster and to a higher extent than corn starch.

Rate and extent of starch degradation in the rumen is determined by plant matrix, diet composition, feed intake, feeding frequency, mechanical and chemical processing, adaptation time, and many other interrelations among several factors (Huntington, 1997). Irrespective of these various influencing aspects ruminal breakdown of starch in itself needs amylolytic enzymes from rumen microorganisms. Kotarski et al. (1992) identified eight amylolytic enzymes produced by rumen microorganisms. The main action of starch hydrolyzation comes from extracellular α- and β-amylases. Alpha-amylase is an endoenzyme acting randomly in the interior of the glucose chains and degrades both amylose and the linear regions of amylopectin, whereas β-amylase is an exoenzyme that degrades amylose and the peripheral regions of amylopectin. The end products from amylose and amylopectin breakdown are maltose and maltotriose, small amounts of free glucose, and a mixture of α-limit dextrins still containing the α-(1-6) glycosidic bonds. Enzymes like maltases, maltose-phosphorylases, and 1,6 glucosidases lead then to the formation of glucose and glucose-1-phosphate. The uptake of the glucose units by the rumen microorganism will then occur very quickly and metabolism via pyruvate results in SCFA, carbon dioxide, and methane (Mills et al., 1999).

Information about ruminal degradation of purified starch of different biological origin is scarce. In a series of different experiments the group around J.W. Cone investigated the *in vitro* degradation of purified starch granules of different biological origin including isolated wheat and corn starch in ruminal fluid over a time span of 6 h (Cone and Wolters, 1990; Cone et al., 1992; Wolters and Cone, 1992). Degradation of isolated wheat starch (20.8%) in ruminal fluid was similar to the degradation of isolated corn starch (19.7%) (Cone and Wolters, 1990). This indicates that the ruminal degradation of starch *per se* is relatively independent of the cereal origin. This assumption is supported by Ataşoğlu and Yurtman (2007) who found that GP from ruminal fluid of sheep incubated with isolated starch granules of wheat was not different from that of corn starch after 2, 4, 6, 8, 10, and 12 h incubation time. Furthermore, Ataşoğlu and Yurtman (2007) found no differences in ammonia production, sugar utilization, microbial

biomass yield, efficiency of microbial protein synthesis, pH, and SCFA production. In contrast, results of GP measurements in the present thesis (Manuscript 2; Manuscript 3) showed that GP of ground corn grains was lower ($P < 0.001$) until 12 h incubation time compared to ground wheat grains (Table 1).

TABLE 1. Pairwise comparison of *in vitro* gas production of corn (Manuscript 2) and wheat grains (Manuscript 3) over different time spans separated by t test (means of n = 20 genotypes per grain type)

		Gas production (ml/200 mg DM)		
Incubation time (h)		Corn	Wheat	P-value
2		5.7	8.7	<0.001
	CI†	*5.5–5.8*	*8.4–9.0*	
4		13.4	21.7	<0.001
	CI	*13.0–13.9*	*21.2–22.2*	
6		25.5	43.4	<0.001
	CI	*24.1–26.8*	*42.1–44.6*	
8		36.8	55.3	<0.001
	CI	*34.9–38.7*	*54.5–56.0*	
12		56.8	64.0	<0.001
	CI	*54.9–58.6*	*63.4–64.6*	
24		73.3	72.5	0.244
	CI	*72.0–74.6*	*71.9–73.1*	
48		81.4	80.4	0.173
	CI	*79.9–82.9*	*79.8–80.9*	
72		83.5	82.9	0.449
	CI	*82.0–85.0*	*82.4–83.5*	

†Confidence Interval

Additionally, the significant differences ($P < 0.05$) in *in situ* starch degradation rate and ED between wheat (Manuscript 3) and corn (Manuscript 2) in the present thesis indicate that other factors than starch granule characteristics *per se* are important in ruminal degradation process. The high difference of about 30 to 36%-units for the EDST between both grain types is in agreement with results of *in situ* and *in vitro* studies for ground raw materials of wheat and corn in the literature (Nocek and Tamminga, 1991; Offner et al., 2003). McAllister et al. (1993) showed that *in vitro* ruminal degradation of isolated corn and barley starch did not differ ($P > 0.05$) whereas starch degradation of ground raw material was higher ($P < 0.05$) for barley compared to corn and that pre-treatment with protease increased ($P < 0.05$) starch degradation

in both raw materials. McAllister et al. (1993) therefore suggested that differences in ruminal starch degradability are more related to the protein and also structural carbohydrates of the endosperm rather than properties of the starch granule itself. This was one motivation to study the starch and CP degradation simultaneously in the present thesis. The influence of the CP content and protein composition of cereal grains on their ruminal degradation characteristics is further evaluated in the following chapter.

3.1.2 PROTEIN

In the present thesis CP content was negatively correlated ($P < 0.01$) with the degradation rates and ED of CP and starch of corn (Manuscript 2; Annex 2–3). For wheat, correlation of CP content with the degradation rate of CP and EDCP was also negative ($P < 0.05$), whereas no relationship ($P > 0.05$) with starch degradation measurements could be detected (Manuscript 3; Annex 2–3). Pairwise comparison of the degradation rate between wheat and corn ($n = 20$ genotypes of each grain type) showed a faster ($P < 0.05$) CP degradation for wheat (21%/h; Manuscript 3) compared to corn (5.1%/h; Manuscript 2). This resulted in a lower ($P < 0.05$) EDCP ($k = 8$%/h) for corn (53%; Manuscript 2) compared to wheat (76%; Manuscript 3). It is therefore necessary to have a closer look on the biochemical basis of the nitrogenous compounds of wheat and corn grains, and how the CP content and protein composition can determine CP and also starch degradation in the rumen.

In cereal grains over 90% of the nitrogenous compounds are found as protein. The protein content of cereal grains is highly variable and values of 4.4–27.0% in DM and 7.0–22.0% of DM were reported in the literature for corn and wheat, respectively (Shewry, 2007). However, the CP of corn grains used in the present thesis ranged between 7.8 and 11.2% of DM with an average value of 9.4% of DM, and 12.5 to 16.2% of DM for wheat with an average value of 13.6% of DM (Rodehutscord et al., 2016). The CP content depends on genetic factors and growing conditions of which the level of N fertilization is of particular importance (Shewry, 2007). The proteins occur in all parts of the grain, but are not distributed uniformly and show different structural and nutritional characteristics. The bran (pericarp, testa, aleurone layer) contains about 18% of the total protein of wheat and about 4% in corn. The germ contains about 8% of the total protein in wheat and about 18% in corn with the remaining protein being located in the starchy endosperm of the grains (Landry and Moureaux, 1980; Shewry et al., 2009).

Depending on the extraction method, values on the amount of non-protein nitrogen (**NPN**) in cereal grains differ between studies. Imafidon and Sosulski (1990) and Shewry (2007) reported values from 1.5 to 2.5% NPN of total N in wheat and corn grains using an ethanol-water

extraction procedure. On the other hand, Baudet et al. (1986) reported NPN values between 4.4 and 6.5% of total N for corn grains when trichloroacetic acid was used for extraction of NPN. In the present thesis the NPN fraction of corn was determined according to Licitra et al. (1996) using tungstic acid as precipitating agent for the protein fraction (Manuscript 2). Values between 7 and 11% NPN of total CP were obtained for the 20 genotypes of corn in the present thesis (Manuscript 2). The library of the Cornell Net Carbohydrate and Protein System model gives NPN values of 11 and 22% of total CP for corn and wheat, respectively (Fox et al., 2003). The NPN fraction of wheat was not determined in the present thesis. Wu and McDonald (1976) determined the NPN fraction of wheat grains using tungstic acid as precipitating agent and found values between 2.5 and 4.1% NPN of total N. About half of the NPN fraction in corn grain consists of free AA with similar amounts being present in the germ and endosperm of the grain (Christianson et al., 1965). Also most of the NPN fraction of wheat consists of free AA (Jennings and Morton, 1963). The major free AA are aspartic acid-asparagine (**Asp**), glutamic acid-glutamine (**Glu**), proline (**Pro**), and alanine (**Ala**) in corn grains and Asp, Glu, glycine (**Gly**), and Ala in wheat grains (Christianson et al., 1965; Martín del Molino et al., 1988; Lawton and Wilson, 2003; Curtis et al., 2009). Information on other NPN fractions in cereal grains is scarce, and information was only found for corn but not for wheat grains. In corn the NPN fraction besides free AA consists of amines, amides, quaternary N compounds like choline and trigonelline, purines, and pyrimidines (Christianson et al., 1960; Christianson et al., 1965).

The proteins can be classified on the basis of morphology, biological function, and chemical composition, but more often cereal proteins are fractionated on the basis of their solubility also called "Osborne fractionation" (Osborne, 1907). Over the years many modifications of the method were established and more or less different classification schemes and nomenclature of protein fractions based on Osborne's method are in use (Wilson, 1985; Shewry et al., 1986; Esen, 1987). The traditional classification scheme distinguishes proteins in four fractions: albumins, globulins, prolamins, and glutelins. Albumins are soluble in water, while globulins are water resistant but extractable in dilute salt solutions (Osborne, 1907). Most of the albumins and globulins are metabolic and structural proteins found in the germ and aleurone layer of the grain. They have a low molecular weight and a globular form (Lásztity, 1996). In wheat about 30% of the total protein is composed of albumins and globulins, whereas this fraction is only about 20% in corn (Wroblewitz et al., 2014). The prolamins are soluble in aqueous alcohol, whereas the glutelins are insoluble in water, salt-solution, and alcohol but are extractable in dilute acetic acid (Osborne, 1907). The prolamins and glutelins are mainly seed storage proteins located in the starchy endosperm. The prolamins are also known as gliadins in wheat and zeins

in corn, and the glutelins are mostly referred to as glutenins in wheat (Shewry, 2002). The prolamins consist of two subtypes: proteins of low molecular weight with single polypeptide chains and intramolecular disulfide bonds, and proteins with many polypeptide chains cross-linked by intermolecular disulfide bonds thus having high molecular weight (Shewry et al., 1995). Intermolecular cross-linking of proteins is more abundant in corn than in wheat, due to specific zein fractions (β- and γ-zeins) which will be discussed in detail in Chapter 3.1.3. The storage proteins make up about 65% and 75% of the total protein in wheat and corn, respectively (Wroblewitz et al., 2014).

Another common fact of the proteins in all cereal grains is that cytoplasmatic and storage proteins show relatively great differences in AA composition. The prolamins and glutelins contain high amounts of Glu and Pro, and in corn also high proportions of Ala and leucine (**Leu**) are found in both fractions, whereas lysine (**Lys**) and other essential AA like arginine (**Arg**), threonine (**Thr**), and tryptophan (**Trp**) occur only in small quantities (Sodek and Wilson, 1971; Wieser et al., 1982). In contrast, the albumins and globulins contain higher amounts of Lys, Arg, Asp, and Gly (Sodek and Wilson, 1971; Shewry et al., 2009).

In the present thesis significant correlations with AA typical for the different Osborne fractions were found for corn (Manuscript 2) and wheat (Manuscript 3). Significantly negative correlations were found between degradation measurements and the AA typical for the prolamin and glutelin fraction. The Glu content showed a strong negative correlation ($P < 0.01$) with the degradation rate of CP (Annex 3) and EDCP (Manuscript 2; Manuscript 3) in both grain types. Proline was also negatively correlated ($P < 0.05$) with the CP degradation rate of corn (Annex 3) and the EDCP in both grain types (Manuscript 2; Manuscript 3). For corn the same AA correlated negatively ($P < 0.05$) with the EDST (Manuscript 2), whereas only Pro showed a negative correlation ($P < 0.05$) with EDST in wheat (Manuscript 3). Positive correlations were found between degradation measurements and the typical AA of albumins and globulins. In both grain types, Lys and Arg were positively correlated ($P < 0.05$) with the CP degradation rate (Annex 3) and the EDCP (Manuscript 2; Manuscript 3). These relationships were also found ($P < 0.05$) with the EDST of corn (Manuscript 2), but not for wheat (Manuscript 3).

These results indicate that the protein composition and different proportions of albumins/globulins to prolamins/glutelins determine rate and extent of CP degradation in both grain types. Starch degradation of corn seems also to be influenced by the protein composition of the grain as indicated by the strong significant correlations with the typical AA

(Manuscript 2). On the other hand, this relationship is not that clear for wheat grains (Manuscript 3). The relationship between CP composition and starch degradation will be discussed in Chapter 3.1.3.

Generally, the rate and extent of protein degradation in the rumen is determined by a variety of factors and it is roughly divided between rumen degradable and undegradable protein. The microbial degradation of protein leads to the formation of peptides and AA that are incorporated into microbial protein or further decomposed to ammonia, SCFA, and carbon dioxide. Regardless of the influences of feeding regime, animal factors, and other nutrients in the diet, the degradability of the protein *per se* is dependent on protein structure and solubility which determine susceptibility and accessibility by ruminal microbes (Tamminga, 1979; Stern et al., 2006).

The N content of ruminant feedstuff was often divided into soluble (NPN, albumins, and globulins) and insoluble in rumen buffer (prolamins and glutelins) with the aim of relating it with the CP degradation in the rumen (Crawford et al., 1978; Krishnamoorthy et al., 1982; Blethen et al., 1990). However, classification of proteins according to buffer solubility in one solvent is insufficient, because soluble proteins are degraded to different extents and insolubility cannot be equated with slow degradation characteristic (Mangan, 1972; Mahadevan et al., 1980; Spencer et al., 1988). Furthermore, different degradation rates of the insoluble proteins and the unavailable part in the insoluble fraction are not considered but play a major role in differences between CP degradation rates of feedstuffs (Madsen and Hvelplund, 1985; Van Soest, 1994). Madsen and Hvelplund (1985) showed that CP from corn and wheat grains had only small differences in buffer solubility, but CP of corn was degraded significantly slower than CP of wheat. Aufrère et al. (1991) found a lower buffer solubility for wheat grains than for corn grains, but solubility was no good predictor of the CP degradation of concentrates.

Osborne classification was also used in studies of rumen degradability of different proteins. Wadhwa et al. (1993) and Romagnolo et al. (1994) found that albumins and globulins of different plant protein sources including cereal grains and by-products were degraded at least numerically faster and to a significantly higher extent than glutelins and prolamins. Messman and Weiss (1994) extracted two prolamin and three glutelin proteins from corn grains before and after ruminal incubation and found that glutelin proteins were degraded within 2 h of incubation, whereas most of the prolamin protein was undegraded and also after 20 h of incubation only little degradation had occurred. Messman and Weiss (1994) also found differences in degradation process between polypeptides of the same protein fraction differing

in molecular size. Fahmy et al. (1991) evaluated the *in vitro* ruminal degradation of purified or semi-purified albumins, globulins, prolamins, and glutelins of wheat compared to corn grains and showed that albumins and globulins in both grains were degraded within 1 h, and that wheat storage proteins degraded faster and to a greater extent than corn storage proteins. This is in accordance with the microscopic examination of the microbial colonization of wheat and corn endosperm after ruminal incubation showing that endosperm of wheat was colonized all over by different rumen microbes within 24 h, whereas the horny endosperm of corn grains was only sparsely colonized even after 48 h (McAllister et al., 1990).

Results from the literature indicate that differences in ruminal CP degradation of wheat and corn are in part due to different proportions of albumins/globulins and prolamins/glutelins. This might be one reason for the faster ($P < 0.05$) *in situ* ruminal degradation of CP of wheat (Manuscript 3) and the higher ED ($P < 0.05$) compared to corn (Manuscript 2) in the present thesis. This assumption is supported by the correlations of AA typical for the different Osborne fractions with the degradation rate and EDCP in corn grains (Manuscript 2) and also with EDCP of wheat grains (Manuscript 3) as was already mentioned above. Moreover, the results of the present thesis are in accordance with *in situ* data of CP degradation comparing wheat and corn grains in one experimental approach, and to the best of the author`s knowledge no study is available showing a faster or higher degradation of CP from corn compared to wheat (Herrera-Saldana et al., 1990; Fahmy et al., 1991; Bacha et al., 1992; Arieli et al., 1995; Michalet-Doreau et al., 1997; O'Mara et al., 1997; Gençoglu et al., 2011).

As mentioned above significant correlations of CP and AA were also found with the starch degradation characteristics of corn grains (Manuscript 2; Annex 2) and, to a lower extent, with the EDST of wheat grains (Manuscript 3; Annex 2). This indicates that an interaction between both nutrients plays a role in the determination of starch degradation in corn and, although less pronounced, in wheat grains.

3.1.3 STARCH-PROTEIN MATRIX

In both, wheat and corn grains the endosperm cells are packed with starch granules embedded in a protein matrix. The interaction between both fractions is a key determinant for the endosperm characteristic which is described using the terms “hardness” and “vitreousness”. Terminology in regard to the words “hardness” and “vitreousness” is often confusing, and in many cases they are used synonymously (Abecassis et al., 1997; Chandrashekar and Mazhar, 1999). However, traditionally hardness is a mechanical property determined by resistance against grinding, crushing, abrading or indentation, whereas vitreousness is an optical property

classically recorded by visual examination of the kernel (Chandrashekar and Mazhar, 1999). The term "vitreous" refers to grains that have a translucent, glassy like appearance, whereas grains with mealy or floury appearance are referred to as being "opaque". In wheat, the endosperm structure is rather uniform, while in corn vitreous as well as opaque endosperm is found within a single kernel as endosperm cells become smaller and the protein matrix is getting thicker with vitreousness rising from the central to the outer endosperm (Delcour and Hoseney, 2010). In both grain types it is generally accepted that variation in hardness and vitreousness is due to differences in the interaction between the starch granules and the protein matrix of the endosperm, although the biochemical basis determining these interactions might be different (Delcour and Hoseney, 2010; Pauly et al., 2013).

Literature values show that increased kernel vitreousness, hardness, and density are negatively associated with the starch degradation of corn grains in the rumen (Philippeau and Michalet-Doreau, 1997; Correa et al., 2002). The literature concerning the association between endosperm characteristic and ruminal starch degradation for wheat grains is not as clear (Swan et al., 2006; Yang et al., 2014). In the present thesis hardness and vitreousness were not determined. But kernel density (**KD**) was used to classify the structure of the grains' endosperm (Manuscript 2; Manuscript 3). The density of an individual kernel is the sum of the densities of its chemical components and the air spaces between the starch-protein matrix. Vitreous and hard kernels are normally more compacted structured than soft or opaque kernels resulting in higher KD (Topin et al., 2008). Kernel density can therefore be used as an analytical measurement tool for the strength of endosperm compression. In the present thesis a strong negative relationship ($P < 0.001$) between KD and the EDST was recorded for corn grains (Manuscript 2), and also for wheat grains (Manuscript 3), but only to a lesser extent ($P < 0.01$). Reasons for this might be found in differences of the nature of the starch-protein matrix in both grains types which are therefore evaluated in detail below.

Wheat grains

In wheat grains, variation in hardness is determined genetically and regulated by at least two proteins: Puroindoline (**Pin**) a and Pin b which are members of the friabilin family (Bhave and Morris, 2008). Greenwell and Schofield (1986) detected a 15kDa protein on the surface of water-washed starch granules from soft wheat but not from hard wheat cultivars, and this apparently single protein was named friabilin. Further studies demonstrated that friabilin consists of different components which are members of the prolamin superfamily (Oda and Schofield, 1997). Beside the Pin proteins, the grain-softness protein 1 belongs to friabilin, but

this protein likely has only minor impact on grain hardness (Morris et al., 2013) and is therefore not further considered. There is also evidence for the occurrence of α-amylase inhibitors associated with friabilin in soft wheat. They have very likely no impact on hardness characteristic but may have an impact on enzymatic microbial degradation in the rumen. It has been found that α-amylase inhibitors from wheat grains are active against α-amylases from other organisms like avian species, insects, mammals, and marine species (Silano et al., 1975; Macri et al., 1977; Gatehouse et al., 1986; Feng et al., 1996; Kataoka and DiMagno, 1999). In contrast, Buonocore et al. (1985) showed that wheat amylase inhibitors were ineffective in inhibiting microbial amylases. However, it appears from the literature that this was the only study with microbial amylases so far. It would therefore be interesting to investigate the effect of different wheat grain α-amylase inhibitors, especially those associated with the friabilin fraction of soft-textured wheat, on amylase activity of rumen microbes to investigate their impact on starch degradation in the rumen. For the Pin proteins antibacterial and antifungal properties have been shown in different *in vitro* and *in vivo* studies, on which will be referred to later in this section.

Puroindolines contain high amounts of cysteine (**Cys**) and a unique Trp-rich domain. They are only present in hexaploid wheat grains (*Triticum aestivum*) but are absent in tetraploid wheat grains (*Triticum durum*) resulting in the very hard endosperm texture of the latter grain genus (Gautier et al., 2000). Differences in endosperm texture of *Triticum aestivum* (varying from very soft cake wheat to hard bread wheat) are due to the expression of both Pin in the specific genotype. When both proteins are in their wild type form, grain texture is soft. When any of the Pin is deficient or changed by mutation, grain texture is hard (Morris, 2002). The mechanism of Pin action is largely due to their interaction with polar lipids (mainly phospholipids and glycolipids) which are associated with the surface of starch (Greenblatt et al., 1995). It is assumed that in the last phase of starch development when the endosperm fills and dries out, the occurrence of Pin blocks the ultimate breakdown of the lipid membrane surrounding the amyloplasts. As a result, the membrane remnants reduce the contact surface between the proteins and starch granules resulting in a loose bondage between starch and protein and in a soft textured endosperm. On the other hand, complete absence or mutation of Pin results in reduced lipid-binding capacity of these proteins. Therefore the amyloplast membrane is broken completely as the kernel dries out forcing gluten proteins onto the surface of starch granules into a cohesive matrix resulting in a hard textured endosperm (Pauly et al., 2013).

Vitreousness, per definition, is only influenced by the number of air-spaces between the starch and protein matrix and there is strong evidence that vitreousness is also determined by genetic

factors, but the controlling mechanisms are not fully elucidated to date (Weightman et al., 2008; Morris and Beecher, 2012). However, it seems that vitreousness is, in contrast to hardness, mainly influenced by environmental factors and tends to increase with drought, rising temperature, and N availability during cultivation (Haddad et al., 2001; Kindred et al., 2008; Weightman et al., 2008).

What is well known for both characteristics is that with increasing hardness and vitreousness the association between the starch granules and the surrounding protein matrix is getting stronger leading to more compacted endosperm characteristics. The KD combines both characteristics to describe the compaction of the endosperm and is therefore the result of varietal origin (hardness and to a lower extent vitreousness) and cultural conditions (vitreousness). For that reason the KD is a good indicator for the whole strength of the starch-protein matrix in the endosperm. If vitreousness is mainly influenced by environmental conditions, this might be one reason why differences between KD measurements in wheat grains were quite low (Manuscript 3), because all the wheat grains used in the present thesis were grown under the same experimental conditions (Rodehutscord et al., 2016). Hence, differences in endosperm structure of the 20 wheat grains used in the present thesis are due to genetic variability, and this might be one reason for the low differences between CP and starch degradation characteristics of wheat grains (Manuscript 3). For this reason, it would be interesting to grow different wheat genotypes in different environments and under different agricultural practices to generate a sample set with wider variability in endosperm characteristics. These samples can then be used to determine their ruminal CP and starch degradation and to investigate the relationship between degradation characteristics and the strength of the starch-protein matrix using measurements like vitreousness, hardness, and KD.

The influence of wheat hardness and/or vitreousness on milling characteristics and dough-quality for cookies, pasta, and bread have been widely documented (Gaines, 1985; Bettge and Morris, 2000; Martin et al., 2001; Groos et al., 2004), but only little is known about the nutritional consequences for humans and farm animals. In farm animals, most effort to understand the metabolism and digestion of different endosperm textures of wheat grains in the gastro-intestinal tract was taken in broiler studies (Salah Uddin et al., 1996; Carré et al., 2005; Amerah et al., 2009) but only little information is available for ruminants.

Swan et al. (2006) studied near isogenic lines of wheat grains only differing in their Pin a and/or Pin b content resulting in different expression of wheat grain hardness ranging from very soft to very hard phenotypes. In a series of three different experiments Swan et al. (2006) found that

starch degradation in the rumen decreased with increasing Pin expression, and that an increase in Pin expression results in a softer endosperm of the grains. It was also shown by Krieg et al. (2015) that hard textured *Triticum durum* (n = 15) had a very fast starch degradation rate (88%/h) compared to the 20 genotypes of *Triticum aestivum* in the present thesis (65%/h; Manuscript 3). This led to a higher ($P < 0.05$) ruminal EDST (93% calculated for k = 8%/h) of *Triticum durum* compared to the 20 genotypes of *Triticum aestivum* in the present thesis (91%, Manuscript 3; Krieg et al., 2015).

A number of studies have established the antifungal and antimicrobial properties of Pin proteins showing that toxicity of Pin proteins seems selective towards microbial cells but have only little haemolytic activity on mammalian cells (Dubreil et al., 1998; Capparelli et al., 2005; Alfred et al., 2013). It can therefore be speculated that Pin protect starch granules from fungal penetration and/or microbial degradation in the rumen decelerating degradation process of soft wheat grains. However, McAllister and Sultana (2011) found that *in situ* ruminal DM and starch degradation rate was highest for a soft wheat variety with the lowest hardness index, and lowest for a *durum* wheat with the hardest kernels of the six and three varieties tested. In the study of McAllister and Sultana (2011) wheat kernels were only halved before rumen incubation, whereas in the study of Swan et al. (2006) wheat kernels were milled prior to incubation. Microscopic examination of soft and/or mealy and hard and/or vitreous wheat endosperm after milling showed that soft endosperm texture fractures easily and protein matrix separates from the starch granule without leaving many protein residues on the surface or without breaking starch granules. But in hard phenotypes, starch granules are damaged after milling (Barlow et al., 1973; Stenvert and Kingswood, 1977) which can facilitate microbial and enzymatic degradation due to a better accessibility of the starch granules. This indicates that starch degradation of grains differing in endosperm characteristics is associated with the surface texture rather than the chemical composition. Nevertheless, it should be further evaluated how the Pin interact with the rumen microbial and fungal ecosystem. And further, if the amount of these proteins in practical feeding situations may influence ruminal degradation and other digestion characteristics.

Corn grains

In contrast to wheat grains, for corn grains the term vitreousness is often used to describe the kernel characteristic without differentiation between hardness as a mechanical property and vitreousness as an optical property. Corn kernels contain both, vitreous and opaque endosperm, and the proportion of both fractions determines the hardness and strength of the whole

endosperm. Differences in the endosperm structure between vitreous and opaque types are due to various factors influencing the packing degree of the different compounds. Cell size is smaller and the cell wall is thinner in vitreous endosperm compared to opaque endosperm (Lending and Larkins, 1989). Furthermore, starch granules are polygonal in shape showing very dense packing in vitreous endosperm, whereas in opaque endosperm starch granules are round with a relative free arrangement having air spaces between them (Robutti et al., 1973). There may be also other endosperm properties like amylose to amylopectin ratio (which was shown to be elevated in vitreous endosperm) or the amount of minor components (like lipids) contributing to the structural differences of both endosperm types (Dombrink-Kurtzman and Knutson, 1997). Nevertheless is it widely accepted that the main influence on endosperm structure of corn is determined by the amount, size, and composition of protein bodies which are more or less tightly packed against the starch granules (Chandrashekar and Mazhar, 1999). The protein bodies are mainly composed of zein, whereas the protein surrounding the protein bodies and starch granules is mainly composed of glutelins (Holding and Larkins, 2006). According to the traditional Osborne fractionation procedure the endosperm of normal corn grains contains approximately 5–8% albumins and globulins, 37–47% zein, and 39–44% are associated with the glutelin fraction (Paulis et al., 1969; Sodek and Wilson, 1971; Lásztity, 1996). In the 1960s and1970s more efficient methods to extract corn endosperm proteins were developed and a fraction not found in wheat or other species of the *Triticae* was identified (Paulis et al., 1969; Landry and Moureaux, 1970). For extraction of these zeins, later referred to as β- and γ-zeins, aqueous alcohol plus a reducing agent like mercaptoethanol is needed which breaks the disulfide bonds cross-linking it to the glutelins of the endosperm (Sodek and Wilson, 1971). Today, zein is divided into four different types (α, β, γ, and δ) according to their solubility behavior and their ability to form disulfide interactions due to their molecular structure (Esen, 1986; Wallace et al., 1990). The most abundant zein group is α-zein (~ 70%) which can be extracted with aqueous alcohol only and is therefore often referred to as "native zein". Gamma-zeins are the second most abundant zein group (~ 20%) followed by β- and δ-zeins with each counting for about 5% of the total zein content (Thompson and Larkins, 1989). Protein bodies are synthesized by membrane-bound polyribosoms and transported into the lumen of the endoplasmatic reticulum, where they form insoluble aggregates (Ibl and Stoger, 2012). When formation of protein bodies starts they are composed of mainly γ-zein with minor amounts of β-zein (Holding, 2014). With further maturation of the kernel, α-zein begins to accumulate. In the final stage, the core of the protein body is filled almost entirely with α-

zein and minor amounts of δ-zein which are completely enclosed by a thin coat of γ- and β-zeins (Lending and Larkins, 1989).

Philippeau et al. (2000) showed that the amount of α-, β-, and δ-zeins was negatively and the amount of true glutelins positively correlated with the starch degradability of 14 corn grains in the rumen. Hancock et al. (1994) showed that δ-zein was highly resistant to microbial degradation in rumen fluid of sheep as degradation process was first visible on sodium dodecyl sulfate polyacrylamide gel electrophoresis after 16 h of incubation. *In situ* evaluation of the rumen stability of a mixture of β- and δ-zeins in transgene alfalfa leaves showed that rumen stability remained intact up to 48 h (Bagga et al., 2004). The resistance of β- and δ-zeins to microbial degradation in the rumen is likely due to their susceptibility to form intra- and intermolecular disulfide cross-linkages (Hancock et al., 1994). Therefore, γ-zeins may also be very resistant to microbial degradation as they have also the ability to form intra- and intermolecular disulfide bonds (Lopes and Larkins, 1991). But until now, studies evaluating the ruminal degradation of γ- and also α-zeins are missing and it would be interesting to evaluate these zein fractions individually in future studies, because they are the most abundant proteins in corn endosperm.

In vitreous endosperm of corn grains, starch granules are trapped in a tight protein matrix containing a high amount of large protein bodies, whereas in soft endosperm protein bodies are smaller and less numerous and only loosely associated with the starch granules (Dombrink-Kurtzman and Bietz, 1993). It can therefore be assumed that the dense packing of the protein-bodies against the starch granules in vitreous endosperm types decelerates microbial enzymatic degradation in the rumen.

The highly significant correlations of the starch degradation rate (Annex 2) and EDST from the corn genotypes used in the present thesis with the typical AA of the zein proteins as already discussed in detail in Manuscript 2 underline the assumption that these proteins determine starch degradation in the rumen. It would therefore be interesting to determine the proportions of different zein fractions of a sample set of different corn grains in future studies, and to investigate their degradation characteristics in the rumen as well as their relationship with starch degradation characteristics and ED in the rumen.

As described in this section, the surface-bound proteins of wheat starch granules are distinct from the surface-bound proteins of corn starch granules. However, in both grain types degradation of starch seems to be influenced by the interaction with the protein in the endosperm; this is more pronounced in corn grains than in wheat grains due to the high ability

of zein proteins to form intra- and intermolecular cross-links that are highly resistant to enzymatic attack. The ability of the zeins to form disulfide cross-links is also responsible for the fact that protein and starch in corn grains are not separated after treatment with water, whereas in wheat grains the bond between protein and starch is broken or at least weakened by water so that both fractions can be easily separated after wetting (Delcour und Hoseney 2010).

3.1.4 MINOR COMPONENTS OF CEREAL GRAINS

In this context, the term "minor" does not mean that these constituents are not important for the characterization of the nutritional value of cereal grains; it is only related to their quantitative proportion compared to the amount of starch and CP in the whole grain. In the present thesis on average 24% of the DM of the examined wheat and corn genotypes were non-starch and non-CP constituents (Table 2) which will hereinafter be referred to as residual DM (**rDM**). The rDM was mainly composed of non-organic compounds, lipids, and non-starch carbohydrates (**NSC**), and composition differed in some traits substantially between corn and wheat grains. The degradation of rDM for each genotype of corn and wheat were calculated from the DM degradation measurements (in g/kg DM) after each incubation time (0, 1, 2, 4, 8, 16, 24, 48 h (additionally 72 h for corn)) minus the starch and CP degradation measurements (in g/kg DM) after the same time points. Then the ruminal degradation parameters of rDM and the ED were determined according to the equations proposed by Ørskov and McDonald (1979): $Deg = a + b \times (1 - e^{-c \times t})$, where **Deg** (%) is the degradation after t hours, $\boldsymbol{a}$ (%) is the rapidly disappearing fraction, $\boldsymbol{b}$ (%) is the potential degradable fraction with the constant rate of degradation $\boldsymbol{c}$ (%/h), and $\boldsymbol{t}$ is the time (h). The ED (%) were then calculated at k = 5%/h as: $ED = a + [(b \times c)/(c + k)]$.

Pairwise comparison of the degradation parameters of rDM (n = 20 genotypes of each grain type) showed that the *a*-fraction and the potential degradable fraction $(a+b)$ were higher ($P < 0.001$) for corn compared to wheat. In contrast the degradation rate was lower ($P < 0.001$) for rDM of corn compared to rDM of wheat. It is therefore necessary to have a closer look at the different constituents of the rDM from corn and wheat grains.

TABLE 2. Total amount (g/kg DM), composition* (g/kg of rDM), and calculated degradation parameters# and ED of rDM† of corn and wheat grains separated by t test (means of n = 20 genotypes per grain type)

	Corn		Wheat		
	Mean	SD	Mean	SD	P-value
Total rDM	235	26.8	237	14.6	0.782
Ash	59	6.3	68	4.2	<0.001
Crude fat	241	65.7	92	8.2	<0.001
aNDFom	384	51.1	498	40.6	<0.001
ADFom	118	14.6	132	14.0	0.004
ADL	19•	2.1	30	5.4	<0.001
Cellulose	99	14.6	102	14.1	0.495
Hemicellulose	266	53.4	367	36.4	<0.001
Organic residues	316	64.4	342	46.2	0.147
Degradation parameters					
a (%)	33	5.2	18	7.2	<0.001
a+*b* (%)	98	1.7	76	2.3	<0.001
c (%/h)	4	0.8	21	4.8	<0.001
ED (%)	63	3.7	64	2.6	0.264

*aNDFom, neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash; ADFom, acid detergent fiber expressed exclusive of residual ash; ADL, acid detergent lignin;
Cellulose = ADFom - ADL; Hemicellulose = aNDFom - ADFom;
Organic residues = 1000 - ash - crude fat - aNDFom.

#Calculated from the equation $Deg = a + b \times (1 - e^{-c \times t})$, where Deg (%) = degradation after t hours; *a* = rapidly disappearing fraction; *b* = potentially degradable fraction; *c* = rate of degradation of *b* and *t* = time (h). Effective degradability was calculated using the equation $ED = a + [(b \times c)/(c + k)]$, with *k* = 5%/h.

†rDM (g/kg DM) = 1000 – starch (g/kg DM) – CP (g/kg DM)

•calculated with a mean value of 4.5 g/kg DM (mean between limit of detection and limit of quantification), because more than 50% of analyzed values were below the limit of quantification (Rodehutscord et al., 2016).

Non-starch carbohydrates

Non-starch carbohydrates include mono-, di-, and oligosaccharides and non-starch polysaccharides (**NSP**)**.** The NSC of wheat and corn are distributed among many tissues. Mono-, di-, and oligosaccharides are present in the aleurone, starchy endosperm, and tissues of the embryonic axis, whereas NSP are found in all tissues but more pronounced in the outer layer of the grains (pericarp and testa). In wheat and corn the quantities of low-molecular weight sugars (mono-, di-, and oligosaccharides) are low with average values of 20 g/kg DM. Fructans are only found in significant quantities in wheat (15–20 g/kg DM) but not in corn and only minor amounts of (1→3;1→4)-β-D-glucans (β-glucans) are found in both grain types (Bach Knudsen, 1997). The common NSP in cereal grains are generally formed by the hexoses

D-glucose, D-galactose, and D-mannose and the pentoses L-arabinose and D-xylose as well as from acidic sugars like D-galacturonic acid and D-glucoronic acid (Choct, 1997). The NSP can be divided in different groups: soluble non-cellulosic and insoluble non-cellulosic polymers, cellulose, and lignin. Wheat and corn grains have naked caryopses and therefore 80–90% of the total NSP are soluble and insoluble non-cellulosic polymers (mainly arabinoxylans) with values of 75 and 99 g/kg DM for corn and wheat, respectively (Bach Knudsen, 1997). For the same reason, cellulose (cellulose (g/kg DM) = acid detergent fiber expressed exclusive of residual ash (**ADFom** in g/kg DM) – acid detergent lignin (**ADL** in g/kg DM); values for ADFom and ADL are published by Rodehutscord et al., 2016) and ADL are present in only minor amounts in both grain types of the present thesis, with values for cellulose of 24 and 23 g/kg DM for wheat and corn, respectively. The amount of ADL was on average 4.5 g/kg DM in corn which is the mean between limit of detection and limit of quantification (more than 50% of the analyzed values were below the limit of quantification), whereas ADL was higher in wheat grains with an average value of 7.1 g/kg DM (Rodehutscord et al., 2016).

The lignin:cellulose ratio describes the extent of lignification of the plant cell walls, and a higher ratio is associated with low ruminal degradation (Van Soest, 1994). The wheat grains of the present thesis had a lignin:cellulose ratio of 0.30, whereas the ratio for corn grains was lower with 0.19. The ratio calculated for wheat grains in the present thesis is therefore at the upper end of the ratios for alfalfa (0.18–0.30) with a ruminal cellulose degradation of 40–60% (Van Soest, 1994), whereas the ratio for corn grains is at the lower end. This might be one reason why potential rDM degradation estimates of wheat in the present thesis were 22%-units lower (76%) compared to corn (98%) (Table 2).

Free sugars and soluble non-cellulosic NSP like fructans and β-glucans are rapidly and almost completely fermented by ruminal microbiota (Lanzas et al., 2007). Because of the minor amounts of these NSC in wheat and corn their nutritional significance is likely to be negligible when evaluating these grain types.

Generally, nutritional availability of non-soluble non-cellulosic NSP (hemicellulose) is more linked with lignin than any other polysaccharide (Van Soest, 1994). It can be speculated that higher lignification in wheat may have protected hemicelluloses form degradation by microbial enzymes resulting in lower potential rDM degradation estimates of wheat compared to corn in the present thesis (Table 2). On the other hand, it has been summarized by Südekum (1994) that degradation of hemicelluloses is closely related to their monosaccharide composition and that ruminal degradability of cell wall glucose and arabinose was higher than that of cell wall xylose

and uronic acids in most experiments. For unlignified or less lignified plant material the xylose:arabinose ratio can be used to describe the availability of hemicelluloses for microbial degradation (Südekum et al., 1992). This ratio is an indicator for the degree of branching of hemicelluloses (Südekum et al., 1992) and generally higher in wheat grains than in corn grains (Bach Knudsen, 1997). This might be another reason for the higher potential rDM degradation estimates of corn compared to wheat grains (Table 2).

Furthermore, the faster starch degradation rate of wheat grains may have induced lower pH in rumen incubated bags filled with wheat compared to corn. If pH was lower in bags filled with wheat and lower than the pH-optimum for cellulolytic bacteria, the number of cellulolytic microbes was likely to be reduced leading to a lower degradation of fiber material from wheat grains. It can also be speculated that a higher amount of catabolites from excessive starch hydrolysis (glucose, maltose) from wheat grains inhibited activity and production of cellulolytic enzymes and therefore fiber degradation in this grain type (Miron et al., 1996). However, degradation rate of rDM was lower for corn grains compared to wheat grains, and reasons for this might be found in other minor components than NSC.

Lipids

The lipid content of wheat and corn grains varies within and between species. The lipid content of wheat is on average lower (2–3%) compared to corn with average values of 4–5% (White and Weber, 2003; Chung and Ohm, 2009). The average crude fat contents of the 20 genotypes of wheat and corn in the present thesis were 2.2 and 5.1%, respectively (Rodehutscord et al., 2016) and therefore comparable to the values reported in the literature. Variation of crude fat content was low in wheat grains with a standard deviation (**SD**) of 0.17% and relatively wide in corn grains with a SD of 2.5% and extreme high values with 10 and even 12% of DM. The high variation in corn grains is due to the inclusion of specialty genotypes (Rodehutscord et al., 2016). Lipid content is a highly heritable trait in corn and therefore breeding programs led to special high-oil corn hybrids with lipid values higher than 6% and average values of 7% with their parental lines having extreme values as high as 17% in the whole kernel (Morrison, 1977).

The lipids found in cereal grains consist of a large number of chemical classes and individual compounds and are unevenly distributed in various parts of the kernel. About 30% of the total germ consists of lipids, whereas relatively low concentrations are found in the endosperm and outer layers (1–2%) of the grains (Morrison, 1977). The lipids in the starchy endosperm can be divided into non-starch lipids, starch surface lipids, and true starch lipids. True starch lipids are lipids inside the starch granule and their occurrence in considerable quantities is very likely to

be unique for cereal grains (Morrison, 1988). The main non-starch lipids of the endosperm are phospholipids and glycolipids, and the true starch lipids are mainly composed of lysophospholipids in wheat and free fatty acids in corn grains. The seed coat contains cutin and waxes that form the cuticular hydrophobic layers. These lipids are central determinants of water diffusion in plant organs and have a high influence on the strength of plant tissues. The aleurone and embryo are mainly composed of storage lipids and have the highest percentage of phospholipids and triglycerides. The main fatty acids of corn and wheat grains are C16:0 (palmitic acid; 10–20%), C18:1(n-9) (oleic acid; 13–35%), and C18:2(n-6) (linoleic acid; 50–62%) accounting together for over 90% of the total fatty acids (Davis et al., 1980; Duckett et al., 2002; Saoussem et al., 2009; Liu, 2011).

In the rumen the triglycerides, glycolipids, and phospholipids of the grains are hydrolyzed very quickly to their constituent fatty acids by microbial lipases, glycosidases, and phospholipases. Arieli et al. (1995) showed that *in situ* fatty acid disappearance rates were 28%/h for corn grains and 36%/h for wheat grains, and therefore relatively similar between both grain types, whereas the disappearance rates of DM, CP, and starch were between 9 and 12%/h for corn grains and between 29 and 34%/h for wheat grains.

Free unsaturated fatty acids from hydrolyzation are hydrogenated by rumen microorganisms to more saturated end products, whereas the remaining constituents like glycerol and sugars are fermented to SCFA. In contrast to the SCFA, the fatty acids released from cereal grains are not absorbed in the rumen, but will pass to the lower gastrointestinal tract and will then be absorbed in the small intestine. Generally, breakdown of dietary lipids to free fatty acids is more rapidly than the biohydrogenation process. Thus, the accumulation of unsaturated fatty acids can have detrimental effects on the rumen microbial population, because unsaturated fatty acids have a stronger antimicrobial effect than saturated fatty acids (Harfoot and Hazlewood, 1997). The toxic effect of unsaturated fatty acids is more pronounced on fibrolytic compared to amylolytic bacteria (Maia et al., 2007). One can speculate that the higher crude fat content of corn compared to wheat and in particular the extreme crude fat content of high-oil hybrids have influenced degradation measurements of DM, starch, CP, and rDM in the present thesis. However, this effect was not observed as indicated by correlation analysis. The crude fat content had no systematic significant relationship to the degradation parameters and ED of DM, starch, and CP of wheat and corn (Manuscript 2; Manuscript 3; Annex 1–3). There were positive correlations ($P < 0.05$) between the crude fat content and the extent of CP degradation of corn after 0 until 4 h incubation time (data not shown). But, these relationships might be due to the fact that most of the crude fat content as well as the easier degradable proteins are located in

the same morphological part of the corn kernel, the germ, rather than a direct influence of crude fat content on CP degradation. Positive correlations ($P < 0.05$) were found between the crude fat content and the ED of rDM in corn grains (Annex 4). This can be due to the fact that the crude fat of corn might have one of the highest degradation rates of all rDM constituents (28%/h; Arieli et al., 1995) as the remaining rDM consists mainly of fiber fractions (Table 2) normally having lower degradation rates in the rumen. This is supported by the correlation between the rDM degradation rate of corn with the crude fat content which tended to be positive ($P = 0.06$), whereas the correlation between the rDM degradation rate and the ADFom content was negative ($P < 0.01$) (Annex 4). However, crude fat content seems to have no or only minor effect on the degradation of starch and CP as well as on whole DM degradation of wheat and corn grains in the rumen. Also, results of dairy feeding experiments with lactating cows showed that the replacement of normal corn with high oil corn exhibited no or only minor effects on production performance as reviewed by Dado (1999) and confirmed by Whitlock et al. (2003).

Minerals

Whole grains contain a considerable amount of minerals. Most of these micronutrients are concentrated in the outer layers of the grain and in the germ. The mineral content of cereal grains has to be considered in ration formulation because they can have a profound effect on the nutritional quality of the diet and adequate nutrient supply to the animal. To the knowledge of the author, so far nothing is known concerning the interaction of intrinsic trace elements of wheat and corn grains with the degradation of starch and CP of these grains in the rumen.

In the present thesis, calcium (**Ca**) showed a positive ($P = 0.04$) but only moderate correlation to the starch and CP degradation rate of corn grains (Annex 2–3). However, an explanation for this relationship could not be found, as most of the Ca is found in the outer layer of corn grains, and in general its concentration is low (O'Dell et al., 1972). The Ca concentration for corn grains in the present thesis was only between 0.03–0.06 g/kg DM (Rodehutscord et al., 2016) and it is therefore likely that this mineral plays no significant role in the CP and starch degradation of corn grains. For wheat grains no significant interactions of the Ca content with the ruminal degradation measurements were detected.

Magnesium (**Mg**) showed negative correlations ($P < 0.05$) with the DM, starch, and CP degradation rates of corn (Annex 1–3). There was also a strong negative correlation ($P = 0.01$) with the EDST of corn (Annex 2). The Mg content of corn in the present thesis was on average 1.45 g/kg DM (Rodehutscord et al., 2016). According to O'Dell et al. (1972) about 90% of the Mg is located in the germ. This is supported by the high correlation of Mg with the crude fat

content of the corn grains in the present thesis ($P < 0.001$). However, Mg showed also a strong positive correlation ($P < 0.001$) with the CP content of the grains, although a correlation between CP content and crude fat content could not be detected ($P > 0.05$). This indicates that the Mg content is also associated with the protein matrix of the endosperm and may therefore be negatively correlated with degradation measurements (Chapter 3.1.2 and 3.1.3). For wheat grains a positive correlation of Mg ($P < 0.01$) with the potential degradable fraction of CP was detected (Annex 3), as well as negative correlations with the *a*-fraction ($P = 0.04$) and the potential degradable fraction ($P = 0.05$) of rDM (Annex 4). The mean Mg content of wheat (1.56 g/kg DM) was higher ($P < 0.05$) than that observed for corn (Rodehutscord et al., 2016). In wheat more than 80% of Mg is located in the bran and aleurone layer of the grain (O'Dell et al., 1972; Brier et al., 2015). This is supported by the positive correlation ($P < 0.05$) of the ADFom content with the Mg content of wheat in the present thesis. This might be one reason for the negative correlations of Mg with the rDM degradation values as the ADFom content shows a negative correlation ($P < 0.05$) with the ED of rDM.

Sodium (**Na**) showed positive relationships ($P < 0.05$) with the degradation of DM and starch of wheat grains (Annex 1–2). In wheat Na content was below the limit of detection in 5 genotypes and between 4.7 and 8.6 mg/kg DM in 15 genotypes (Rodehutscord et al., 2016). Therefore correlation analysis was performed only with the remaining 15 genotypes. In corn, Na was below the limit of detection in all genotypes (Rodehutscord et al., 2016). Correlations of Na with other physical and chemical characteristics (except zinc (**Zn**)) were not detected. Probably due to the low concentrations in both grain types, information about the localization of Na in wheat could not be found in the literature.

Iron (**Fe**) showed negative correlations ($P < 0.05$) with the DM, starch, and CP degradation rate of corn grains, as well as with the EDST of corn grains ($P < 0.05$) (Annex 1–3). On the other hand a positive correlation ($P < 0.05$) with the *a*-fraction of rDM was detected (Annex 4). In corn over 80% of the Fe is located in the germ (O'Dell et al., 1972). This is in line with the positive correlation ($P = 0.01$) of Fe with the crude fat content in the present thesis. However, also a strong positive correlation ($P < 0.001$) was detected with the CP content of the grains which may be the underlying mechanism for the negative correlations with the degradation values in the rumen (Chapter 3.1.2; Chapter 3.1.3). In wheat grains Fe showed no relationship to any of the degradation characteristics of DM, starch, CP, and rDM (Annex 1–4).

In the present thesis, potassium (**K**) showed positive relationships ($P < 0.05$) with the degradation of DM, CP, and rDM in corn grains and also with the degradation of starch in wheat

grains (Annex 1–4). Most of the K in corn grains is localized in the germ (O'Dell et al., 1972). Therefore, genotypes with a larger germ may have higher values of K and a higher amount of easier degradable proteins (albumins and globulins). This assumption is supported by the high correlation of the K concentration of the 20 genotypes with the crude fat content in corn grains ($P = 0.001$). However, this relationship was not observed for wheat grains, were K was positively related to the starch degradation of the grains ($P < 0.05$).

Negative significant correlations were found between the Zn content and the degradation of DM, CP, and starch of corn grains (Annex 1–3). Morphologically most of the Zn is located in the aleurone layer (Bänziger and Long, 2000) but considerable amounts are also found in the endosperm of the grains (Bityutskii et al., 2002). In the present thesis, the Zn content was also positively associated with the CP content of corn. This relationship might be due to the presence of so called Zn fingers associated with the proteins of corn grains. Zinc fingers are small protein domains in which Zn contributes to the stability of the protein as it binds other components like nucleic acids, protein, and several small molecules (Krishna, 2003). Small proteins are often stabilized by binding to metal ions, most frequently Zn, and therefore contribute to the strength of the protein matrix. Corn grains contain a prolamin-box binding factor with a highly conserved Zn finger deoxyribonucleic acid-binding domain that plays a dominant role in regulating the expression of endosperm development and of storage protein synthesis (Noguero et al., 2013). However, it is not known whether these bonds play a significant role in the degradation of nutrients in the rumen. But, the negative relationship between Zn and the ruminal degradation of corn might be due to the interaction between degradation and endosperm protein as discussed in Chapter 3.1.2 and 3.1.3, and Zn may partially determine the strength of the starch-protein matrix and therefore ruminal degradation of corn grains.

Manganese, copper, and phosphate showed no correlation ($P > 0.05$) with any of the ruminal degradation characteristics of DM, starch, CP, and rDM of wheat and corn grains.

Bioactive compounds or secondary plant substances

Although the term “biologically active” is often used in the academic world together with the term “functional food” no official definition is available at the moment. Biologically active compounds are most often defined as substances that interact with cell tissue in the body and have any effect beyond basic nutrition (Bradford, 2014; Gupta et al., 2015). They are associated with health benefits, but they can also be considered as antinutrients when taking into account animal performance traits. Secondary plant substances can be roughly divided in terpenes, phenols, and alkaloids and many compounds of this heterogeneous groups show antimicrobial

activity and are active against bacteria, protozoa, and fungi (Bodas et al., 2012). In whole cereal grains most of the bioactive compounds are found in the outer layers of the grains.

Phenolic substances like phenolic acids, flavonoids, tannins, coumarins, and alkylresorcinols are the main antimicrobial agents of the plant secondary substances. In corn grain the main phenolic compounds are phenolic acids, whereas in wheat grains besides phenolic acids alkylresorcinols are found in high quantities (Naczk and Shahidi, 2006). Alkylresorcinols are molecules that contain an aromatic (phenolic) ring with two hydroxyl groups in the *meta* position (1,3-dihydroxybenzene derivatives) and an odd numbered mainly saturated alkyl chain which is attached at position 5 of the benzene ring. They are found in bacteria, fungi, and also in higher plants, from which cereal grains like rye, triticale, and wheat show high contents (> 500 µg/g of DM), whereas lower concentrations were found in barley and very small amounts could be detected in the pericarp wax of corn grains (Ross et al., 2003). Alkylresorcinols have been shown to have antioxidant (rather weak), antimutagenic, and antimicrobial activity, as well as the ability to interact with proteins *in vitro* and stimulate or inhibit metabolic enzymes (Bondia-Pons et al., 2009). It has also been shown that alkylresorcinols form complexes with starch during thermal treatment (Ross et al., 2003).

Wieringa (1967) detected that alkylresorcinols had detrimental effects on growth rates of rats and pigs when fed in untypical high concentrations in the diet. A review of Ross et al. (2004) about a number of studies on the effects of rye in relationship with the alkylresorcinols concentration or the effect of purified alkylresorcinols when fed to rats, poultry or pigs revealed contradictory results. But in most cases the decreased growth of animals fed on rye was attributed to the water-soluble pentosans rather than the alkylresorcinol content (Ross et al., 2004). To the best of the author's knowledge, no study is available investigating the alkylresorcinols in context with ruminant nutrition. What may be interesting concerning ruminal degradation are the antibacterial and antifungal activities of alkylresorcinols (Kozubek and Tyman, 1999). Ratcliffe (1929) showed that oral administration of alkylresorcinols lowered gram-positive bacteria and protozoa to almost absence in the cecum of rats and chicken. However, the influence of alkylresorcinols on the microbiota of farm animals was not further investigated thereafter. The depressive effect on gram-positive bacteria and protozoa is likely to be associated with an unproportional growth of gram-negative bacteria that colonize the available niches. Most of the starch fermenting bacteria in the rumen are gram-negative (Kotarski et al., 1992) and a shift of the microbial community in the bags used for *in situ* incubations might be partly responsible for the rapid starch degradation rates of wheat (Manuscript 3) and the even more rapid starch degradation rates of rye grains (116%/h; J Krieg,

personal communication). Furthermore, if protozoa are decreased in consequence of an increased alkylresorcinol content in wheat and rye grains starch degradation can be increased as it is know that protozoa slow ruminal starch degradation process by ingesting complete starch granules and inhibiting microbial growth (Mendoza et al., 1993).

Alkylresorcinols are located mainly in the intermediate layer of the caryopsis of the grains (Landberg et al., 2008). Therefore, the lower degradation of the rDM of wheat might be partly due to the higher alkylresorcinol content of wheat rDM compared to corn rDM in the present thesis. It has been shown that the adhesion of cell wall-degrading bacteria is negatively influenced by the phenolic content of cell walls (Chesson, 1988). Therefore cell-wall degrading bacteria might be more active on the outer layers of corn grains because of their low phenolic concentration compared to wheat grains.

To examine the plausibility of the hypothesis mentioned above, it would be interesting to investigate the effect of different concentrations of alkylresorcinols of cereal grains on the ruminal bacterial community in further studies. The influence of alkylresorcinols on rumen fermentation should first of all be investigated by *in vitro* screening. For this purpose, cereal grains only differing in alkylresorcinol content should be investigated with different dosages. Target organism may be gram-positive bacteria and protozoa, because these groups have been shown to be very sensitive to alkylresorcinol content (Ratcliffe, 1929). After *in vitro* examination results must be verified *in vivo* under consideration of feed intake, ruminal degradation of nutrients and end product formation, microbial protein synthesis, and alteration and adaption of the microbial community.

3.1.5 PHYSICAL CHARACTERISTICS OF WHEAT AND CORN

The most noticeable differences between the structures of cereal grain species occur in shape, size, and mass. Also within species considerable variation in the morphology of the grain occur and can be related to its nutritive value. It might therefore be possible to associate the feeding value of cereals with visual, mechanical, and physical measurements which are linked to the individual structure of the grain.

Test weight, thousand seed weight, and falling number

Test weight (**TW**), thousand seed weight (**TSW**), and falling number (**FN)** are standard variables often used by the feed industry as indicators of nutritive value.

The TW is defined as the weight of a given volume of grains including the voids and is given in kg/hl. It is a rough measurement of the density of grains and in the present thesis TW

correlated well with the KD showing r = 0.81 and r = 0.88 ($P < 0.001$) for wheat and corn, respectively. In the literature no relationship between TW and nutritional values of wheat for ruminants could be detected. Correlations between TW and predicted metabolizable energy (**ME**) or apparent digestibility of wheat containing diets differing substantially in TW were not significant (Wilkinson et al., 2003). Also, *in vitro* studies evaluating GP, ME or rumen degradable starch found no interaction between TW and any of these measures (Moss et al., 1999; Moss and Givens, 2002; Wilkinson et al., 2003). In contrast, EDCP and EDST of wheat and also of corn grains in the present thesis were negatively associated ($P < 0.05$) with the TW of the genotypes (Manuscript 2; Manuscript 3). No study was found evaluating the feeding value of corn grains differing in TW. In the present thesis TW showed also a positive correlation with the plateau of GP ($P = 0.003$) and a negative correlation with the GP rate of corn ($P < 0.05$), whereas this relationship was not detected for wheat grains. These results indicate that TW alone might be used for a rough estimation of ED of starch and CP in the rumen.

The TSW gives an indication of the average weight of the individual kernels, and it is assumed that it usually correlates well with KD (Paulsen et al., 2003). Such relationship was not obvious when the correlations between TSW and KD were evaluated for the genotypes of wheat ($P = 0.984$) and corn ($P = 0.828$) in the present thesis. Philippeau et al. (1999a) showed that TSW was lower for vitreous corn grains compared to dent types and that ruminal starch degradability could be accurately predicted ($r^2 = 0.91$; root mean square error (**RMSE**) = 0.4) when TSW was combined with KD. In the present thesis these variables were also selected for the prediction of EDCP ($k = 5\%/h$) of corn, but adjusted r^2 (**adj r^2**) was lower and RMSE was higher (Manuscript 2) compared to the results obtained by Philippeau et al. (1999). For wheat grains no significant relation between TSW and any nutritive value for ruminants was found in the literature (Moss and Givens, 2002). Further, correlation analysis showed no substantial interactions between any of the measured *in situ* or *in vitro* values with TSW of wheat and corn grains evaluated in the present thesis. The TSW seems therefore no suitable sole criterion for classifying degradation characteristics.

Falling number test is used to determine the α-amylase activity, and FN below 180 sec indicate preharvest sprouting of wheat in which carbohydrates are converted to complex sugar compounds by enzyme activity (Carson and Edwards, 2009). The FN of all wheat grains in the present thesis were higher than the critical value (265–401 sec; Rodehutscord et al., 2016) and no relationship with any *in vitro* or *in situ* measurement or parameter was detected. These results are in accordance with Moss and Givens (2002) who found that FN was not related to any nutritive value for ruminants determined by *in vitro* methods. It can be concluded that, if FN

ranges between the normal values for unsprouted wheat, this parameter gives no indication about the ruminal degradation characteristics of wheat grains.

Vitreousness and hardness

Vitreousness and hardness are physical characteristics related to the strength of the starch-protein matrix in the endosperm of the grains. The relationship between CP and starch degradation of wheat and corn grains with both criteria were described and discussed in detail in Chapter 3.1.3 and in Manuscript 2 and 3.

3.2 METHODICAL ASPECTS

To study the degradation of nutrients in the rumen different *in vivo, in situ,* and *in vitro* techniques are available. Most of these techniques were developed to study the degradation of forages in the rumen and they are more or less suited to study the nutrient degradation of concentrates.

3.2.1 *IN VIVO* TECHNIQUES

In vivo techniques to determine the degradation of nutrients in the rumen need fistulated animals to collect digesta from the rumen, omasum, abomasum or duodenum (Harmon and Richards, 1997; Huhtanen et al., 1997). The degradation of starch and CP in the rumen can then be determined by rumen evacuation technique (Reid, 1965) or in flow measurement studies using digestibility markers (Owens and Hanson, 1992). These techniques are often considered as the reference methods to study the rumen degradability of feeds (Kitessa et al., 1999). Problems of the *in vivo* determination of ruminal starch and CP degradation of cereal grains are due to unrepresentative sampling and the distribution of marker concentrations in relation to the particle size fractions. Even with the use of double and triple marker methods no satisfactory solutions have been found until now, and the limitations of these techniques are reviewed in detail by Firkins et al. (1998), Hogan and Flinn (1999), and Huhtanen and Sveinbjörnsson (2006).

3.2.2 *IN SITU* TECHNIQUE

Compared to the *in vivo* methods, the *in situ* technique is simpler and gives a rapid estimation of the rate and extent of degradation of feedstuff in the rumen. With the *in situ* technique a small amount of the feedstuff is incubated in the rumen of a living animal using bags with a defined pore size and time-series sampling to obtain kinetic data. Hence, the biggest advantage of the

in situ method is that the feedstuff can be tested within the target organ and animal-diet interactions can be taken into account. However, the advantages and limitations of this method depend mainly on the incubated feedstuff and nutrient of interest, and standardization of the procedural steps is essential to obtain valid results. Methodical studies and reviews addressing several or single factors of the *in situ* method with recommendations for a standardized way of application were frequently published (Ørskov et al., 1980; Nocek, 1985; Michalet-Doreau and Ould-Bah, 1992; Madsen and Hvelplund, 1994; Vanzant et al., 1998; López et al., 1999; López, 2005; Südekum, 2005; Krizsan et al., 2015). According to these authors, the variables that need to be standardized can be roughly categorized into animal factors, dietary factors, bag characteristics, sample preparation, procedural factors of the ruminal incubation, treatment of bag residuals, and also evaluation of results and use of mathematical models. Some of these factors can be defined easily. For example, the diet consumed by the animal used for *in situ* incubations should contain the feedstuff or nutrient of interest in considerable amounts to ensure adaptation of the ruminal microbial community. That recommendation applies irrespective of the sample material, whereas other critical points of the technique can vary dependent on the targeted substrate and nutrient. For example, microbial contamination of bag residues resulting in an underestimation of N degradation should be considered when evaluating the protein degradation of forage material, whereas it seems of minor importance when evaluating protein concentrates or soft cereal grains (Wanderley et al., 1993; Krawielitzki et al., 2006; Rodríguez and González, 2006; Steingass et al., 2013).

Generally, three main important limitations can be identified, firstly, no breakdown of the samples due to chewing or rumination; secondly, the feedstuff is not able to leave the rumen when the appropriate particle size is reached; and thirdly, material that is small enough to leave the bag is not necessarily completely degraded but can be transported quickly out of the rumen with the liquid phase (Ørskov et al., 1980). The last point can be of major importance when evaluating the degradation of starch from cereal grains in the rumen. Starch particles that leave the bag undegraded are called secondary starch particle losses and are found throughout the literature as a hypothesis for the fast *in situ* degradation rates of soft cereal grains and the discrepancy of *in situ* EDST with *in vivo* measurements (Nocek and Tamminga, 1991; Huhtanen and Sveinbjörnsson, 2006). Evaluation of secondary starch particle losses in the present thesis (Manuscript 1) showed that this assumption is valid for wheat and barley grains under *in vitro* conditions using bags with pore sizes of 50 and 30 µm. On the other hand these losses could not be detected when evaluating corn grains. Reasons for this are again found in

differences of the endosperm characteristics of soft and hard grains which were already discussed in detail in Chapter 3.1.3 and Manuscript 1.

In contrast to the initial washout losses (*a*-fraction) secondary starch particle losses during incubation cannot be accounted for by simple washing methods and correction through determining the soluble fraction of the feedstuff (Madsen and Hvelplund, 1994). Secondary starch particle losses are mainly due to the interaction between bag pore size and sample material (López, 2005). It is therefore necessary to choose an appropriate pore size that can be defined as the minimum pore size required for unrestricted microbial accessibility, and the maximum pore size required to avoid secondary starch particle losses during incubation process.

Microbial accessibility

It has been shown that a bag pore size of 20 µm is suitable to prevent secondary starch particle losses *in vitro* when incubating soft cereal grains (Manuscript 1). However, microbial numbers were not examined and the slower *in situ* degradation of starch in bags with 20 µm pore size compared to 50 µm pore size might be also due to differences in microbial colonization of the bags. The size of the majority of the rumen bacteria is within the range of 0.4–1.0 µm in diameter and 1–3 µm length (Hungate, 1966). Most of the ruminal bacteria are therefore small enough to enter bags with pore sizes as small as 5–6 µm. A small amount of large bacteria with cell sizes up to 50 µm are present in the rumen but little is known about their ecological role, and there is evidence that they are highly specific to green leave surfaces (Clarke, 1979). They may ferment glucose, fructose, and disaccharides like sucrose and lactose but contribution to the fermentation of polysaccharides and proteins seems to be negligible (Orpin, 1976; Stewart et al., 1997).

Lindberg et al. (1984) measured adenosine triphosphate (**ATP**) concentrations as an indicator of microbial activity in bags with 10, 20, and 36 µm pore size incubated over 2, 4, and 8 h either filled with barley, hay or rapeseed meal. Lindberg et al. (1984) found significant differences between all three pore sizes showing lower ATP concentrations with smaller pore size. The difference in ATP between 20 and 36 µm was very high with barley grain after 2 and 4 h incubation time compared to hay and rapeseed meal and only negligible in all samples after 8 h incubation time. Lindberg et al. (1984) concluded that the differences in ATP between pore sizes might be due to lower numbers of protozoa with smaller pore size. Protozoa can be up to 500 µm in size (Williams and Coleman, 1997) and are therefore bigger than the pore size of bags used for *in situ* measurements. However, microbial activity is normally higher and starch

degradation faster in the absence of protozoa. An explanation for this is that protozoa can slow ruminal degradation rates by engulfing bacteria or by ingesting starch granules therefore decreasing rapid degradation by bacteria (Mendoza et al., 1993). On the other hand, ruminal CP degradation of feedstuffs is usually higher in faunated than in defaunated animals (Jouany, 1996; Eugène et al., 2004) due to extra- and intracellular protozoal proteolysis, and due to a better accessibility of the dietary protein to microbial proteases when protozoa are present in the rumen (Ushida et al., 1986). A consequence of defaunation in the case of corn grains may be that ruminal starch degradation would be decreased compared to faunated animals because of the high influence of the protein matrix on corn starch degradation in the rumen (Manuscript 2; Chapter 3.1.3). Consequently starch degradation of corn may be decreased when protozoal numbers in the bags are low.

Van Zwieten et al. (2008) found that *in vitro* corn starch disappearance from bags incubated with faunated rumen fluid was numerically slower compared to starch degradation outside the bags, whereas this was not the case for defaunated rumen fluid. Moreover starch degradation outside the bags was not different between faunated and defaunated rumen fluid. Therefore the results of this study allow no clear conclusion concerning the influence of protozoa on corn grain degradation. In the present thesis starch disappearance of corn was significantly reduced when incubated with smaller bag pore sizes *in vitro*, whereas the DM and starch degradation rates of corn determined *in situ* were not significantly different using bags with pore sizes of 50 and 20 µm (Manuscript 1). These results indicate that the effect of the bag pore size on the degradation of corn grains is different with either method and this may be due to differences in the microbial community *in vitro* and *in situ*.

To investigate these assumptions it would be interesting to further examine the effect of protozoa on the degradation of the protein and starch fraction of corn grains in detail and to evaluate the microbial community in bags of different pore sizes compared to the rumen environment.

Most of the protozoa ingest particles and therefore poorly utilize soluble compounds (Coleman, 1986). Except the holotrich protozoa that use mainly readily available soluble carbohydrates from fresh grasses or sugar cane (Williams and Coleman, 1997). However, Ushida and Jouany (1985) showed that the protozoal effect on the degradability of different proteins was greater when protein solubility was low. As mentioned in Chapter 3.2.2 studies on buffer solubility of CP from wheat and corn are not suitable to predict CP degradation in the rumen, nevertheless in most of these studies buffer solubility of wheat CP was higher than that of corn CP (Aufrère

et al., 1991; Susmel et al., 1993). It is therefore possible, that the CP degradation of wheat is less affected by the protozoal population compared to corn.

It would be interesting to investigate the effect of the protozoal population on the CP degradation of different grain types and also other feeds with higher protein content. For the purposes of future experiments in this field *in vitro* or *in situ* studies with faunated and defaunated animals may offer a lot of experimental possibilities.

Release of fermentation end products

Beside the aim to ensure an adequate microbial accessibility into the bags it is also important ensuring fermentation end products can leave the bag unhindered to prevent accumulation of SCFA, ammonia, and gas. These substances may change the fermentation conditions in the bag resulting in a micro-environment in the bag that differs completely compared to the surrounding rumen environment. In the present thesis gas accumulation was recorded in bags with pore sizes of 6 and 20 µm (Manuscript 1), and therefore the exchange between the interior of the bags and the rumen environment was not ensured throughout the incubation process. Reasons for this were discussed in Manuscript 1, but a solution could not be proposed, and it was therefore not possible to use a pore size smaller than 50 µm to study the *in situ* degradation characteristics of wheat grains (Manuscript 3). In this section, some opportunities that need to be further investigated and may help solving the methodical problems with pore sizes of 20 µm will be presented.

On the basis of the literature (Weakley et al., 1983; Marinucci et al., 1992) one theory for the occurrence of gas accumulation in the bags was the formation of high amounts of bacterial slime in the bags that blocked the pores hampering exchange with the surrounding rumen content (Manuscript 1). The formation of bacterial slime in the rumen is frequently mentioned in connection with feedlot bloat. Frothy bloat is a digestive disorder which is characterized by an accumulation of gas in the rumen and reticulum when the fermentation of readily degradable feed components results in the formation of stable foam that prevents the release of gas by eructation. It is often distinguished between pasture bloat that occurs when high amount of fast degradable legumes are fed, and feedlot bloat that is related to diets containing high amounts of cereal grains (Wang et al., 2012). A major factor in the occurrence of feedlot bloat is the excessive production of microbial slime that contributes to an increased viscosity of ruminal fluid (Nagarajak et al., 1997). Microbial slime belongs to a group of extracellular polysaccharides that are synthesized by a variety of microbial cells. The purpose of the slime production appears to be protective e.g. against predation by protozoa, antibiotics, toxic

compounds or osmotic stress. Extracellular slime has also a role in surface adhesion and the formation of biofilms. The chemical composition and structure of microbial slime is not only dependent on the producing microorganism but can also vary within different environmental conditions (e.g. temperature, pH, electrolyte and macromolecule concentration) (Bazaka et al., 2011). It has been shown that the extent of slime production varies between different rumen bacteria and research has shown a high diversity in the bacterial population associated with feedlot bloat (Cheng et al., 1998). Other factors like finely ground feed particles and lyses of bacterial cells associated with the release of cell contents (endotoxins, carbohydrates) can further enhance the viscosity of the rumen fluid (Wang et al., 2012).

In connection with the results of the present thesis one hypothesis is that during the incubation of cereal grains in the rumen similar microbial activities as in the case of feedlot bloat are responsible for the bloating of bags with small pore sizes when incubated *in vitro* with ruminal fluid or *in situ* in the rumen of a fistulated animal (Manuscript 1). It would therefore be interesting to characterize the slimy residue on the inside of the bags regarding its polysaccharide and protein composition and its ability of increasing the viscosity of solutions as suggested by Gutierrez et al. (1961). If the formation of microbial slime is responsible for bloating of the bags used for *in situ* incubations some of the strategies that mitigate feedlot bloat may be suitable to prevent microbial slime production. According to Wang et al. (2012) the incidence of feedlot bloat can be decreased by different strategies. The use of feed additives and anti-foam agents to reduce the viscosity of the ruminal fluid in the bag and to enhance the exchange with the surrounding ruminal fluid may be suitable in combination with the *in situ* technique. However, the inclusion of exogenous surface active substances into the bags may interact with the substrate and/or the microbial community in a way that influences the degradation characteristics of the feedstuff. In a first step it would therefore be necessary to identify potential substances that can be used in combination with the *in situ* technique. In a second step the ability of these substances to enhance the exchange between the bag and the ruminal environment by taking into account their influence on the evaluation of the degradation characteristics of the incubated feedstuff should be evaluated.

Although it can be assumed that the starch degradation kinetics of the 20 genotypes of wheat in the present thesis were substantially affected by the occurrence of secondary starch particle losses (Manuscript 1), the EDST (k = 5%/h) was in good agreement with *in vivo* values from the literature (Manuscript 3). It seems therefore possible to determine the EDST from wheat *in situ* with bags with pore sizes of 50 µm without any remarkable overestimation. This is in accordance with a study of De Jonge et al. (2015) who used a modified rinsing method to reduce

the washout fraction of starch when evaluating cereal grains *in situ* and found that the ED determined with the modified rinsing method was lower compared to *in vivo* ruminal starch degradation in the literature. De Jonge et al. (2015) therefore concluded that the overestimation of the EDST associated with washing machine rinsing or secondary starch particle losses seems to compensate unfavorable fermentation conditions caused by limited microbial accessibility and end product accumulation in the bags compared to *in vivo* conditions.

Suboptimal fermentation conditions would also lead to an underestimation of *in situ* starch degradation of corn grains compared to *in vivo* data as supported by different author groups (Ewing and Johnson, 1987; Nocek and Tamminga, 1991; Offner and Sauvant, 2004). However *in vivo* starch degradation of corn grains reviewed by Patton et al. (2012) and Ferraretto et al. (2013) were 54.6 and 54.1%, respectively and therefore in the same range as the average value of the EDST calculated for k = 8%/h (55%), and on average lower calculated for k = 5%/h (65%) for the 20 genotypes in the present thesis (Manuscript 2). This was similar for the results obtained for corn in Manuscript 1 incubated with a pore size of 50 µm (65%; k = 5%/h vs. 51%; k = 8%/h), whereas for a pore size of 20 µm *in situ* EDST was lower using k = 8%/h (40%), but similar calculated for k = 5%/h (55%) compared to *in vivo* EDST (Patton et al., 2012; Ferraretto et al., 2013). The *in situ* technique as applied in the present thesis seems therefore suitable for the prediction of the EDST for corn (Manuscript 2) and wheat (Manuscript 3) in the rumen.

The kinetic values determined with the *in situ* and GP technique showed that results are comparable for corn (Manuscript 2), whereas no relationship was detected between both methods in the case of wheat (Manuscript 3). That is another indicator that kinetic parameters of wheat determined with the *in situ* method are affected by secondary starch particle losses and therefore not reliable, although the ED seems relatively unaffected by this methodical error. In many cases the starch degradation rate in the rumen is of major importance with regard to health problems in high producing dairy herds, and it is therefore necessary to have reliable values for grain types with a high incidence for acidosis or feedlot bloat. As secondary starch particle losses cannot occur with the GP or other *in vitro* batch methods these techniques might be more suitable to predict the kinetics of microbial fermentation in the rumen as it was already suggested by different authors (Dewhurst et al., 1995; Cone et al., 2002).

3.2.3 *IN VITRO* TECHNIQUES

Many different *in vitro* techniques are available to study the kinetics of ruminal degradation of feedstuffs in the rumen, and they can be categorized into techniques using solubility, incubation

of the feed with different enzymes, and incubation with a buffer solution and ruminal fluid or feces (López, 2005). The microbial fermentation can then be measured directly by the DM or nutrient disappearance in the *in vitro* container (Tilley and Terry, 1963; Czerkawski and Breckenridge, 1977) or characterized indirectly by the GP measurement of feedstuff fermentation (Menke and Steingass, 1988) over different time spans. The latter one can be regarded as one of the fastest and most simple methods and its good standardization maintains a high accuracy and a low susceptibility to errors (Getachew et al., 2005). The GP technique was used in the present thesis to determine the kinetic of the fermentation for each genotype of corn (Manuscript 2) and wheat (Manuscript 3) because a large number of samples can be compared in quite a short time, and the application under controlled conditions is advantageous when the intrinsic properties of the incubated substrates is of primary interest (Mertens, 2005). Gas production measures reflect the microbial fermentation of all organic compounds of the incubated feed. In the case of wheat and corn the primary nutrient is starch and it has been recently shown that the *in vivo* starch degradation of different cereal grains can be predicted accurately by the GP of the whole material (Tahir et al., 2013). The GP technique has therefore a great potential to predict the starch degradation of cereal grains in the rumen. Although the GP rate cannot give direct predictions of the degradation rate under *in vivo* conditions, it is suitable to rank the degradation kinetics of different feeds according to their intrinsic properties.

In the present thesis differences in fermentation kinetics of the genotypes of corn (Manuscript 2) and wheat (Manuscript 3) could be detected using the GP method. The variation in the potential GP (ml/200 mg DM) of the 20 genotypes of corn (Manuscript 2) was higher (mean ± SD: 86 ± 3.1) than the variation between wheat genotypes (Manuscript 3; mean ± SD: 82 ± 1.2). This was also the case for the GP rate, which showed lower variation between the genotypes of wheat (10.5–12.3%/h) compared to the 20 genotypes of corn (6.0–8.5%/h). Results are therefore in accordance with *in vivo* values for wheat and corn. As shown in Manuscript 3 *in vivo* values of ruminal starch degradation of wheat grains in the literature varied only slightly (mean ± SD: 90% ± 3.6) also between different studies, whereas variation was higher for corn (mean ± SE: 55% ± 18.5) as reviewed by Patton et al. (2012).

The comparison of GP kinetic studies with *in vivo* data to determine degradation characteristics of grains in the rumen is scarce and most research was done in comparison with the *in situ* technique. Hindle et al. (2005) determined the *in vivo* degradation of starch from wheat and corn meal, and pure potato starch and compared the results with their *in situ* degradation kinetics and *in vitro* GP profile. Hindle et al. (2005) concluded that the *in vivo* ruminal starch degradation was estimated rather well for wheat by the *in situ* technique (*in vivo:* 89% vs. *in*

situ: 86%), but was underestimated for corn (*in vivo:* 75% vs. *in situ*: 53%), and potato starch (*in vivo*: 84% vs. *in situ*: 66%). However, Hindle et al. (2005) noticed that the *in situ* kinetic and the GP kinetic behaved rather similar as indicated by the long lag time for potato starch. Umucalilar et al. (2002) examined the relationships between *in situ* EDDM (k = 5%/h) and the GP after 6, 24, and 48 h for corn and wheat and found significant correlations between EDDM of corn with *in vitro* GP at all time points, whereas correlation of EDDM with GP for wheat grains was only significant after 48 h. Rymer and Givens (2002) stated that a relationship between total GP and DM degradability *in situ* is hardly surprising because both are endpoint measures. However, relationships between GP values and kinetics of the *in situ* degradation are more difficult, and Rymer and Givens (2002) found no relationship between *in situ* ED of organic matter and the time to maximum GP rate for corn. Michalet-Doreau et al. (1997) found a faster fermentation for wheat compared to corn with both methods, and that treatment with formaldehyde decreased fermentation rate both *in situ* and *in vitro*.

According to the knowledge of the author, direct comparisons between GP kinetic and *in situ* degradation kinetic of DM, starch, and CP degradation with different samples of one grain type were not reported in the literature. Good relationships between the kinetic of *in situ* degradation and GP technique were found for forages (Cone et al., 1998; López et al., 1998; Jančík et al., 2011) but not for corn silage (Valentin et al., 1999) and different concentrate feedstuffs (Cone et al., 2002). In the present thesis GP rates were on average slightly higher compared with their corresponding *in situ* DM degradation rates (*in vitro*: 7.1%/h vs. *in situ*: 6.0%/h) for corn (Manuscript 2) and substantially lower (*in vitro*: 11.4%/h vs. *in situ*: 39.5%/h) for wheat (Manuscript 3). The GP rate correlated well with the *in situ* degradation rates of DM, starch, and even CP ($P < 0.001$) for corn (Manuscript 2; Annex 1–3) but showed no relationship to the degradation rates of DM, CP, and starch for wheat (Manuscript 3; Annex 1–3). There was also a positive relationship ($P < 0.05$) between the GP rate and the *a*-fraction of DM and starch degradation (Annex 1–3) as well as with the EDCP and EDST of corn (Manuscript 2). On the other hand the GP rate of wheat showed only a low negative correlation ($P = 0.03$) with the *a*-fraction of starch (Annex 2).

The comparison of the kinetic values determined with the GP and *in situ* method and comparison of the ED with *in vivo* data from the literature indicate that the *in situ* and *in vitro* method are suitable to study the dynamic processes of nutrient degradation for corn. It is therefore possible to use GP kinetic studies for a fast screening of ruminal degradation characteristics of corn grains. On the other hand, results for wheat lead to the conclusion that it is possible to determine *in vivo* total ruminal degradation of starch by *in situ* measurements, but

dynamic processes of nutrient degradation are not well described by studying *in situ* kinetics of wheat as indicated by secondary starch particle losses (Manuscript 1) and the missing relationship with the GP kinetics (Manuscript 3). In case of wheat grains, the GP technique may therefore be more suitable to study the dynamics of microbial fermentation. But this supposition needs to be further evaluated.

3.3 PREDICTION OF *IN SITU* DEGRADATION CHARACTERISTICS

Despite the problems associated with the use of the *in situ* technique to study the degradation of soft cereal grains in the rumen, *in situ* measurements are the basis of many feed evaluation systems (Tamminga et al., 1994; NRC, 2001). As already mentioned, this technique requires rumen-fistulated animals and is time-consuming and labor intensive. It is therefore not suitable for a fast screening of different samples in plant breeding and animal feed industries. In comparison, the GP technique can compare many samples within a maximum time of 96 h without any additional chemical analyses. It is therefore a fast alternative to predict the degradation characteristics in the rumen. However, fistulated animals to obtain rumen fluid are still required, and characterization of the ruminal degradation by easy measurable chemical and physical characteristics would be preferable.

One aim of the present thesis was to investigate the possibility to predict the *in situ* degradation of CP and starch for different wheat and corn samples with chemical and physical characteristics of the grains alone, or in combination with the GP method. Few attempts to predict the *in situ* degradation of grains by easier measurable characteristics are found in the literature. It was shown in Manuscript 2 that prediction equations for EDST and EDCP from KD (Correa et al., 2002) and EDDM (Ramos et al., 2009) were in good agreement with the measured *in situ* values for corn in the present thesis. In a study of Philippeau et al. (1999a) vitreousness in comparison with apparent grain density and TSW gave the most accurate prediction of starch degradation of corn in the rumen ($r^2 = 0.97$; RMSE = 0.4). The measurement of vitreousness is very time-consuming, and using apparent grain density and TSW alone showed also a high accuracy for the prediction of EDST of corn ($r^2 = 0.91$; RMSE = 0.8) (Philippeau et al., 1999a). Unfortunately, Philippeau et al. (1999a) did not publish the whole equation, and it was therefore not possible to use the equation for the corn grains in the present thesis and to compare the results with EDST values determined *in situ.*

Umucalilar et al. (2002) calculated a regression equation to predict the EDDM of soft cereal grains including wheat from GP measurements ($r^2 = 0.30$; $P < 0.05$). Results of the EDDM (k = 5%/h) predicted with the published equation from Umucalilar et al. (2002) for wheat grains

of the present thesis were not correlated ($P < 0.05$) with the measured *in situ* EDDM of wheat (Manuscript 3). Other studies, which try to predict *in situ* degradation characteristics of wheat in the rumen are not known by the author.

In the present thesis the best prediction equations for EDCP and EDST from physical, chemical, and GP data were given for corn (Manuscript 2) and wheat (Manuscript 3).

The use of physical measurements were sufficient to predict the EDCP and EDST of corn with an adj $r^2 > 0.6$ (Manuscript 2), whereas for wheat additional information about crude nutrient composition and AA was necessary to achieve a comparable accuracy (Manuscript 3). The combination of chemical and physical characteristics with GP measurements improved the prediction for both grain types (Manuscript 2; Manuscript 3). The EDDM alone was a good predictor for the EDST and EDCP of corn (adj $r^2 > 0.9$) (Manuscript 2). But for wheat combination of EDDM with crude nutrients and AA was necessary to show similar accuracy for the prediction of EDST and EDCP (Manuscript 3). Therefore, the combination of EDDM and chemical and physical characteristics led to the best prediction equations for both nutrients and grain types (Manuscript 2; Manuscript 3). The models to predict EDCP and EDST of corn contained physical and chemical characteristics that can be associated with the structure of the starch-protein matrix (Manuscript 2, Chapter 3.1.3), whereas for wheat the selected variables could not be linked that clearly (Manuscript 3).

The models based on the same pool of variables to predict EDCP and EDST of both grain types showed always a higher adj r^2 for corn compared to the models obtained for wheat. The models to predict ED of wheat were more complex compared to the models obtained for corn where even one or two explanatory variables were sufficient to achieve a good accuracy of prediction. The RMSE was in most cases lower for the prediction equations of wheat, because of the higher variation in ED of corn compared to the ED of wheat (Manuscript 2; Manuscript 3).

For the prediction of EDCP of wheat very different variables associated with a high analytical effort were chosen to obtain an adj $r^2 > 0.7$ (Manuscript 3). However, as described in Manuscript 3, the variance in CP degradation of different wheat genotypes can be regarded as negligible in practical feeding situations of ruminants. On the other hand, the EDST of wheat and the EDST and EDCP of corn should be taken into account in practical feeding situations and can be predicted accurately by the equations published in the present thesis (Manuscript 2; Manuscript 3).

Although the best prediction for both grain types and nutrients were obtained using EDDM, the new equations published in the present thesis do not necessarily rely on *in situ* incubation for

the accurate prediction of nutrient degradation. These equations are therefore applicable for screening a large number of samples in the plant breeding and animal feed industries. However, investigation into NIRS calibrations seems promising for the prediction of EDCP and EDST of corn and wheat (J Krieg et al., unpublished data) and could accelerate the characterization of the ruminal degradation of different corn and wheat varieties.

3.4 PERSPECTIVES FOR FUTURE RESEARCH

The studies of the present thesis show that there are considerable differences in the degradation characteristics between and within corn and wheat grains. A lot of chemical and physical characteristics were used to describe the samples of the present thesis, and it has been shown that correlations between ruminal degradation characteristics and laboratory measurements are in line with the present literature (e.g. the high influence of the starch-protein matrix of corn). However, the influence of some intrinsic components of the evaluated grains on ruminal degradation remains open and should be subject of further investigations:

- It has been discussed in Chapter 3.1.3 that members of the friabilin family (α-amylase inhibitors, Pin proteins) of wheat may interact with the rumen microbial ecosystem and therefore influence wheat grain degradation in the rumen. However, the role of these substances has not yet been investigated sufficiently.
- In Chapter 3.1.3 the influence of the zein proteins on the ruminal degradation characteristics of corn have been discussed and it has been shown that there is a lack of information about the degradation of γ- and α-zeins which are the most abundant proteins in corn endosperm.
- Another chemical group that deserves further attention are alkylresorcinols. These phenolic substances occur in considerable amounts in wheat and have been shown to have antimicrobial activity (Kozubek and Tyman, 1999). However their effect on the rumen microbial community has not been evaluated to date (Chapter 3.1.4).

The examination of the relationship of the cereal components mentioned above with the fermentation characteristics of grains is a prerequisite for understanding the interaction between intrinsic characteristics of the kernel and the rumen microbial community. Once the mechanisms and their relevance are understood these substances can be used to modify the degradation of cereal grains in the rumen and to select genotypes with desired degradation characteristics. To study the intrinsic characteristics mentioned above the application under controlled conditions would be advantageous to mimic the influence of animal factors, and it is therefore recommended to use *in vitro* systems in a first step. For this purpose cereal grains only differing in the targeted component should be investigated with regard to their interaction

with the rumen microbiota and on fermentation characteristics of the grains. After *in vitro* examination results must be verified *in vivo* under consideration of feed intake, ruminal degradation of nutrients and end product formation, microbial protein synthesis, and alteration and adaption of the microbial community.

With the experiments of the present thesis it was possible to prove that secondary starch particle losses occur when soft cereal grains are incubated with bags of 50 µm pore size in *in situ* studies and that a pore size of 20 µm might be suitable to prevent secondary starch particle losses. However, some methodical questions arise when changing the pore size of the bags used for incubation and these should be addressed in further experiments:

- The methodical problems of gas accumulation associated with pore sizes of 20 µm must be solved before further evaluation of this pore size. This includes the characterization of the slimy residue in the bags that seems responsible for hampering exchange with the surrounding rumen environment to consider possible solutions.
- Another important aspect is to examine the microbial community in bags with 20 µm pore size compared to the pore size of 50 µm and to the surrounding rumen environment.
- There is also a shortage on information about the effect of protozoa on the protein and starch degradation of different grain types and other feeds, and future studies with faunated and defaunated animals may offer a lot of experimental possibilities.

To validate the results examined with the *in situ* technique it is important to examine wheat and corn grains differing in nutrient composition under *in vivo* conditions. Due to the problems associated with flow measurement studies (omasal flow measurement, duodenal flow measurement) the rumen evacuation technique might be preferable to study the starch and CP degradation of cereal grains in the rumen (Huhtanen and Sveinbjörnsson, 2006).

Based on the feeding values examined for different genotypes of wheat and corn grains feeding trials should be conducted to examine the effect of the genotypic variation in ruminal fermentation characteristics on animal health and animal performance parameters.

The equations to predict EDST and EDCP of wheat and corn published in the present thesis are applicable for screening a high number of samples in the plant breeding and animal feed industries. However, investigation into NIRS calibrations is necessary to further accelerate the ruminal characterization of different corn and wheat varieties.

A major objective of the GrainUp project was to identify the variation in digestibility measurements between genotypes of different grain types (Rodehutscord et al., 2016). The

20 genotypes of wheat were grown at a single plot with the same growing conditions for all genotypes. Therefore, they showed rather similar physical and chemical characteristics. On the other hand, corn grains were partly grown at different locations, and inclusion of specialty corn genotypes resulted in a great variation in several nutritional characteristics (Rodehutscord et al., 2016). This might be one reason why degradation characteristics of corn showed higher variation compared to wheat grains. For correlation analysis and to obtain prediction equations for ruminal degradation characteristics, some variation in physical and chemical characteristics of samples is necessary. This might be one reason why degradation characteristics of corn showed more significant correlations with physical and chemical characteristics and better prediction equations for EDST and EDCP compared to wheat. Further ruminal degradation studies with a proper number of wheat grains with a greater variability in selected characteristics (e.g. protein content and composition, vitreousness, hardness, KD, etc.) are necessary. To obtain a more variable sample set, genotypes could be grown on different locations and under application of different agronomical practices (e.g. amount of N fertilization).

3.5 CONCLUSIONS

- The *in situ* technique has a high potential to determine the rate and extent of CP and starch from cereal grains in the rumen and to rank feeds according to their degradation characteristics. However, incubating soft cereal grains with bags having 50 and 30 µm pore size leads to substantial secondary starch particle losses during incubation. These losses result in an overestimation of the degradation rate of DM and starch and influence the calculation of the ED in the rumen. A pore size of 20 µm prevents secondary starch particle losses from the bags, but gas accumulation occurs in such bags, and thus their use cannot be recommended. Further research is necessary to solve these problems. No secondary starch particle losses occur when corn is incubated with 50 µm pore size, and can therefore be studied using bags with this pore size. In spite of all the advantages of the *in situ* technique, caution must be taken when applying and interpreting ruminal cereal grain degradation measurements, as there are substrate-related errors which must be taken into account.
- The ruminal degradation of corn grains determined with the *in situ* technique is highly variable between different genotypes. The variation is well explainable by relationships between endosperm characteristics and laboratory measurements. The *in situ* and *in vitro* fermentation kinetics show a very good correlation between both methods. It is therefore possible to apply *in vitro* GP kinetic measurements as a fast screening method to rank

different corn grain varieties according to their fermentation characteristics in the rumen. The EDCP and EDST can be predicted from chemical characteristics alone or in combination with the GP measurements. Since corn grains can constitute a high proportion of dairy rations the EDCP and EDST should be taken into account in practical ration formulation. The equations published in the present thesis can be used in the plant breeding industry to take into account ruminal degradation characteristics when creating new corn grain varieties.

- Wheat grain genotypes show considerable variability in the estimates of the degradation parameters for CP and starch. The variation in the degradation parameters is not reflected in the ED due to the high degradation rates of both nutrients. Degradation measurements are related to the AA composition of the grains which indicates that the ruminal degradation is influenced by the protein composition of the grains. The *in situ* and *in vitro* fermentation kinetics show no good agreement between both methods. However, the EDCP and EDST can be predicted from chemical characteristics alone or in combination with the GP measurements. As starch is the primary nutrient of wheat grains and can comprise substantial portions of dairy rations the total amount of starch and its ED must be taken into account. On the other hand, the variance in CP degradation can be regarded as negligible in practical feeding situations of ruminants, and it is therefore sufficient to assume an average value for ruminal EDCP in diet formulation.

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CHAPTER IV

INCLUDED MANUSCRIPTS

MANUSCRIPT 1

In vitro and *in situ* evaluation of secondary starch particle losses from nylon bags during the incubation of different cereal grains

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ABSTRACT

In two *in vitro* and one *in situ* experiment the loss of secondary starch particles from bags used for the *in situ* incubation of different cereal grains was studied. Lactating Jersey cows fitted with rumen cannulae were used for rumen fluid collection and *in situ* degradation studies in randomly assigned repeated measures designs, each including three replicates per treatment. An *in vitro* time course study was conducted to determine whether secondary starch particle losses occur during ruminal incubation. Ground wheat (sieve size: 2 mm) was weighed and placed in bags with a pore size of 50 µm, then washed, dried, and incubated for 0.5, 1, 2, 3, 5, 8, 16, and 32 h in a modified RUSITEC-system. Bag residues and samples of freeze-dried fermenter fluids were analyzed for starch content using an enzymatic method. With the same technique used for the first *in vitro* experiment, but with an incubation time of only 8 h, ground wheat, barley, and corn grains were incubated in bags with pore sizes of 50, 30 (with the exception of corn), 20, and 6 µm. In the *in situ* experiment, ground wheat, barley, corn, and oats were rumen-incubated in bags with pore sizes of 50, 20, and 6 µm for different time periods. Then, the grains and bag residues were analyzed to determine their starch content, and the degradation characteristics of the grains were calculated. The *in vitro* trials showed that incubating wheat and barley in bags with pore sizes of 50 and 30 µm leads to a substantial degree of secondary starch particle loss during incubation. These losses were not detectable using bags with pore sizes of 20 and 6 µm. No secondary starch losses occurred in corn, regardless of pore size; thus, corn can be studied *in situ* even when using bags with 50-µm pore size. Because of the high washout losses the *in situ* method is not suitable for the measurement of starch degradation in oats using the pore sizes tested in the present study. Due to the methodological problems associated with pore sizes <50 µm, no recommendations can be provided for the evaluation of wheat and barley. Thus, caution must be taken when the *in situ* technique is used for ruminal grain starch degradation measurements, as there are substrate-related errors possible that must be taken into account.

MANUSCRIPT 2

Variation in ruminal *in situ* degradation of crude protein and starch from maize grains compared to *in vitro* gas production kinetics and physical and chemical characteristics

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ABSTRACT

The objectives of this study were (1) to evaluate *in situ* ruminal dry matter (**DM**), crude protein (**CP**), and starch degradation characteristics and *in vitro* gas production (**GP**) kinetics using a set of 20 different corn grain genotypes, and (2) to predict the effective degradation (**ED**) of CP and starch from chemical and physical characteristics alone or in combination with *in vitro* GP measurements. Corn grains were characterized by crude nutrient composition, amino acid content, and CP fractions according to the Cornell Net Carbohydrate and Protein System, as well as physical characteristics such as thousand seed weight, test weight, and kernel density. Ruminal *in situ* degradation was measured in three lactating Jersey cows fitted with a rumen cannula. Ground grains (sieve size: 2 mm) were incubated in nylon bags for 1, 2, 4, 8, 16, 24, 48, and 72 h. Bag residues were analyzed for CP and starch content. *In situ* degradation kinetics were determined and the ED of DM, CP, and starch calculated using a ruminal passage rate of 5 and 8%/h. The GP of the ground grains (sieve size: 1 mm) was recorded after 2, 4, 6, 8, 12, 24, 48, and 72 h incubation in buffered rumen fluid and fitted to an exponential equation to determine GP kinetics. Correlations and stepwise multiple linear regressions were evaluated for the prediction of ED calculated for a passage rate of 5%/h (**ED5**) for CP (**EDCP5**) and starch (**EDST5**). The *in situ* parameters and ED5 of samples varied widely between genotypes with average values (± SD) of 64% ± 4.2, 62% ± 4.1, and 65% ± 5.2 for ED5 of DM, EDCP5, and EDST5 and were approximately 10 percentage points lower for a passage rate of 8%/h. Degradation rates varied between 4.8 and 7.4%/h, 4.1 and 6.5%/h, and 5.3 and 8.9%/h for DM, CP, and starch, respectively. These rates were in the same range as GP rates (6.0 to 8.3%/h). The EDCP5 and EDST5 were closely related to CP concentration and could be evaluated in detail using CP fractions and specific amino acids. *In vitro* GP at different incubation times and GP rates correlated with EDCP5 and EDST5 and predicted EDCP5 (adj $r^2 = 0.97$) and EDST5 (adj $r^2 = 0.89$) well in combination with the chemical characteristics of the samples. Thus, equations can be used to obtain quick and cost effective information on ruminal degradation of CP and starch from corn grains.

MANUSCRIPT 3

In situ starch and crude protein degradation in the rumen and *in vitro* gas production kinetics of wheat genotypes

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ABSTRACT

The present study was conducted to determine the variation of *in situ* ruminal degradation characteristics of dry matter (DM), crude protein (CP), and starch (ST), and effective degradation (ED) of wheat genotypes. Further, these *in situ* values were associated with their corresponding *in vitro* gas production (GP) kinetics and laboratory measurements using correlation and multiple linear regression analyses. Twenty genotypes of wheat grains were characterized by crude nutrient composition, amino acid (AA) composition, and physical characteristics. Ruminal degradation kinetics were determined by *in situ* degradation of DM, CP, and ST, and subsequent evaluation of *in vitro* GP relative to time courses. *In situ* and GP measurements were fitted to an exponential equation and ED was calculated using different passage rates in the rumen of 5%/h (ED5) and 8%/h (ED8). Correlations were evaluated and stepwise multiple linear regression analyses were applied to predict ED8 of CP (EDCP8) and ST (EDST8). Estimated degradation parameters (*a*, *b*, and *c*) varied considerably between wheat genotypes irrespective of the nutrient tested. Variance in degradation parameters was not reflected in the variation of the ED, due to high degradation rates (*c*). The assumed passage rate also had only minor impact on the estimation of the ED. Estimated GP parameters varied only slightly among wheat genotypes. Regression models explained up to 80 and 99% of the variance in EDCP8 and EDST8, respectively, and associations between EDST8 and EDCP8 and chemical and physical characteristics of grains were detected. As ST is the primary nutrient in wheat grains and can comprise substantial portions of dairy rations, the total amount of ST as and its ED in the rumen should be taken into account when wheat is incorporated into dairy rations. Conversely, due to the low variance in wheat grain CP degradation the differences between genotypes can largely be neglected in practical ration formulation for ruminants.

CHAPTER V

SUMMARY

5 SUMMARY

One of the major challenges in ruminant nutrition is meeting the energetic needs of the modern dairy cow by taking into account the demand for a minimal amount of structural fiber to realize the high milk production potential while ensuring a stable and healthy rumen environment. Ration formulation is therefore a walk on the tightrope between an adequate amount of structural fiber from forages and the inclusion of a maximum amount of energy from concentrates like cereal grains. Thus it is all the more important to have reliable information about the amount, rate, and extent of ruminal cereal starch degradation. Due to the high amounts of cereals used in dairy cow rations, a considerable proportion of the dietary crude protein (**CP**) can be provided by cereal sources. Hence it is necessary to have profound information on the ruminal degradation of CP as part of the overall feeding value of the grains.

The major objectives of the present thesis were to characterize the ruminal CP and starch degradation of different genotypes of corn and wheat grains and to predict the effective degradation (**ED**) of CP and starch with easily measurable characteristics.

The *in situ* method is the standard technique to study the ruminal degradation of feeds in many feed evaluation systems. This technique was originally applied to study forages and it was therefore necessary to clarify methodical details related to the measurements of *in situ* starch degradation from cereal grains (Manuscript 1). Two *in vitro* and one *in situ* approach were conducted to study the loss of secondary starch particles from bags with different pore sizes used for the *in situ* incubation of different cereal grains. In the first *in vitro* study ground wheat was weighed and placed in bags with a pore size of 50 µm, then washed, dried, and incubated over different time spans (0.5, 1, 2, 3, 5, 8, 16, 32 h) in a modified rumen simulation technique (**RUSITEC**). Bag residues and freeze-dried fermenter fluids were analyzed for their enzymatic starch content. In the second *in vitro* study ground wheat, barley, and corn were incubated with bags of pore sizes of 50, 30 (except corn), 20, and 6 µm using the same techniques as for the first experiment, but with an incubation time of only 8 h. In the *in situ* study ground wheat, barley, corn, and oats were rumen incubated over different time spans using bags with pore sizes of 50, 20, and 6 µm. The starch content of the grains and bag residues was analyzed enzymatically and the degradation characteristics of starch were calculated for each grain type and pore size.

It was shown for the first time that incubating wheat and barley in bags with 50 and 30 µm pore size lead to a substantial amount of secondary starch particle losses during incubation process

in vitro. These losses were not detectable when the grains were incubated with bags having pore sizes of 20 and 6 µm. Independent of the bags' pore size no secondary starch particle losses were found by the incubation of corn. Thus corn can be studied *in situ* even with bags with 50 µm pore size. Oats showed very high washout losses with all pore sizes tested in the present thesis and therefore none of them is suitable to study the starch degradation measurements of oats. Because of methodical problems of gas accumulation in bags having pore sizes < 50 µm, no recommendations can be provided for the *in situ* evaluation of wheat and barley. Further research is necessary to solve these problems.

In the second and third study of the present thesis ruminal *in situ* degradation of 20 corn grain genotypes (Manuscript 2) and 20 wheat grain genotypes (Manuscript 3) was measured in three lactating Jersey cows. In both experiments the same techniques were used to characterize the ruminal degradation of CP and starch. Ground grains (2 mm) were rumen incubated in bags (50 µm pore size) over 1, 2, 4, 8, 16, 24, and 48 h (additionally 72 h for corn grains). Grains and bag residues were analyzed for their CP and starch content. The degradation parameters and the ED were calculated for dry matter (**DM**), CP, and starch for passage rates (***k***) of 5 and 8%/h. Gas production (**GP**) of ground grains (1 mm) was recorded after incubation over 2, 4, 6, 8, 12, 24, 48, and 72 h in buffered ruminal fluid and fitted to an exponential equation to determine GP parameters. To predict ED of CP and starch correlations with physical and chemical characteristics and *in vitro* measurements were evaluated and stepwise multiple linear regression analyses were applied.

The *in situ* parameters (soluble fraction, potential degradable fraction, and degradation rate) varied widely between genotypes of corn and wheat grains. The ED (k = 5%/h) of DM, CP, and starch showed a high variation for corn grain genotypes with average values (± standard deviation (**SD**)) of 64 ± 4.2%, 62 ± 4.1%, and 65 ± 5.0%, respectively. Due to the high degradation rates, the ED (k = 5%/h) of wheat grains were similar between genotypes with average values (± SD) of 85 ± 1.6% for DM, 82 ± 1.5% for CP, and 94 ± 1.4% for starch. The GP rate was in good agreement with the *in situ* values for corn grains, whereas no systematic relationship between both methods was observed for wheat grains.

Evaluation of correlation analysis showed significant relationships ($P < 0.05$) between calculated ED of CP and several amino acids (**AA**) for both grain types. This indicates that the protein composition of the grains influences CP degradation in the rumen. Similar relationships were found between the same AA and ED of starch of corn grains which highlights the impact of the protein composition on ruminal starch degradation for this grain type.

For both grain types, the ED of starch and CP could be predicted accurately from physical and chemical characteristics alone or in combination with GP measurements. Thus, the equations presented in the present thesis can be used to obtain rapid and cost effective information on ruminal degradation of CP and starch for corn and wheat grains.

The results of the present thesis show that there is considerable variation of ruminal CP and starch degradation from different genotypes of corn and also – albeit to a lesser extent – for wheat grains. Differences in ED of starch should be taken into account when formulating rations containing significant amounts of corn and wheat grains. In the case of corn grains differences in ED of CP should also be accounted for.

CHAPTER VI

ZUSAMMENFASSUNG

6 ZUSAMMENFASSUNG

Eine der größten Herausforderungen der Wiederkäuerernährung ist es, den Energiebedarf der modernen Milchkuh zu decken und gleichzeitig die Versorgung mit einer Mindestmenge an Strukturfaser sicherzustellen. Nur so kann das hohe Milchleistungspotential ausgeschöpft und eine stabile Pansengesundheit sichergestellt werden. Die Rationsgestaltung ist daher ein Balanceakt zwischen dem adäquaten Einsatz von strukturiertem Grobfutter und der Bereitstellung einer maximalen Energiemenge durch den Einsatz von Kraftfuttermitteln wie Getreide. Aus diesem Grund ist es besonders wichtig, verlässliche Informationen zum Anteil, der Geschwindigkeit und dem Ausmaß des ruminalen Stärkeabbaus von Getreide zu haben. Aufgrund der hohen Einsatzmengen von Getreide in Milchkuhrationen kann ein bedeutender Anteil des Rohproteins (**XP**) in der Ration aus Getreide stammen. Daher ist es notwendig über genaue Informationen zum ruminalen XP-Abbau von Getreide als Bestandteil des Gesamtfutterwertes zu verfügen.

Die Hauptziele der vorliegenden Arbeit waren es, den XP- und Stärkeabbau unterschiedlicher Genotypen von Mais- und Weizenkörnern im Pansen zu charakterisieren und den effektiven Abbau (**ED**) des XP und der Stärke mit einfach messbaren Parametern zu schätzen.

Die *in situ* Methode ist in vielen Futterbewertungssystemen die Standard-Methode um den ruminalen Abbau von Futtermitteln zu untersuchen. Diese Methode wurde ursprünglich für Grobfuttermittel angewendet und es war daher notwendig methodische Details in Bezug auf den Einsatz der *in situ* Methode zur Messung des Stärkeabbaus von Getreide genauer zu untersuchen (Manuskript 1). Um sekundäre Stärkepartikelverluste bei der *in situ* Inkubation verschiedener Getreidearten in Beuteln mit unterschiedlicher Porengröße zu untersuchen, wurden zwei *in vitro* und eine *in situ* Untersuchung durchgeführt. In der ersten *in vitro* Studie wurden gemahlene Weizenkörner in Beutel mit einer Porengröße von 50 µm eingewogen, gewaschen, getrocknet und dann über verschiedene Zeitspannen (0,5; 1; 2; 3; 5; 8; 16; 32 h) in einem modifizierten Pansensimulationssystem (**RUSITEC**) inkubiert. Die Beutelrückstände und gefriergetrockneten Fermenterflüssigkeiten wurden anschließend enzymatisch auf ihren Stärkegehalt hin untersucht. In der zweiten *in vitro* Untersuchung wurden gemahlene Weizen-, Gerste- und Maiskörner mit Beuteln einer Porengröße von 50, 30 (außer Mais), 20 und 6 µm mit der gleichen Technik wie bei der ersten Untersuchung inkubiert, wobei hier nur eine Inkubationszeit von 8 h untersucht wurde. Bei der *in situ* Untersuchung wurden gemahlene Weizen-, Gerste-, Mais- und Haferkörner über verschiedene Zeitspannen in Beuteln mit einer Porengröße von 50, 20 und 6 µm im Pansen inkubiert. Der Stärkegehalt der Getreide und

Beutelrückstände wurde enzymatisch ermittelt und anschließend die Abbaucharakteristik der Stärke für jede Getreideart und Porengröße angepasst.

Es konnte zum ersten Mal gezeigt werden, dass es bei der Inkubation von Weizen und Gerste in Beuteln mit einer Porengröße von 50 und 30 µm zu einer bedeutenden Menge an sekundären Stärkepartikelverlusten im *in vitro* Inkubationsverlauf kommt. Diese Verluste waren nicht nachweisbar, wenn die Getreide mit Beuteln einer Porengröße von 20 und 6 µm inkubiert wurden. Unabhängig von der Porengröße der Beutel konnten keine sekundären Stärkepartikelverluste bei der Inkubation von Mais festgestellt werden. Aus diesem Grund können für *in situ* Untersuchungen von Mais auch Beutel mit einer Porengröße von 50 µm verwendet werden. Bei Haferkörnern wurden bei allen getesteten Porengrößen sehr hohe Waschverluste festgestellt, weshalb keine von diesen Porengrößen zur Untersuchung des Stärkeabbaus von Hafer empfohlen werden kann. Aufgrund methodischer Probleme durch die Akkumulation von Gas in Beuteln mit einer Porengröße < 50 µm, können keine Empfehlungen hinsichtlich der *in situ* Untersuchung von Weizen und Gerste gegeben werden. Zur Lösung dieses Problems sind weitere Untersuchungen daher unbedingt notwendig.

In der zweiten und dritten Studie der vorliegenden Arbeit wurde jeweils der *in situ* Abbau von je 20 Genotypen von Mais (Manuskript 2) und Weizen (Manuskript 3) im Pansen von drei laktierenden Jersey Kühen untersucht. In beiden Untersuchungen wurden die gleichen Techniken angewendet um den XP- und Stärkeabbau im Pansen zu charakterisieren. Die gemahlenen Körner (2 mm) wurden in Beuteln (Porengröße: 50 µm) über 1; 2; 4; 8; 16; 24 und 48 h (bei Mais zusätzlich 72 h) im Pansen inkubiert. Die Getreide und Beutelrückstände wurden auf ihren XP- und Stärkegehalt untersucht. Anschließend wurden die Abbauparameter und der ED für die Trockenmasse (**TM**), das XP und die Stärke für Passageraten (***k***) von 5 und 8%/h berechnet. Die Gasbildung (**GB**) der gemahlenen Körner (1 mm) wurde nach Inkubation in gepuffertem Pansensaft über 2; 4; 6; 8; 12; 24; 48 und 72 h erfasst und an eine Exponentialfunktion angepasst um die GB-Parameter zu bestimmen. Um den ED des XP und der Stärke zu schätzen wurden Korrelationen mit physikalischen Parametern und chemischen Inhaltsstoffen sowie mit den *in vitro* Werten untersucht und schrittweise multiple lineare Regressionsanalysen angewendet.

Die *in situ* Parameter zeigten sehr große Unterschiede zwischen den einzelnen Genotypen von Mais und Weizen. Der ED (k = 5%/h) zeigte große Unterschiede für verschiedene Maisgenotypen, mit Mittelwerten (± Standardabweichung (**SD**)) von 64 ± 4,2% für die TM, 62 ± 4,1% für das XP, und 65 ± 5,0% für die Stärke. Aufgrund der hohen Abbauraten, war der

ED (k = 5%/h) verschiedener Weizengenotypen sehr ähnlich, mit Mittelwerten (± SD) von 85 ± 1,6% für die TM, 82 ± 1,5% für das XP und 94 ± 1,4% für die Stärke. Bei Mais konnte eine gute Übereinstimmung der GB-rate mit den *in situ* ermittelten Werten festgestellt werden, während bei Weizen kein systematischer Zusammenhang zwischen beiden Methoden festgestellt werden konnte.

Die Auswertung der Korrelationsanalyse zeigte signifikante Beziehungen zwischen dem kalkulierten ED des XP und verschiedenen Aminosäuren (**AS**) für beide Getreidearten. Das deutet daraufhin, dass die Proteinzusammensetzung der Getreide einen Einfluss auf den XP-Abbau im Pansen hat. Bei Mais zeigten dieselben AS eine ähnliche Beziehung zum ED der Stärke, das verdeutlicht den Einfluss der Proteinzusammensetzung auf den Stärkeabbau im Pansen bei dieser Getreideart.

Für beide Getreidearten konnten der ED der Stärke und des XP mit ausreichender Genauigkeit aus physikalischen und chemischen Eigenschaften, oder in Kombination mit den GB-Werten, geschätzt werden. Die Schätzgleichungen der vorliegenden Arbeit können daher dafür verwendet werden, schnell und kostengünstig Informationen zum ruminalen XP- und Stärkeabbau für Mais und Weizen zu erhalten.

Die Ergebnisse der vorliegenden Arbeit zeigen eine bedeutende Variation des ruminalen XP- und Stärkeabbaus zwischen verschiedenen Maisgenotypen. In etwas geringerem Ausmaß gilt dies auch für Weizen. Bei höheren Mengen von Mais oder Weizen in der Ration, sollten die Unterschiede im ED der Stärke bei der Rationsformulierung berücksichtigt werden. Bei Mais sollte auch der ED des XP beachtet werden.

Annex

ANNEX 1. Correlation coefficients of DM degradation characteristics with physical and chemical characteristics and *in vitro* measurements of wheat and corn (n = 20 genotypes per grain type)

		Wheat					Corn				
		a	a+b	c	ED5	ED8	a	a+b	c	ED5	ED8
TSW‡	[g/1000 seeds]	0.10	0.05	0.02	0.05	0.05	-0.33	0.37	-0.05	-0.20	-0.22
	p	*0.69*	*0.85*	*0.95*	*0.82*	*0.82*	*0.15*	*0.11*	*0.85*	*0.41*	*0.35*
TW♦	[kg/hl]	-0.37	-0.23	-0.54	-0.61	-0.62	-0.89	0.05	-0.47	-0.77	-0.79
	P	*0.11*	*0.32*	*0.01*	*<0.01*	*<0.01*	*<0.01*	*0.82*	*0.04*	*<0.01*	*<0.01*
FN§	[per s]	-0.01	-0.34	-0.02	-0.18	-0.14					
	P	*0.97*	*0.14*	*0.94*	*0.44*	*0.56*					
Crude ash	[g/kg DM]	0.04	-0.11	-0.49	-0.36	-0.36	0.06	-0.17	-0.12	-0.04	-0.02
	P	*0.85*	*0.64*	*0.03*	*0.12*	*0.12*	*0.81*	*0.48*	*0.61*	*0.88*	*0.92*
Crude protein	[g/kg DM]	-0.08	-0.19	-0.45	-0.39	-0.39	-0.68	0.13	-0.71	-0.77	-0.77
	P	*0.72*	*0.42*	*0.05*	*0.09*	*0.09*	*<0.01*	*0.58*	*<0.01*	*<0.01*	*<0.01*
Crude fibre	[g/kg DM]	0.10	-0.62	-0.06	-0.30	-0.22	0.34	0.13	0.16	0.28	0.29
	P	*0.68*	*<0.01*	*0.79*	*0.20*	*0.35*	*0.14*	*0.57*	*0.51*	*0.23*	*0.22*
Crude fat	[g/kg DM]	-0.04	-0.40	-0.02	-0.26	-0.21	0.33	0.19	-0.26	0.07	0.10
	p	*0.85*	*0.08*	*0.95*	*0.27*	*0.37*	*0.16*	*0.41*	*0.27*	*0.78*	*0.67*
aNDFom*	[g/kg DM]	0.03	-0.46	-0.18	-0.32	-0.27	0.59	-0.08	0.46	0.58	0.59
	P	*0.91*	*0.04*	*0.44*	*0.16*	*0.25*	*<0.01*	*0.75*	*0.04*	*<0.01*	*<0.01*
ADFom#	[g/kg DM]	-0.01	-0.50	-0.05	-0.33	-0.27	-0.42	0.04	-0.32	-0.43	-0.44
	P	*0.95*	*0.03*	*0.83*	*0.15*	*0.25*	*0.07*	*0.86*	*0.17*	*0.06*	*0.05*
ADL†	[g/kg DM]	-0.25	-0.30	-0.15	-0.36	-0.34					
	P	*0.30*	*0.20*	*0.52*	*0.11*	*0.14*					
Starch¥	[g/kg DM]	0.19	0.36	0.51	0.55	0.53	-0.02	-0.16	0.33	0.15	0.13
	P	*0.42*	*0.12*	*0.02*	*0.01*	*0.02*	*0.93*	*0.49*	*0.15*	*0.51*	*0.57*
Gross energy	[MJ/kg DM]	0.02	-0.02	-0.29	-0.18	-0.18	0.27	0.21	-0.33	-0.01	0.02
	P	*0.93*	*0.93*	*0.21*	*0.46*	*0.44*	*0.26*	*0.38*	*0.15*	*0.96*	*0.92*
Arg	[g/16g N]	0.33	0.34	0.41	0.53	0.51	0.68	0.07	0.30	0.58	0.60
	P	*0.16*	*0.14*	*0.07*	*0.02*	*0.02*	*<0.01*	*0.77*	*0.20*	*<0.01*	*<0.01*
His	[g/16g N]	0.27	-0.03	0.02	0.06	0.08	0.21	-0.20	0.23	0.24	0.24
	P	*0.24*	*0.90*	*0.92*	*0.79*	*0.73*	*0.37*	*0.40*	*0.33*	*0.31*	*0.31*
Ile	[g/16g N]	0.01	0.17	0.37	0.31	0.30	-0.42	0.33	-0.35	-0.44	-0.44
	P	*0.97*	*0.46*	*0.11*	*0.18*	*0.19*	*0.06*	*0.15*	*0.13*	*0.05*	*0.05*
Leu	[g/16g N]	-0.15	0.12	0.41	0.21	0.20	-0.89	0.13	-0.57	-0.82	-0.84
	P	*0.51*	*0.61*	*0.07*	*0.37*	*0.39*	*<0.01*	*0.57*	*<0.01*	*<0.01*	*<0.01*
Lys	[g/16g N]	0.18	0.19	0.48	0.45	0.46	0.81	0.03	0.48	0.75	0.76
	P	*0.45*	*0.42*	*0.03*	*0.04*	*0.04*	*<0.01*	*0.90*	*0.03*	*<0.01*	*<0.01*
Met	[g/16g N]	0.29	0.31	0.29	0.42	0.40	0.40	-0.11	0.11	0.29	0.31
	P	*0.22*	*0.18*	*0.22*	*0.07*	*0.08*	*0.08*	*0.64*	*0.63*	*0.22*	*0.19*
Phe	[g/16g N]	-0.50	-0.09	-0.12	-0.33	-0.34	-0.88	0.30	-0.63	-0.84	-0.85
	P	*0.02*	*0.70*	*0.62*	*0.16*	*0.14*	*<0.01*	*0.20*	*<0.01*	*<0.01*	*<0.01*
Thr	[g/16g N]	0.18	0.07	0.27	0.26	0.27	0.60	0.24	0.14	0.43	0.46
	P	*0.44*	*0.77*	*0.25*	*0.27*	*0.25*	*<0.01*	*0.31*	*0.55*	*0.06*	*0.04*
Trp	[g/16g N]	0.16	0.08	0.09	0.15	0.15	0.83	0.01	0.49	0.75	0.77
	P	*0.50*	*0.73*	*0.70*	*0.53*	*0.53*	*<0.01*	*0.97*	*0.03*	*<0.01*	*<0.01*
Val	[g/16g N]	0.14	0.28	0.43	0.44	0.43	-0.02	0.44	-0.30	-0.17	-0.15
	P	*0.56*	*0.24*	*0.06*	*0.05*	*0.06*	*0.94*	*0.05*	*0.20*	*0.47*	*0.52*
Ala	[g/16g N]	0.28	0.18	0.45	0.47	0.48	-0.75	0.25	-0.42	-0.64	-0.67
	P	*0.24*	*0.45*	*0.05*	*0.03*	*0.03*	*<0.01*	*0.30*	*0.06*	*<0.01*	*<0.01*
Asp	[g/16g N]	0.05	0.19	0.33	0.34	0.33	0.73	0.10	0.56	0.74	0.74
	P	*0.82*	*0.41*	*0.15*	*0.14*	*0.15*	*<0.01*	*0.68*	*<0.01*	*<0.01*	*<0.01*
Cys	[g/16g N]	0.43	0.25	0.20	0.37	0.37	0.47	-0.31	0.42	0.49	0.49
	P	*0.06*	*0.30*	*0.39*	*0.10*	*0.11*	*0.04*	*0.19*	*0.07*	*0.03*	*0.03*
Glu	[g/16g N]	-0.34	-0.39	-0.40	-0.57	-0.55	-0.86	0.06	-0.49	-0.76	-0.78
	P	*0.15*	*0.09*	*0.08*	*<0.01*	*0.01*	*<0.01*	*0.81*	*0.03*	*<0.01*	*<0.01*

		Wheat					Corn				
		a	a+b	c	ED5	ED8	a	a+b	c	ED5	ED8
Gly	[g/16g N]	0.22	-0.16	0.24	0.17	0.21	0.71	0.12	0.26	0.56	0.59
	P	*0.34*	*0.50*	*0.31*	*0.47*	*0.37*	*<0.01*	*0.61*	*0.27*	*0.01*	*<0.01*
Pro	[g/16g N]	-0.36	-0.51	-0.40	-0.61	-0.58	-0.61	0.14	-0.54	-0.63	-0.64
	P	*0.12*	*0.02*	*0.08*	*<0.01*	*<0.01*	*<0.01*	*0.54*	*0.01*	*<0.01*	*<0.01*
Ser	[g/16g N]	-0.11	-0.28	-0.19	-0.33	-0.31	-0.57	0.27	-0.56	-0.60	-0.60
	P	*0.65*	*0.24*	*0.42*	*0.15*	*0.18*	*<0.01*	*0.25*	*<0.01*	*<0.01*	*<0.01*
Tyr	[g/16g N]	-0.02	-0.48	0.01	-0.20	-0.14	-0.63	0.34	-0.54	-0.61	-0.63
	P	*0.94*	*0.03*	*0.97*	*0.40*	*0.57*	*<0.01*	*0.14*	*0.01*	*<0.01*	*<0.01*
Ca	[g/kg DM]	-0.02	0.24	-0.09	0.01	-0.03	0.21	-0.35	0.44	0.33	0.32
	P	*0.95*	*0.31*	*0.69*	*0.97*	*0.91*	*0.37*	*0.13*	*0.05*	*0.15*	*0.17*
Mg	[g/kg DM]	0.18	0.08	0.08	0.11	0.11	-0.19	0.18	-0.61	-0.43	-0.40
	P	*0.46*	*0.73*	*0.73*	*0.65*	*0.65*	*0.41*	*0.46*	*<0.01*	*0.06*	*0.08*
K	[g/kg DM]	0.46	0.45	0.16	0.48	0.45	0.57	-0.16	0.13	0.38	0.41
	P	*0.04*	*0.05*	*0.49*	*0.03*	*0.04*	*<0.01*	*0.50*	*0.58*	*0.10*	*0.07*
Na	[mg/kg DM]	0.65	0.22	0.35	0.53	0.56					
	P	*<0.01*	*0.44*	*0.20*	*0.04*	*0.03*					
Fe	[mg/kg DM]	-0.14	-0.22	-0.18	-0.25	-0.24	-0.21	0.14	-0.49	-0.38	-0.36
	P	*0.55*	*0.36*	*0.46*	*0.28*	*0.31*	*0.38*	*0.56*	*0.03*	*0.10*	*0.12*
Mn	[mg/kg DM]	-0.10	-0.21	-0.30	-0.32	-0.31	-0.14	0.17	-0.14	-0.17	-0.16
	P	*0.68*	*0.38*	*0.20*	*0.17*	*0.18*	*0.56*	*0.47*	*0.57*	*0.49*	*0.49*
Zn	[mg/kg DM]	0.25	0.07	0.17	0.22	0.23	-0.40	0.23	-0.44	-0.48	-0.47
	P	*0.30*	*0.78*	*0.48*	*0.35*	*0.33*	*0.08*	*0.34*	*0.05*	*0.03*	*0.04*
Cu	[mg/kg DM]	0.22	-0.01	-0.02	0.03	0.04	-0.08	-0.08	-0.39	-0.29	-0.26
	P	*0.36*	*0.97*	*0.95*	*0.89*	*0.86*	*0.75*	*0.75*	*0.09*	*0.22*	*0.27*
P	[g/kg DM]	0.03	-0.13	-0.17	-0.20	-0.18	-0.11	-0.11	-0.29	-0.21	-0.20
	P	*0.91*	*0.57*	*0.48*	*0.41*	*0.44*	*0.65*	*0.64*	*0.22*	*0.37*	*0.40*
GP2•	[ml/200 mg DM]	-0.52	-0.19	-0.09	-0.32	-0.33	-0.02	-0.16	0.34	0.16	0.14
	P	*0.02*	*0.43*	*0.69*	*0.16*	*0.16*	*0.93*	*0.51*	*0.14*	*0.49*	*0.55*
GP4•	[ml/200 mg DM]	-0.40	-0.52	-0.03	-0.37	-0.33	0.40	-0.32	0.80	0.65	0.62
	P	*0.08*	*0.02*	*0.91*	*0.10*	*0.16*	*0.08*	*0.17*	*<0.01*	*<0.01*	*<0.01*
GP6•	[ml/200 mg DM]	-0.39	-0.44	-0.11	-0.39	-0.36	0.48	-0.34	0.79	0.68	0.66
	P	*0.09*	*0.05*	*0.65*	*0.09*	*0.12*	*0.03*	*0.14*	*<0.01*	*<0.01*	*<0.01*
GP8•	[ml/200 mg DM]	-0.24	-0.09	0.08	-0.06	-0.06	0.36	-0.36	0.74	0.59	0.56
	P	*0.30*	*0.70*	*0.74*	*0.80*	*0.81*	*0.12*	*0.12*	*<0.01*	*<0.01*	*<0.01*
GP12•	[ml/200 mg DM]	0.03	0.42	0.16	0.32	0.27	0.21	-0.31	0.66	0.46	0.43
	P	*0.91*	*0.07*	*0.50*	*0.17*	*0.24*	*0.38*	*0.18*	*<0.01*	*0.04*	*0.06*
GP24•	[ml/200 mg DM]	-0.18	0.18	-0.06	-0.01	-0.05	-0.08	-0.28	0.41	0.15	0.12
	P	*0.46*	*0.46*	*0.81*	*0.96*	*0.85*	*0.73*	*0.23*	*0.07*	*0.52*	*0.61*
GP48•	[ml/200 mg DM]	0.03	0.36	0.11	0.26	0.22	-0.26	-0.20	0.27	-0.02	-0.05
	P	*0.91*	*0.12*	*0.63*	*0.26*	*0.35*	*0.27*	*0.39*	*0.25*	*0.92*	*0.82*
GP72•	[ml/200 mg DM]	0.09	0.33	0.22	0.35	0.32	-0.31	-0.18	0.21	-0.08	-0.11
	P	*0.71*	*0.16*	*0.35*	*0.14*	*0.18*	*0.18*	*0.46*	*0.37*	*0.73*	*0.64*
GPb£	[ml/200 mg DM]	0.09	0.43	0.14	0.33	0.28	-0.39	-0.14	0.09	-0.19	-0.22
	P	*0.70*	*0.06*	*0.57*	*0.16*	*0.23*	*0.09*	*0.57*	*0.69*	*0.42*	*0.36*
GPc¤	[%/h]	-0.38	-0.40	-0.10	-0.37	-0.34	0.63	-0.32	0.79	0.78	0.77
	P	*0.10*	*0.08*	*0.68*	*0.11*	*0.14*	*<0.01*	*0.17*	*<0.01*	*<0.01*	*<0.01*

‡TSW, thousand seed weight; •TW, test weight; §FN, falling number; *aNDFom, neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash; #ADFom, acid detergent fiber expressed exclusive of residual ash; †ADL, acid detergent lignin; ¥Starch, determined enzymatically; •GPx, Gas production after x h incubation time; £GPb, potential gas production; ¤GPc, rate of gas production of GPb.

ANNEX 2. Correlation coefficients of starch degradation characteristics with physical and chemical characteristics and *in vitro* measurements of wheat and corn (n = 20 genotypes per grain type)

		Wheat					Corn				
		a	a+b	c	ED5	ED8	a	a+b	c	ED5	ED8
TSW‡	[g/1000 seeds]	0.25	0.10	0.09	0.21	0.20	-0.19	0.35	-0.08	-0.15	-0.15
	p	*0.29*	*0.69*	*0.69*	*0.38*	*0.40*	*0.43*	*0.13*	*0.75*	*0.54*	*0.52*
TW♦	[kg/hl]	-0.31	-0.09	-0.32	-0.53	-0.51	-0.80	-0.22	-0.38	-0.66	-0.69
	P	*0.18*	*0.70*	*0.17*	*0.02*	*0.02*	*<0.01*	*0.36*	*0.10*	*<0.01*	*<0.01*
FN§	[per s]	-0.16	-0.28	0.16	-0.16	-0.11					
	P	*0.51*	*0.22*	*0.49*	*0.51*	*0.64*					
Crude ash	[g/kg DM]	0.23	0.27	-0.40	-0.14	-0.17	-0.13	-0.23	-0.08	-0.12	-0.12
	P	*0.34*	*0.25*	*0.08*	*0.57*	*0.47*	*0.60*	*0.33*	*0.73*	*0.62*	*0.61*
Crude protein	[g/kg DM]	0.02	-0.17	-0.17	-0.21	-0.18	-0.77	-0.25	-0.68	-0.83	-0.83
	P	*0.93*	*0.48*	*0.47*	*0.38*	*0.45*	*<0.01*	*0.28*	*<0.01*	*<0.01*	*<0.01*
Crude fibre	[g/kg DM]	0.13	-0.40	0.25	0.02	0.09	0.22	0.28	0.23	0.24	0.24
	P	*0.58*	*0.08*	*0.28*	*0.92*	*0.72*	*0.36*	*0.24*	*0.33*	*0.30*	*0.31*
Crude fat	[g/kg DM]	-0.05	-0.09	0.03	-0.12	-0.10	0.06	0.07	-0.34	-0.14	-0.11
	p	*0.82*	*0.70*	*0.89*	*0.61*	*0.67*	*0.80*	*0.78*	*0.14*	*0.57*	*0.64*
aNDFom*	[g/kg DM]	0.13	-0.07	-0.13	-0.11	-0.09	0.52	0.17	0.46	0.54	0.54
	P	*0.58*	*0.78*	*0.58*	*0.65*	*0.70*	*0.02*	*0.48*	*0.04*	*0.01*	*0.01*
ADFom#	[g/kg DM]	0.18	0.05	0.08	0.01	0.01	-0.58	-0.02	-0.19	-0.47	-0.48
	P	*0.45*	*0.82*	*0.73*	*0.97*	*0.97*	*<0.01*	*0.93*	*0.42*	*0.04*	*0.03*
ADL†	[g/kg DM]	-0.15	-0.09	-0.26	-0.36	-0.34					
	P	*0.52*	*0.72*	*0.28*	*0.12*	*0.14*					
Starch¥	[g/kg DM]	0.19	0.12	0.38	0.46	0.44	0.29	0.01	0.35	0.35	0.35
	P	*0.43*	*0.61*	*0.1*	*0.04*	*0.06*	*0.21*	*0.97*	*0.13*	*0.13*	*0.13*
Gross energy	[MJ/kg DM]	0.13	0.02	-0.09	0.00	-0.01	0.00	0.05	-0.40	-0.21	-0.19
	P	*0.57*	*0.92*	*0.70*	*0.98*	*0.98*	*0.99*	*0.82*	*0.08*	*0.37*	*0.43*
Arg	[g/16g N]	0.28	0.34	0.16	0.41	0.36	0.50	0.14	0.24	0.44	0.46
	P	*0.23*	*0.15*	*0.50*	*0.07*	*0.12*	*0.02*	*0.55*	*0.30*	*0.05*	*0.04*
His	[g/16g N]	0.34	0.18	-0.05	0.13	0.11	0.18	-0.17	0.24	0.23	0.23
	P	*0.15*	*0.44*	*0.84*	*0.58*	*0.65*	*0.44*	*0.47*	*0.32*	*0.32*	*0.33*
Ile	[g/16g N]	-0.14	-0.06	0.30	0.18	0.19	-0.41	0.28	-0.27	-0.40	-0.40
	P	*0.55*	*0.81*	*0.19*	*0.44*	*0.43*	*0.07*	*0.23*	*0.25*	*0.08*	*0.08*
Leu	[g/16g N]	-0.17	0.25	0.23	0.18	0.14	-0.80	-0.16	-0.49	-0.70	-0.71
	P	*0.48*	*0.29*	*0.33*	*0.45*	*0.56*	*<0.01*	*0.50*	*0.03*	*<0.01*	*<0.01*
Lys	[g/16g N]	0.05	0.14	0.19	0.28	0.25	0.64	0.21	0.41	0.60	0.62
	P	*0.82*	*0.56*	*0.43*	*0.24*	*0.28*	*<0.01*	*0.37*	*0.08*	*<0.01*	*<0.01*
Met	[g/16g N]	0.20	0.14	0.20	0.32	0.30	0.18	-0.01	0.03	0.10	0.11
	P	*0.39*	*0.55*	*0.41*	*0.17*	*0.20*	*0.46*	*0.96*	*0.89*	*0.67*	*0.63*
Phe	[g/16g N]	-0.35	0.04	-0.11	-0.27	-0.27	-0.82	-0.03	-0.56	-0.78	-0.79
	P	*0.13*	*0.88*	*0.63*	*0.26*	*0.24*	*<0.01*	*0.91*	*0.01*	*<0.01*	*<0.01*
Thr	[g/16g N]	0.09	0.12	0.15	0.21	0.19	0.43	0.40	0.05	0.27	0.29
	P	*0.71*	*0.62*	*0.52*	*0.37*	*0.42*	*0.06*	*0.08*	*0.84*	*0.26*	*0.21*
Trp	[g/16g N]	0.23	0.17	-0.11	0.15	0.12	0.73	0.28	0.44	0.67	0.69
	P	*0.33*	*0.47*	*0.66*	*0.54*	*0.61*	*<0.01*	*0.22*	*0.05*	*<0.01*	*<0.01*
Val	[g/16g N]	0.01	0.13	0.31	0.33	0.30	-0.12	0.41	-0.32	-0.25	-0.23
	P	*0.97*	*0.57*	*0.18*	*0.16*	*0.19*	*0.63*	*0.07*	*0.17*	*0.29*	*0.32*
Ala	[g/16g N]	0.11	0.07	0.22	0.31	0.29	-0.66	-0.02	-0.40	-0.56	-0.57
	P	*0.64*	*0.76*	*0.35*	*0.19*	*0.21*	*<0.01*	*0.93*	*0.08*	*<0.01*	*<0.01*
Asp	[g/16g N]	-0.09	0.08	0.01	0.08	0.06	0.66	0.44	0.53	0.70	0.71
	P	*0.70*	*0.75*	*0.98*	*0.74*	*0.79*	*<0.01*	*0.05*	*0.02*	*<0.01*	*<0.01*
Cys	[g/16g N]	0.38	0.23	0.16	0.38	0.35	0.43	-0.17	0.39	0.43	0.43
	P	*0.10*	*0.33*	*0.50*	*0.10*	*0.14*	*0.06*	*0.48*	*0.09*	*0.06*	*0.06*
Glu	[g/16g N]	-0.18	-0.17	-0.15	-0.34	-0.32	-0.72	-0.22	-0.43	-0.61	-0.62
	P	*0.44*	*0.48*	*0.52*	*0.14*	*0.18*	*<0.01*	*0.36*	*0.06*	*<0.01*	*<0.01*

		Wheat					Corn				
		a	a+b	c	ED5	ED8	a	a+b	c	ED5	ED8
Gly	[g/16g N]	0.25	0.11	0.16	0.32	0.30	0.53	0.28	0.18	0.41	0.43
	P	*0.28*	*0.64*	*0.49*	*0.17*	*0.20*	*0.02*	*0.23*	*0.45*	*0.07*	*0.06*
Pro	[g/16g N]	-0.29	-0.23	-0.30	-0.47	-0.44	-0.67	-0.27	-0.51	-0.58	-0.58
	P	*0.21*	*0.34*	*0.20*	*0.04*	*0.05*	*<0.01*	*0.25*	*0.02*	*<0.01*	*<0.01*
Ser	[g/16g N]	0.10	0.03	-0.03	-0.08	-0.08	-0.62	-0.11	-0.60	-0.56	-0.55
	P	*0.68*	*0.88*	*0.89*	*0.75*	*0.75*	*<0.01*	*0.65*	*<0.01*	*<0.01*	*<0.01*
Tyr	[g/16g N]	-0.06	-0.27	0.05	-0.07	-0.03	-0.63	0.02	-0.55	-0.63	-0.63
	P	*0.80*	*0.26*	*0.83*	*0.77*	*0.89*	*<0.01*	*0.94*	*0.01*	*<0.01*	*<0.01*
Ca	[g/kg DM]	0.11	0.34	-0.05	0.04	-0.01	0.34	-0.08	0.46	0.41	0.41
	P	*0.66*	*0.15*	*0.83*	*0.87*	*0.98*	*0.14*	*0.73*	*0.04*	*0.07*	*0.07*
Mg	[g/kg DM]	0.41	0.24	0.22	0.38	0.35	-0.39	-0.11	-0.65	-0.56	-0.54
	P	*0.07*	*0.30*	*0.35*	*0.10*	*0.13*	*0.09*	*0.64*	*<0.01*	*0.01*	*0.01*
K	[g/kg DM]	0.59	0.31	0.20	0.56	0.52	0.33	-0.03	0.10	0.22	0.24
	P	*<0.01*	*0.18*	*0.39*	*0.01*	*0.02*	*0.16*	*0.91*	*0.66*	*0.34*	*0.30*
Na	[mg/kg DM]	0.53	-0.17	0.56	0.61	0.63					
	P	*0.04*	*0.55*	*0.03*	*0.02*	*0.01*					
Fe	[mg/kg DM]	-0.21	-0.49	-0.07	-0.33	-0.26	-0.38	-0.04	-0.51	-0.50	-0.49
	P	*0.38*	*0.03*	*0.76*	*0.15*	*0.27*	*0.10*	*0.85*	*0.02*	*0.02*	*0.03*
Mn	[mg/kg DM]	-0.19	-0.25	-0.08	-0.32	-0.28	-0.08	0.20	-0.18	-0.17	-0.15
	P	*0.42*	*0.28*	*0.72*	*0.17*	*0.23*	*0.74*	*0.39*	*0.46*	*0.49*	*0.52*
Zn	[mg/kg DM]	0.37	0.13	0.37	0.45	0.43	-0.46	0.08	-0.44	-0.52	-0.52
	P	*0.11*	*0.60*	*0.11*	*0.05*	*0.06*	*0.04*	*0.74*	*0.05*	*0.02*	*0.02*
Cu	[mg/kg DM]	0.24	-0.22	0.07	0.02	0.06	-0.14	-0.17	-0.38	-0.32	-0.29
	P	*0.31*	*0.34*	*0.75*	*0.92*	*0.79*	*0.55*	*0.47*	*0.10*	*0.18*	*0.21*
P	[g/kg DM]	0.18	0.14	0.02	0.08	0.06	-0.26	-0.33	-0.29	-0.29	-0.29
	P	*0.45*	*0.54*	*0.95*	*0.73*	*0.79*	*0.26*	*0.16*	*0.22*	*0.21*	*0.21*
GP2•	[ml/200 mg DM]	-0.64	0.05	-0.26	-0.48	-0.49	0.21	-0.01	0.34	0.31	0.30
	P	*<0.01*	*0.84*	*0.27*	*0.03*	*0.03*	*0.36*	*0.96*	*0.15*	*0.18*	*0.20*
GP4•	[ml/200 mg DM]	-0.59	-0.62	-0.02	-0.49	-0.40	0.55	0.01	0.79	0.73	0.72
	P	*<0.01*	*<0.01*	*0.92*	*0.03*	*0.08*	*0.01*	*1.00*	*<0.01*	*<0.01*	*<0.01*
GP6•	[ml/200 mg DM]	-0.52	-0.62	-0.13	-0.54	-0.45	0.59	0.01	0.81	0.77	0.75
	P	*0.02*	*<0.01*	*0.59*	*0.01*	*0.05*	*<0.01*	*0.95*	*<0.01*	*<0.01*	*<0.01*
GP8•	[ml/200 mg DM]	-0.43	-0.32	-0.10	-0.33	-0.29	0.50	-0.03	0.78	0.70	0.68
	P	*0.06*	*0.18*	*0.66*	*0.15*	*0.22*	*0.03*	*0.88*	*<0.01*	*<0.01*	*<0.01*
GP12•	[ml/200 mg DM]	-0.17	0.28	-0.11	0.00	-0.05	0.36	-0.04	0.70	0.58	0.55
	P	*0.47*	*0.23*	*0.65*	*0.99*	*0.83*	*0.12*	*0.86*	*<0.01*	*<0.01*	*0.01*
GP24•	[ml/200 mg DM]	-0.39	0.07	-0.25	-0.33	-0.34	0.08	-0.02	0.50	0.28	0.26
	P	*0.09*	*0.77*	*0.29*	*0.15*	*0.14*	*0.75*	*0.95*	*0.03*	*0.22*	*0.27*
GP48•	[ml/200 mg DM]	-0.18	0.21	0.00	-0.02	-0.05	-0.09	-0.02	0.37	0.13	0.10
	P	*0.44*	*0.38*	*0.99*	*0.94*	*0.83*	*0.72*	*0.93*	*0.11*	*0.59*	*0.67*
GP72•	[ml/200 mg DM]	-0.09	0.20	0.15	0.13	0.10	-0.16	-0.02	0.33	0.07	0.04
	P	*0.72*	*0.40*	*0.53*	*0.58*	*0.68*	*0.51*	*0.94*	*0.16*	*0.79*	*0.88*
GPb£	[ml/200 mg DM]	-0.08	0.32	0.01	0.08	0.03	-0.24	-0.01	0.20	-0.05	-0.08
	P	*0.72*	*0.16*	*0.96*	*0.75*	*0.91*	*0.31*	*0.98*	*0.39*	*0.83*	*0.75*
GPc¤	[%/h]	-0.49	-0.52	-0.17	-0.50	-0.43	0.72	0.00	0.77	0.83	0.82
	P	*0.03*	*0.02*	*0.48*	*0.03*	*0.06*	*<0.01*	*0.99*	*<0.01*	*<0.01*	*<0.01*

‡TSW, thousand seed weight; ♦TW, test weight; §FN, falling number; *aNDFom, neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash; #ADFom, acid detergent fiber expressed exclusive of residual ash; †ADL, acid detergent lignin; ¥Starch, determined enzymatically; •GPx, Gas production after x h incubation time; £GPb, potential gas production; ¤GPc, rate of gas production of GPb.

ANNEX 3. Correlation coefficients of CP degradation characteristics with physical and chemical characteristics and *in vitro* measurements of wheat and corn (n = 20 genotypes per grain type)

		Wheat					Corn				
		a	a+b	c	ED5	ED8	a	a+b	c	ED5	ED8
TSW‡	[g/1000 seeds]	-0.36	0.34	-0.04	-0.06	-0.12	-0.49	0.05	-0.06	-0.28	-0.31
	p	*0.12*	*0.14*	*0.88*	*0.79*	*0.61*	*0.03*	*0.85*	*0.80*	*0.24*	*0.18*
TW♦	[kg/hl]	-0.36	0.30	-0.61	-0.56	-0.58	-0.88	0.29	-0.65	-0.83	-0.85
	P	*0.12*	*0.19*	*<0.01*	*<0.01*	*<0.01*	*<0.01*	*0.21*	*<0.01*	*<0.01*	*<0.01*
FN§	[per s]	0.28	-0.27	-0.18	-0.13	-0.07					
	P	*0.24*	*0.26*	*0.45*	*0.59*	*0.76*					
Crude ash	[g/kg DM]	-0.22	0.19	-0.40	-0.42	-0.42	0.17	0.00	-0.13	0.01	0.03
	P	*0.34*	*0.42*	*0.08*	*0.06*	*0.07*	*0.47*	*1.00*	*0.58*	*0.97*	*0.90*
Crude protein	[g/kg DM]	-0.36	0.60	-0.60	-0.45	-0.51	-0.49	0.41	-0.81	-0.75	-0.71
	P	*0.12*	*<0.01*	*<0.01*	*0.05*	*0.02*	*0.03*	*0.07*	*<0.01*	*<0.01*	*<0.01*
Crude fibre	[g/kg DM]	-0.34	0.12	-0.37	-0.49	-0.48	0.43	-0.14	0.34	0.42	0.43
	P	*0.14*	*0.62*	*0.11*	*0.03*	*0.03*	*0.06*	*0.57*	*0.14*	*0.06*	*0.06*
Crude fat	[g/kg DM]	0.24	-0.18	-0.19	-0.15	-0.10	0.65	0.15	-0.05	0.32	0.37
	p	*0.31*	*0.46*	*0.42*	*0.53*	*0.67*	*<0.01*	*0.54*	*0.84*	*0.17*	*0.11*
aNDFom*	[g/kg DM]	0.05	-0.07	-0.08	-0.09	-0.07	0.52	-0.37	0.65	0.65	0.64
	P	*0.83*	*0.78*	*0.73*	*0.72*	*0.77*	*0.02*	*0.11*	*<0.01*	*<0.01*	*<0.01*
ADFom#	[g/kg DM]	-0.28	0.06	-0.21	-0.37	-0.36	-0.27	0.09	-0.32	-0.34	-0.33
	P	*0.24*	*0.79*	*0.38*	*0.11*	*0.12*	*0.26*	*0.72*	*0.17*	*0.14*	*0.15*
ADL†	[g/kg DM]	0.02	-0.37	0.02	-0.17	-0.11					
	P	*0.95*	*0.11*	*0.93*	*0.48*	*0.66*					
Starch¥	[g/kg DM]	0.12	-0.06	0.32	0.32	0.31	-0.34	-0.12	0.15	-0.09	-0.13
	P	*0.6*	*0.82*	*0.17*	*0.18*	*0.19*	*0.14*	*0.6*	*0.54*	*0.69*	*0.59*
Gross energy	[MJ/kg DM]	-0.33	0.59	-0.42	-0.29	-0.36	0.59	0.15	-0.13	0.24	0.29
	P	*0.16*	*<0.01*	*0.07*	*0.22*	*0.12*	*<0.01*	*0.53*	*0.59*	*0.32*	*0.22*
Arg	[g/16g N]	0.59	-0.33	0.56	0.63	0.65	0.87	-0.07	0.53	0.76	0.78
	P	*<0.01*	*0.15*	*<0.01*	*<0.01*	*<0.01*	*<0.01*	*0.77*	*0.02*	*<0.01*	*<0.01*
His	[g/16g N]	0.17	-0.35	0.21	0.13	0.17	0.24	-0.04	0.24	0.26	0.26
	P	*0.46*	*0.13*	*0.37*	*0.59*	*0.47*	*0.31*	*0.86*	*0.30*	*0.27*	*0.27*
Ile	[g/16g N]	0.31	0.02	0.21	0.31	0.30	-0.51	0.01	-0.36	-0.47	-0.47
	P	*0.18*	*0.95*	*0.37*	*0.19*	*0.20*	*0.02*	*0.97*	*0.12*	*0.04*	*0.04*
Leu	[g/16g N]	0.13	-0.11	0.25	0.18	0.19	-0.93	0.34	-0.80	-0.94	-0.95
	P	*0.58*	*0.65*	*0.29*	*0.46*	*0.43*	*<0.01*	*0.14*	*<0.01*	*<0.01*	*<0.01*
Lys	[g/16g N]	0.64	-0.52	0.59	0.61	0.66	0.92	-0.22	0.72	0.91	0.92
	P	*<0.01*	*0.02*	*<0.01*	*<0.01*	*<0.01*	*<0.01*	*0.35*	*<0.01*	*<0.01*	*<0.01*
Met	[g/16g N]	0.45	-0.07	0.22	0.37	0.38	0.57	-0.04	0.32	0.49	0.51
	P	*0.05*	*0.77*	*0.35*	*0.10*	*0.10*	*<0.01*	*0.87*	*0.16*	*0.03*	*0.02*
Phe	[g/16g N]	-0.74	0.24	-0.23	-0.50	-0.53	-0.86	0.36	-0.77	-0.90	-0.91
	P	*<0.01*	*0.31*	*0.33*	*0.02*	*0.02*	*<0.01*	*0.12*	*<0.01*	*<0.01*	*<0.01*
Thr	[g/16g N]	0.52	-0.31	0.19	0.30	0.34	0.68	-0.14	0.48	0.56	0.56
	P	*0.02*	*0.19*	*0.41*	*0.20*	*0.14*	*<0.01*	*0.54*	*0.03*	*<0.01*	*<0.01*
Trp	[g/16g N]	0.13	-0.12	0.32	0.23	0.24	0.87	-0.13	0.70	0.86	0.87
	P	*0.59*	*0.61*	*0.18*	*0.32*	*0.31*	*<0.01*	*0.59*	*<0.01*	*<0.01*	*<0.01*
Val	[g/16g N]	0.42	-0.07	0.30	0.41	0.41	0.01	0.06	-0.11	-0.07	-0.06
	P	*0.07*	*0.77*	*0.19*	*0.07*	*0.07*	*0.98*	*0.79*	*0.64*	*0.78*	*0.82*
Ala	[g/16g N]	0.68	-0.50	0.54	0.60	0.65	-0.80	0.27	-0.62	-0.73	-0.75
	P	*<0.01*	*0.03*	*0.01*	*<0.01*	*<0.01*	*<0.01*	*0.25*	*<0.01*	*<0.01*	*<0.01*
Asp	[g/16g N]	0.63	-0.56	0.54	0.57	0.63	0.67	-0.34	0.74	0.82	0.82
	P	*<0.01*	*0.01*	*0.01*	*<0.01*	*<0.01*	*<0.01*	*0.14*	*<0.01*	*<0.01*	*<0.01*
Cys	[g/16g N]	0.46	-0.11	0.25	0.37	0.38	0.49	-0.10	0.50	0.51	0.51
	P	*0.04*	*0.66*	*0.28*	*0.11*	*0.10*	*0.03*	*0.67*	*0.02*	*0.02*	*0.02*
Glu	[g/16g N]	-0.72	0.37	-0.57	-0.73	-0.76	-0.95	0.34	-0.73	-0.86	-0.88
	P	*<0.01*	*0.11*	*<0.01*	*<0.01*	*<0.01*	*<0.01*	*0.14*	*<0.01*	*<0.01*	*<0.01*
Gly	[g/16g N]	0.43	-0.18	0.11	0.22	0.25	0.86	-0.16	0.56	0.77	0.79
	P	*0.06*	*0.46*	*0.65*	*0.35*	*0.29*	*<0.01*	*0.51*	*0.01*	*<0.01*	*<0.01*

		Wheat					Corn				
		a	a+b	c	ED5	ED8	a	a+b	c	ED5	ED8
Pro	[g/16g N]	-0.36	-0.03	-0.37	-0.53	-0.52	-0.47	0.46	-0.64	-0.54	-0.53
	P	*0.11*	*0.89*	*0.11*	*0.02*	*0.02*	*0.04*	*0.04*	*<0.01*	*0.01*	*0.02*
Ser	[g/16g N]	-0.48	0.17	-0.41	-0.54	-0.54	-0.36	0.52	-0.62	-0.42	-0.41
	P	*0.03*	*0.48*	*0.07*	*0.01*	*0.01*	*0.12*	*0.02*	*<0.01*	*0.06*	*0.07*
Tyr	[g/16g N]	0.29	-0.16	-0.11	-0.02	0.01	-0.50	0.51	-0.57	-0.54	-0.54
	P	*0.21*	*0.50*	*0.63*	*0.93*	*0.96*	*0.02*	*0.02*	*<0.01*	*0.01*	*0.01*
Ca	[g/kg DM]	-0.25	0.27	-0.09	-0.11	-0.14	-0.05	-0.36	0.46	0.24	0.20
	P	*0.29*	*0.26*	*0.71*	*0.64*	*0.54*	*0.83*	*0.12*	*0.04*	*0.31*	*0.39*
Mg	[g/kg DM]	-0.30	0.64	-0.24	-0.16	-0.23	0.12	0.32	-0.53	-0.24	-0.20
	P	*0.20*	*<0.01*	*0.31*	*0.51*	*0.33*	*0.61*	*0.17*	*0.02*	*0.31*	*0.41*
K	[g/kg DM]	-0.03	0.50	0.14	0.29	0.21	0.62	-0.27	0.28	0.47	0.50
	P	*0.90*	*0.03*	*0.57*	*0.21*	*0.37*	*<0.01*	*0.25*	*0.24*	*0.04*	*0.03*
Na	[mg/kg DM]	0.42	0.33	0.02	0.41	0.36					
	P	*0.12*	*0.23*	*0.96*	*0.13*	*0.19*					
Fe	[mg/kg DM]	0.05	0.10	-0.21	-0.10	-0.10	0.00	0.25	-0.45	-0.27	-0.24
	P	*0.83*	*0.67*	*0.38*	*0.69*	*0.67*	*1.00*	*0.28*	*0.04*	*0.25*	*0.32*
Mn	[mg/kg DM]	-0.01	0.14	-0.40	-0.29	-0.28	-0.23	0.08	-0.06	-0.16	-0.17
	P	*0.97*	*0.57*	*0.08*	*0.22*	*0.22*	*0.34*	*0.73*	*0.81*	*0.49*	*0.47*
Zn	[mg/kg DM]	-0.28	0.56	-0.13	-0.08	-0.15	-0.34	0.25	-0.49	-0.48	-0.47
	P	*0.23*	*0.01*	*0.59*	*0.75*	*0.53*	*0.14*	*0.28*	*0.03*	*0.03*	*0.04*
Cu	[mg/kg DM]	0.27	0.03	-0.21	-0.05	-0.03	0.03	0.14	-0.35	-0.22	-0.19
	P	*0.24*	*0.91*	*0.38*	*0.84*	*0.89*	*0.90*	*0.56*	*0.13*	*0.35*	*0.43*
P	[g/kg DM]	-0.30	0.47	-0.36	-0.35	-0.39	0.04	0.15	-0.33	-0.17	-0.15
	P	*0.21*	*0.04*	*0.11*	*0.13*	*0.09*	*0.88*	*0.52*	*0.16*	*0.47*	*0.54*
GP2•	[ml/200 mg DM]	0.21	-0.48	-0.01	-0.08	-0.01	-0.26	0.02	0.21	-0.02	-0.05
	P	*0.38*	*0.03*	*0.97*	*0.72*	*0.97*	*0.27*	*0.94*	*0.38*	*0.94*	*0.83*
GP4•	[ml/200 mg DM]	0.15	-0.43	-0.10	-0.16	-0.09	0.08	-0.26	0.68	0.43	0.39
	P	*0.51*	*0.06*	*0.67*	*0.51*	*0.71*	*0.75*	*0.28*	*<0.01*	*0.06*	*0.09*
GP6•	[ml/200 mg DM]	0.06	-0.37	-0.15	-0.21	-0.15	0.15	-0.33	0.68	0.46	0.43
	P	*0.80*	*0.11*	*0.53*	*0.37*	*0.53*	*0.52*	*0.15*	*<0.01*	*0.04*	*0.06*
GP8•	[ml/200 mg DM]	0.30	-0.55	0.23	0.16	0.23	0.03	-0.32	0.59	0.35	0.31
	P	*0.19*	*0.01*	*0.34*	*0.50*	*0.34*	*0.90*	*0.18*	*<0.01*	*0.13*	*0.18*
GP12•	[ml/200 mg DM]	0.52	-0.54	0.51	0.52	0.57	-0.10	-0.37	0.49	0.23	0.19
	P	*0.02*	*0.01*	*0.02*	*0.02*	*<0.01*	*0.69*	*0.11*	*0.03*	*0.32*	*0.42*
GP24•	[ml/200 mg DM]	0.41	-0.59	0.25	0.22	0.29	-0.39	-0.40	0.23	-0.08	-0.12
	P	*0.08*	*<0.01*	*0.30*	*0.36*	*0.22*	*0.09*	*0.08*	*0.33*	*0.74*	*0.62*
GP48•	[ml/200 mg DM]	0.37	-0.41	0.33	0.35	0.39	-0.55	-0.26	0.07	-0.25	-0.29
	P	*0.11*	*0.07*	*0.15*	*0.13*	*0.09*	*0.01*	*0.27*	*0.78*	*0.28*	*0.21*
GP72•	[ml/200 mg DM]	0.29	-0.28	0.36	0.39	0.40	-0.57	-0.22	0.01	-0.30	-0.34
	P	*0.22*	*0.24*	*0.12*	*0.09*	*0.08*	*<0.01*	*0.34*	*0.97*	*0.20*	*0.15*
GPb£	[ml/200 mg DM]	0.35	-0.34	0.37	0.39	0.42	-0.62	-0.22	-0.09	-0.38	-0.42
	P	*0.13*	*0.14*	*0.11*	*0.09*	*0.07*	*<0.01*	*0.35*	*0.70*	*0.10*	*0.07*
GPc¤	[%/h]	0.11	-0.38	-0.10	-0.16	-0.10	0.39	-0.31	0.74	0.63	0.60
	P	*0.64*	*0.10*	*0.67*	*0.49*	*0.67*	*0.09*	*0.19*	*<0.01*	*<0.01*	*<0.01*

‡TSW, thousand seed weight; ♦TW, test weight; §FN, falling number; *aNDFom, neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash; #ADFom, acid detergent fiber expressed exclusive of residual ash; †ADL, acid detergent lignin; ¥Starch, determined enzymatically; •GPx, Gas production after x h incubation time; £GPb, potential gas production; ¤GPc, rate of gas production of GPb.

ANNEX 4. Correlation coefficients of rDM degradation characteristics with physical and chemical characteristics and *in vitro* measurements of wheat and corn (n = 20 genotypes per grain type)

		Wheat					Corn				
		a	a+b	c	ED5	ED8	a	a+b	c	ED5	ED8
TSW‡	[g/1000 seeds]	-0.24	-0.22	0.43	0.02	0.07	-0.41	0.14	-0.01	-0.24	-0.28
	p	*0.30*	*0.36*	*0.06*	*0.92*	*0.77*	*0.08*	*0.54*	*0.98*	*0.31*	*0.23*
TW♦	[kg/hl]	-0.19	-0.31	0.03	-0.34	-0.32	-0.19	0.72	-0.76	-0.56	-0.52
	P	*0.42*	*0.18*	*0.91*	*0.15*	*0.17*	*0.41*	*<0.01*	*<0.01*	*0.01*	*0.02*
FN§	[per s]	0.27	0.07	-0.45	-0.12	-0.14					
	P	*0.24*	*0.76*	*0.05*	*0.61*	*0.55*					
Crude ash	[g/kg DM]	-0.24	-0.38	0.21	-0.27	-0.23	0.52	0.13	-0.10	0.30	0.35
	P	*0.30*	*0.09*	*0.36*	*0.25*	*0.33*	*0.02*	*0.58*	*0.66*	*0.19*	*0.13*
Crude protein	[g/kg DM]	-0.21	-0.34	0.33	-0.16	-0.10	0.27	0.55	-0.37	-0.03	0.02
	P	*0.38*	*0.15*	*0.16*	*0.51*	*0.67*	*0.24*	*0.01*	*0.11*	*0.90*	*0.95*
Crude fibre	[g/kg DM]	-0.08	-0.47	-0.06	-0.42	-0.37	0.30	-0.21	-0.09	0.12	0.17
	P	*0.75*	*0.04*	*0.82*	*0.07*	*0.11*	*0.19*	*0.36*	*0.70*	*0.60*	*0.49*
Crude fat	[g/kg DM]	0.01	-0.22	-0.22	-0.33	-0.32	0.70	-0.30	0.43	0.71	0.73
	p	*0.97*	*0.35*	*0.36*	*0.16*	*0.17*	*<0.01*	*0.19*	*0.06*	*<0.01*	*<0.01*
aNDFom*	[g/kg DM]	-0.17	-0.51	0.23	-0.32	-0.24	0.17	-0.50	0.32	0.31	0.30
	P	*0.48*	*0.02*	*0.32*	*0.17*	*0.31*	*0.47*	*0.02*	*0.17*	*0.18*	*0.19*
ADFom#	[g/kg DM]	-0.32	-0.59	0.09	-0.55	-0.50	0.50	0.45	-0.59	-0.03	0.06
	P	*0.17*	*<0.01*	*0.71*	*0.01*	*0.02*	*0.02*	*0.04*	*<0.01*	*0.89*	*0.80*
ADL†	[g/kg DM]	-0.22	-0.22	0.13	-0.21	-0.20					
	P	*0.34*	*0.34*	*0.58*	*0.38*	*0.41*					
Starch¥	[g/kg DM]	-0.11	0.11	-0.24	-0.11	-0.17	-0.84	0.05	-0.15	-0.65	-0.71
	P	*0.66*	*0.65*	*0.32*	*0.64*	*0.46*	*<0.01*	*0.84*	*0.53*	*<0.01*	*<0.01*
Gross energy	[MJ/kg DM]	-0.23	-0.33	0.13	-0.31	-0.28	0.70	-0.27	0.39	0.68	0.71
	P	*0.33*	*0.15*	*0.57*	*0.19*	*0.23*	*<0.01*	*0.25*	*0.09*	*<0.01*	*<0.01*
Arg	[g/16g N]	0.20	0.31	-0.06	0.31	0.29	0.42	-0.59	0.52	0.57	0.57
	P	*0.40*	*0.18*	*0.81*	*0.19*	*0.22*	*0.07*	*<0.01*	*0.02*	*<0.01*	*<0.01*
His	[g/16g N]	-0.04	-0.20	-0.05	-0.19	-0.17	0.08	0.22	0.01	0.10	0.09
	P	*0.86*	*0.40*	*0.83*	*0.42*	*0.46*	*0.74*	*0.35*	*0.97*	*0.69*	*0.71*
Ile	[g/16g N]	0.31	0.45	-0.14	0.38	0.35	0.06	0.27	-0.36	-0.19	-0.15
	P	*0.18*	*0.05*	*0.56*	*0.10*	*0.13*	*0.79*	*0.25*	*0.12*	*0.41*	*0.52*
Leu	[g/16g N]	0.01	0.14	-0.16	-0.01	-0.06	-0.20	0.66	-0.66	-0.52	-0.49
	P	*0.99*	*0.55*	*0.49*	*0.96*	*0.82*	*0.41*	*<0.01*	*<0.01*	*0.02*	*0.03*
Lys	[g/16g N]	0.31	0.43	-0.18	0.37	0.34	0.41	-0.71	0.66	0.66	0.65
	P	*0.18*	*0.06*	*0.45*	*0.11*	*0.15*	*0.08*	*<0.01*	*<0.01*	*<0.01*	*<0.01*
Met	[g/16g N]	0.23	0.34	-0.23	0.20	0.16	0.62	-0.14	0.38	0.68	0.69
	P	*0.33*	*0.15*	*0.33*	*0.40*	*0.50*	*<0.01*	*0.55*	*0.10*	*<0.01*	*<0.01*
Phe	[g/16g N]	-0.36	-0.18	0.28	-0.13	-0.13	-0.15	0.53	-0.60	-0.47	-0.43
	P	*0.12*	*0.44*	*0.23*	*0.57*	*0.58*	*0.54*	*0.02*	*<0.01*	*0.04*	*0.06*
Thr	[g/16g N]	0.22	0.20	-0.35	0.03	-0.01	0.46	-0.39	0.53	0.65	0.64
	P	*0.34*	*0.40*	*0.13*	*0.92*	*0.96*	*0.04*	*0.09*	*0.02*	*<0.01*	*<0.01*
Trp	[g/16g N]	0.01	0.05	0.27	0.22	0.24	0.23	-0.67	0.53	0.44	0.43
	P	*0.97*	*0.85*	*0.25*	*0.36*	*0.30*	*0.32*	*<0.01*	*0.02*	*0.05*	*0.06*
Val	[g/16g N]	0.28	0.41	-0.21	0.30	0.26	0.35	-0.03	0.06	0.27	0.30
	P	*0.23*	*0.08*	*0.38*	*0.20*	*0.26*	*0.13*	*0.92*	*0.79*	*0.25*	*0.20*
Ala	[g/16g N]	0.42	0.47	-0.23	0.41	0.38	-0.22	0.38	-0.31	-0.31	-0.31
	P	*0.07*	*0.04*	*0.32*	*0.07*	*0.10*	*0.36*	*0.10*	*0.19*	*0.19*	*0.19*
Asp	[g/16g N]	0.35	0.53	-0.08	0.52	0.49	0.18	-0.56	0.46	0.40	0.38
	P	*0.13*	*0.02*	*0.73*	*0.02*	*0.03*	*0.45*	*0.01*	*0.04*	*0.08*	*0.10*
Cys	[g/16g N]	0.21	0.14	-0.26	0.02	0.01	0.05	-0.11	0.24	0.19	0.17
	P	*0.38*	*0.56*	*0.28*	*0.92*	*0.99*	*0.83*	*0.65*	*0.31*	*0.41*	*0.46*
Glu	[g/16g N]	-0.37	-0.51	0.19	-0.45	-0.42	-0.34	0.67	-0.61	-0.57	-0.56
	P	*0.11*	*0.02*	*0.43*	*0.05*	*0.07*	*0.14*	*<0.01*	*<0.01*	*<0.01*	*0.01*

		Wheat					Corn				
		a	a+b	c	ED5	ED8	a	a+b	c	ED5	ED8
Gly	[g/16g N]	-0.05	-0.24	-0.08	-0.24	-0.23	0.42	-0.56	0.57	0.61	0.61
	P	*0.84*	*0.31*	*0.73*	*0.30*	*0.34*	*0.07*	*<0.01*	*<0.01*	*<0.01*	*<0.01*
Pro	[g/16g N]	-0.14	-0.34	0.15	-0.23	-0.19	0.16	0.49	-0.34	-0.09	-0.05
	P	*0.56*	*0.15*	*0.52*	*0.33*	*0.43*	*0.50*	*0.03*	*0.14*	*0.72*	*0.84*
Ser	[g/16g N]	-0.50	-0.65	0.00	-0.74	-0.72	0.09	0.19	-0.15	-0.02	0.00
	P	*0.03*	*<0.01*	*0.99*	*<0.01*	*<0.01*	*0.72*	*0.42*	*0.53*	*0.94*	*0.99*
Tyr	[g/16g N]	0.11	-0.17	0.02	-0.06	-0.01	-0.02	0.35	-0.32	-0.18	-0.16
	P	*0.64*	*0.47*	*0.93*	*0.82*	*0.96*	*0.94*	*0.13*	*0.16*	*0.45*	*0.50*
Ca	[g/kg DM]	-0.24	-0.04	0.01	-0.13	-0.16	-0.34	-0.05	-0.06	-0.23	-0.26
	P	*0.31*	*0.85*	*0.99*	*0.57*	*0.49*	*0.15*	*0.83*	*0.80*	*0.33*	*0.27*
Mg	[g/kg DM]	-0.47	-0.44	0.33	-0.34	-0.31	0.50	0.11	0.03	0.35	0.39
	P	*0.04*	*0.05*	*0.16*	*0.14*	*0.18*	*0.02*	*0.64*	*0.89*	*0.13*	*0.09*
K	[g/kg DM]	-0.18	-0.04	0.21	0.03	0.03	0.69	-0.22	0.35	0.70	0.72
	P	*0.46*	*0.87*	*0.38*	*0.90*	*0.89*	*<0.01*	*0.35*	*0.13*	*<0.01*	*<0.01*
Na	[mg/kg DM]	0.31	0.12	-0.06	0.22	0.26					
	P	*0.26*	*0.66*	*0.84*	*0.43*	*0.36*					
Fe	[mg/kg DM]	0.06	0.05	0.17	0.17	0.19	0.47	0.26	-0.04	0.34	0.37
	P	*0.81*	*0.82*	*0.47*	*0.48*	*0.43*	*0.03*	*0.27*	*0.87*	*0.14*	*0.11*
Mn	[mg/kg DM]	0.21	0.12	0.01	0.19	0.21	-0.21	0.02	0.07	-0.08	-0.11
	P	*0.37*	*0.60*	*0.99*	*0.42*	*0.37*	*0.38*	*0.93*	*0.78*	*0.74*	*0.65*
Zn	[mg/kg DM]	-0.22	-0.29	0.12	-0.24	-0.22	0.18	0.39	-0.17	0.05	0.07
	P	*0.35*	*0.22*	*0.62*	*0.30*	*0.35*	*0.45*	*0.09*	*0.48*	*0.82*	*0.77*
Cu	[mg/kg DM]	-0.14	-0.10	-0.09	-0.21	-0.23	0.22	0.36	-0.16	0.02	0.06
	P	*0.56*	*0.67*	*0.69*	*0.37*	*0.32*	*0.36*	*0.12*	*0.51*	*0.93*	*0.82*
P	[g/kg DM]	-0.26	-0.42	0.17	-0.34	-0.31	0.43	0.19	-0.06	0.28	0.31
	P	*0.27*	*0.07*	*0.47*	*0.14*	*0.19*	*0.06*	*0.43*	*0.79*	*0.22*	*0.18*
GP2•	[ml/200 mg DM]	0.21	0.26	-0.29	0.10	0.06	-0.67	-0.05	-0.02	-0.46	-0.52
	P	*0.36*	*0.26*	*0.21*	*0.68*	*0.80*	*<0.01*	*0.83*	*0.93*	*0.04*	*0.02*
GP4•	[ml/200 mg DM]	0.24	0.11	-0.22	0.06	0.06	-0.37	-0.33	0.28	-0.07	-0.12
	P	*0.31*	*0.63*	*0.35*	*0.80*	*0.81*	*0.11*	*0.15*	*0.23*	*0.77*	*0.61*
GP6•	[ml/200 mg DM]	0.13	0.10	-0.05	0.10	0.10	-0.29	-0.40	0.22	-0.07	-0.10
	P	*0.58*	*0.68*	*0.83*	*0.67*	*0.66*	*0.22*	*0.08*	*0.34*	*0.78*	*0.67*
GP8•	[ml/200 mg DM]	0.32	0.43	-0.10	0.42	0.40	-0.35	-0.28	0.09	-0.18	-0.22
	P	*0.16*	*0.06*	*0.69*	*0.06*	*0.08*	*0.13*	*0.23*	*0.70*	*0.44*	*0.36*
GP12•	[ml/200 mg DM]	0.49	0.68	-0.29	0.56	0.50	-0.36	-0.21	0.04	-0.22	-0.25
	P	*0.03*	*<0.01*	*0.22*	*0.01*	*0.02*	*0.11*	*0.37*	*0.88*	*0.36*	*0.29*
GP24•	[ml/200 mg DM]	0.46	0.59	-0.35	0.43	0.38	-0.34	0.17	-0.31	-0.37	-0.39
	P	*0.04*	*<0.01*	*0.13*	*0.06*	*0.10*	*0.14*	*0.47*	*0.19*	*0.10*	*0.09*
GP48•	[ml/200 mg DM]	0.49	0.65	-0.40	0.47	0.40	-0.38	0.29	-0.47	-0.50	-0.50
	P	*0.03*	*<0.01*	*0.08*	*0.04*	*0.08*	*0.10*	*0.21*	*0.04*	*0.02*	*0.02*
GP72•	[ml/200 mg DM]	0.41	0.53	-0.34	0.40	0.35	-0.31	0.33	-0.54	-0.50	-0.49
	P	*0.07*	*0.02*	*0.15*	*0.08*	*0.13*	*0.18*	*0.16*	*0.01*	*0.02*	*0.03*
GPb£	[ml/200 mg DM]	0.44	0.60	-0.37	0.43	0.37	-0.30	0.42	-0.59	-0.52	-0.50
	P	*0.05*	*<0.01*	*0.11*	*0.06*	*0.11*	*0.20*	*0.07*	*<0.01*	*0.02*	*0.02*
GPc¤	[%/h]	0.11	0.09	-0.02	0.10	0.11	-0.24	-0.57	0.47	0.10	0.05
	P	*0.64*	*0.72*	*0.95*	*0.67*	*0.66*	*0.32*	*<0.01*	*0.04*	*0.67*	*0.82*

‡TSW, thousand seed weight; •TW, test weight; §FN, falling number; *aNDFom, neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash; #ADFom, acid detergent fiber expressed exclusive of residual ash; †ADL, acid detergent lignin; ¥Starch, determined enzymatically; •GPx, Gas production after x h incubation time; £GPb, potential gas production; ¤GPc, rate of gas production of GPb.

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