

**Processed Grains as a Supplement
to Lactating Dairy Cows**

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Abstract

In this thesis the effect of different ways of thermal processing (pelleting, expanding, toasting) of barley and maize on the degradative behaviour of their starch and protein in the rumen of lactating dairy cows are described. *In situ* studies showed that all thermal processing methods increased the ruminal starch and protein availability of maize, while all thermal processing methods decreased ruminal starch availability but only pelleting increased ruminal protein availability of barley. Based on *in vivo* experiments compared to untreated grains, expander treatment increased the apparent rumen and total tract digestibility of maize starch but did not affect the digestibility of barley starch. Supplementing grazing dairy cows with pelleted and pressure toasted maize and barley slightly (not significantly) decreased the dry matter intake of grass in the first grazing event in the morning after milking, and it decreased ruminal clearance of nitrogen. Supplementing pasture grass with pelleted and pressure toasted cereal grains decreased the pH, the $\text{NH}_3\text{-N}$ level in the rumen, the ammonia to total VFA (TVFA) ratio, the isobutyrate proportion, the acetate to propionate ratio and the non-glucogenic to glucogenic ratio in the rumen. Simultaneously it increased TVFA concentrations, propionate, butyrate and valerate proportions as a percentage of the TVFA. All processed grains did affect production responses in dairy cows, by elevating milk protein and decreasing milk fat production, and milk urea nitrogen but no significant differences between these two heat treatments were found. It is concluded that the need of synchrony is specially important with diets based on fresh grass, in which markedly asynchronous rates of release of energy and nitrogen occur in the rumen. It appears that to generate a better ruminal N and organic matter synchrony to improve microbial N yield and N utilization feeding different types of cereal grain that differ by nature in rate and extent of ruminal degradation has more effect than using one of this processing methods on the same type of grain. Responses obtained from cereal grain supplementation are very dependent on the quality and degradation characteristics of the pasture consumed, which changes through the grazing season.

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PREFACE

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CHAPTER 1

General Introduction

Ruminants such as dairy cattle have a natural tendency to graze and depend on forages as their major source of nutrients. Cattle historically grazed about three times daily and consumed almost entirely grass and similar forages. They normally select some leaves, buds and plants stems as well as grass (Van Soest, 1994). Under north-western European conditions fresh grass is still one of the cheapest important sources of energy and protein for ruminants (Peyraud, 2001), but the nutrient supply of grazing cows is insufficient for milk productions higher than 30 kg/day (Van Vuuren, 1990).

In The Netherlands where the dairy husbandry is characterised by a high production level per cow, approximately 30 to 50% forage consumed by dairy cows is fresh grass, primarily perennial ryegrass (*Lolium perenne*). In order to achieve a high digestibility, grass has to be offered in a young and leafy stage with a low proportion of stems with less lignified cell walls, when nitrogen content is high. Researchers have shown by both *in vitro* and *in situ* techniques that fresh forages contain a high proportion of their protein (approximately 60-80%) as ruminally degradable protein (Cammell *et al.*, 1983; Abdalla *et al.*, 1988; Van Vuuren *et al.*, 1991). This should result in high levels of ammonia in the rumen. A large part of the ammonia will leave the rumen by absorption through the rumen wall mainly as a consequence of a ruminal imbalance between degraded protein and energy supply for optimal microbial capture of the ammonia-N (Ulyatt *et al.*, 1988). Excess ammonia is metabolised in the liver to urea and excreted in the urine, which has a negative effect on the cow's energy balance (Twigge and Van Gils, 1988). Another source of nitrogen loss in the urine can be an inefficient conversion of absorbed amino acids into milk and body proteins (Tamminga, 1992).

The grazing season under north-western European farms lasts from April to October, and the plant composition changes with time. Young grass contains lower dry matter and higher organic matter, than more mature grass, which contains less nitrogen but more sugars and cell-wall constituents (Hodgson, 1990; Osbourn, 1980). The concentration, composition and rate and extent of degradation in the rumen of protein and carbohydrates in grass vary significantly. This variation is influenced by the maturity of the grass, its nitrogen fertilisation (Van Vuuren, 1992), the season, growing conditions (environmental factors like radiation, rainfall and air temperature) and number of days of regrowth (Minson, 1990). Differences in the rate of degradation of non-structural carbohydrates result in a quite

variable availability in the rumen. Thus, energy made available to the rumen microbes may not be synchronised with the availability of rumen degradable grass protein ingested during grazing. Hence the capture of rumen degradable grass protein in microbial biomass may not be at its maximum efficiency. Due to this, around 50% of the crude protein ingested with the grass may be lost. This not only means a severe loss of protein, it may also cause environmental pollution as ammonia volatilisation and nitrate leaching (Tamminga, 1992). From an environmental point of view the importance of nitrogen in acidifying ecosystems and causing eutrophication in natural waters is clearly recognised (OECD, 1982) and intergovernmental efforts in Europe are underway to reduce nitrogen emissions. Agricultural systems are targeted for tighter regulation (Dietz and Hoogervorst, 1991; Novotny, 1999).

With proper nutrition management a reduction in nitrogen loss by grazing dairy cows can be achieved by reducing the nitrogen content of pasture grass or with a supplement and so replace part of the grass by low protein feeds. Maize silage or cereal grains are rich in either rapidly degradable structural or non-structural carbohydrates, mainly starch (Valk *et al.*, 1990, Valk, 1994). As a result the microbial protein synthesis may be improved by the supply of energy, mainly carbohydrates. Consequently ammonia concentration decreases in the rumen, and nitrogen excretion will be less.

The quantities of concentrates, which may be fed to dairy cows as a supplement to grass, are limited due to possible disturbances of rumen fermentation (Van Vuuren, 1986). However important differences have been observed in the magnitude of ruminal digestion between different sources of cereal starch (Nocek and Tamminga, 1991). Generally rapidly degraded sources of cereal starch are wheat, barley and oats, while maize (corn), milo, rice and millet are rather resistant. In between are starches in legumes like peas and beans.

Whole grain with an intact pericarp is largely or entirely resistant to digestion by ruminants because whole kernels are resistant to bacterial attachment (Beauchemin *et al.*, 1994). Conversely, grain is processed by the application of various combinations of heat, moisture, time and mechanical action. These processing treatments alter kernel structure, thus, enhancing the release of starch granules from the protein matrix and disrupting their order (i.e. of crystallinity) during gelatinization, resulting in increased susceptibility to enzyme activity (Hoover and Vasathan, 1994). Non-thermal processes (roller and

hammermill) and thermal processes (*dry*: roasting, popping, micronizing and *wet*: autoclaving, steam-flaking, steam pelleting, expanding, extruding, toasting) can be used to manipulate rate of degradation and hence ruminal availability (Owens *et al.*, 1986; Theurer, 1986). However Huntington (1997) summarised that rate and extent of starch digestion in the rumen are determined by several factors (source of dietary starch, diet composition, amount of feed per unit time, mechanical alterations, chemical alterations and degree of adaptation of microbiota to the diet).

Cereal grain processing presents a means by which it is possible to manipulate the ratio of rumen available protein to fermentable carbohydrate, and achieve some increase in efficiency of nitrogen utilisation.

From this background, the main objective of the studies described in this thesis is to investigate the effect of different ways of thermal processing of barley and maize on the degradative behaviour of their starch in the rumen of dairy cows in such a way that the N-loss in the rumen is lowered by increasing the efficiency of microbial protein synthesis.

The more specific objectives were:

1. To measure rate of rumen degradation of different starch sources using digestibility measurements, evacuation of rumen contents and the *in sacco* procedure to investigate the effects of grain type and expander treatment as means of heat-processing on rumen starch degradation.
2. To test the hypothesis that pressure toasting and pelleting have similar effects in cereal grains and legume seeds.
3. To test the hypothesis that supplementing grazing cows with differently processed grains can help to synchronize rumen fermentation, and reduce ammonia level in the rumen.
4. To measure the effects of processing feed grains, fed as supplement to grazing cows, on rate of grass intake and on rumen fermentation terms of pH, ammonia and VFA concentrations.
5. To estimate VFA productions using ^{13}C labeled acetate as marker.
6. To determine rate of starch and protein degradation of processed feed *in sacco*.
7. To investigate the effect of processed grains as a supplement on performance in dairy cows.

Outline of the thesis

Figure 1 gives a schematical overview of the contents of this thesis. The general aspects of differences in cereal starch, its importance in dairy diets and the different processing methods of cereal grains and the effect of processing on Salmonella reduction, on ruminal and intestinal starch digestion, on rumen fermentation and dry matter intake and on production are reviewed in Chapter 2. Examination of the available literature clearly shows that the effects of thermo-mechanical treatments has potential to manipulate the rumen degradability of the cereal starch. Thermal processing methods have been widely studied, with those of expander processing or pressure toasting still being relatively recent, therefore in this thesis the following grain processing were studied:

- Expander treatment and subsequently pelleting
- Pressure toasting
- Pelleting
- Pressure toasting and subsequently pelleting

In Chapter 3 the rumen degradability, intestinal digestibility and ruminal fermentation of ground and expander processed subsequently pelleted maize grain and barley grain were measured. Measurements included *in situ* nylon bag (pore size of 36 μm and 15 μm) incubations and rumen evacuations in ruminally and intestinally cannulated lactating dairy cows, fed grass silage in the barn. This experiment was conducted at the Danish Institute of Animal Science (DIAS) at Foulum, Denmark.

The research described in Chapter 4 to 7 focused on grazing conditions and executed at the experimental farm "De Ossekampen" of Wageningen University.

Rook *et al.* (1994) have observed two major grazing bouts for lactating cows: one in the morning and the largest in the afternoon. All the studies in these chapters were concentrated on the first 3 hours long grazing bout after the morning milking, and the following starvation. The objectives in Chapter 4 were to measure the effects of pelleted or pressure toasted and subsequently pelleted maize and barley grain as supplement to grazing dairy cows on perennial ryegrass intake, rumen fill and rumen pool sizes, and ru-

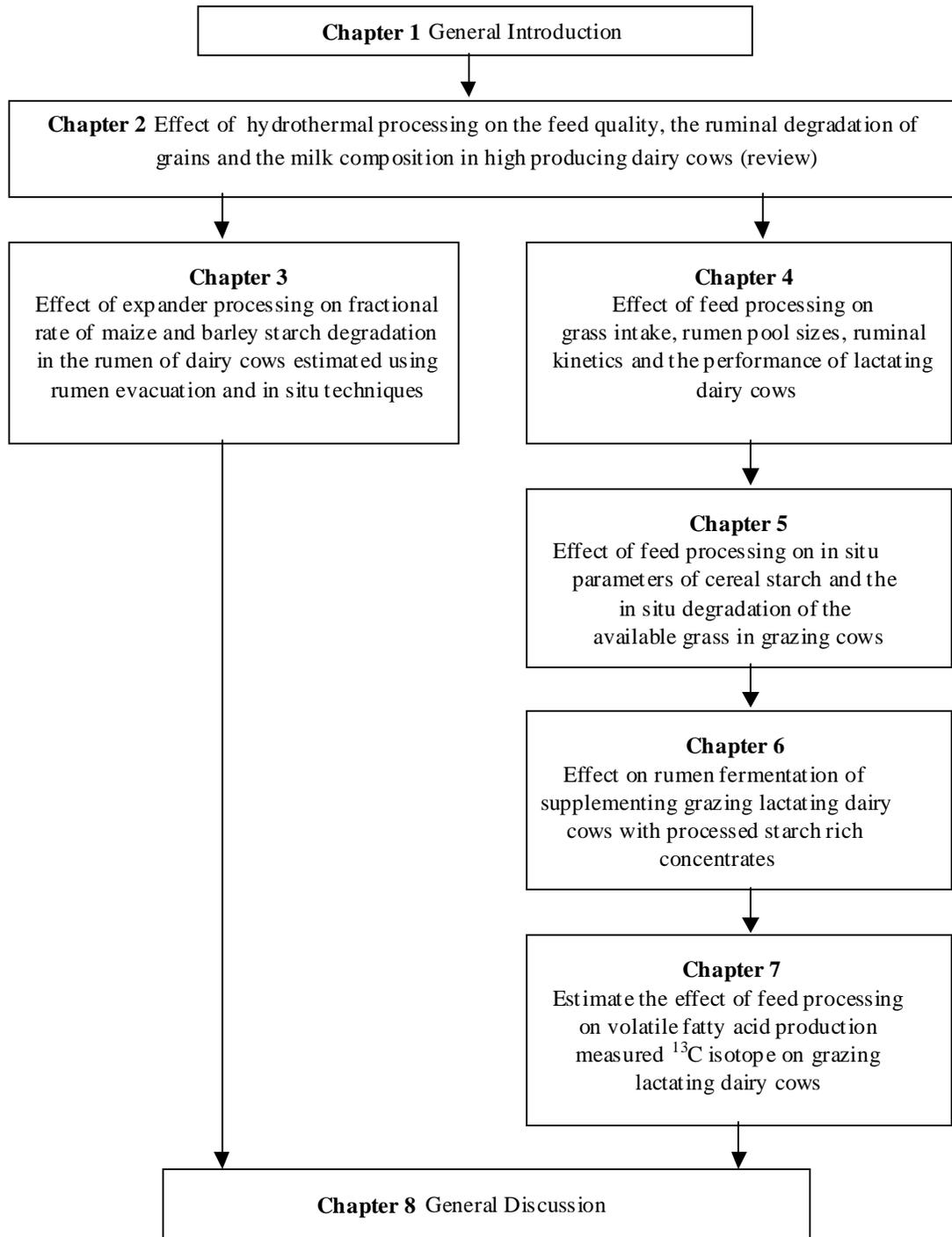


Figure 1. A schematical representation of the outline of the thesis

ruminal kinetics using the rumen evacuation technique. In this chapter the effect of processed cereal grains on milk production and milk composition was also studied.

In Chapter 5 the *in sacco* (pore size of 40 μm) organic matter, starch, protein and neutral detergent fibre (NDF) degradation of processed grains and available grass and the nitrogen to organic matter ratio in the organic matter released from the nylon bags were studied with concentrates and grasses. The effects of grain supplementation next to grass on rumen fermentation were studied in terms of pH, ammonia, and volatile fatty acid (VFA) concentrations (Chapter 6) and VFA production, using ^{13}C labelled acetate as a marker (Chapter 7).

The General Discussion (Chapter 8), gives an integrated résumé of the results of all experiments, possibilities of synchronisation of ruminal available organic matter and nitrogen sources using a synchronisation index (Sinclair *et al.*, 1993) and gives an evaluation of the topics discussed in the other chapters.

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CHAPTER 2

Effect of Hydrothermal Processing on the Feed Quality, the Ruminal Degradation of Grains and the Milk Composition in High Producing Dairy Cows

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Abstract

Starch is the major energy component of cereal grains. Proportions of starch fermented in the rumen can be predicted satisfactorily for a variety of grains and processing methods. In practice, since the mid-1980's high temperature, short time conditioning (HTST) has been described as a wet (hydro-) thermal process of a wide range of machines capable of feed pasteurisation and as an effective means to kill pathogenic micro organisms. In the feed manufacturing industry the processes spread firstly in monogastric animal nutrition but more recently also in ruminant nutrition. Wet (hydro-) thermal process causes gelatinization of grain starch, denaturization of protein, bonding of lipids, inactivation of anti-nutritional factors, pasteurisation of processed material. Based on the literature dates using wet (hydro-) thermal processing, effectively controls Salmonella in feed, increase of the percentage of bypass protein, increases ruminal starch degradability of cereal grains, affects the site of digestion of the starch and of the protein, simultaneously improving intestinal rate of degradation, increase of the total digestibility of grain starch, feed structure corresponding to the needs of the animals as well as to the TMR feeding systems, increase of the milk yield and improve the pellet quality if the feed is to be pelleted. Economical by we have to note that hydrothermal treatments are approximately 2-3 times more expensive than dry rolling.

Keywords: Heat treatment; Salmonella; Barley; Maize; Corn; Starch

Introduction

Processing of cereal grains has become important in the feed industry since it increases energy availability from cereals by improving ruminal and total tract digestibility (Reynolds *et al.*, 1996). Particle size reduction (Thomas *et al.*, 1988, Cerneau and Michalet-Doreau, 1991), starch gelatinization, retrogradation and dextrination which improve accessibility of enzymes to the starch granules, may shift the site of digestion of protein and starch from the rumen to the small intestine (Hale, 1973; Ørskov, 1986; Theurer, 1986; Owens *et al.*, 1986) and so results in an improved supply of amino acids and glucose to animal metabolism (Nocek and Tamminga, 1991). Elevated glucose

absorption represents one mechanism by which increased postruminal starch digestion might increase milk, and milk component yield (Sutton, 1985, MacRae *et al.*, 1988). Increased net energy density of cereals is also beneficial because high yielding dairy cows frequently are unable to consume sufficient net energy during early lactation to meet their requirements. Increased digestion of feed protein and starch in the small intestine results in less nitrogen and carbohydrate loss to the environment because a high flow of starch to the large intestine stimulates microbial growth and causes a net N influx to the large intestine thereby increasing N in faeces.

Research into the effects of chemical treatment such as NaOH, formaldehyde, ammoniation, (Miron *et al.*, 1997; Mayne and Doherty; 1996, Robinson and Kennelly, 1988; Fluharty and Loerch, 1989; Oke *et al.*, 1991; McAllister, 1992; Theurer, 1986) and physical processing (i.e. breaking, cracking, grinding, rolling) dried grains on utilisation of the starch in cereal grains has been studied extensively. However these reviews hardly have focussed on the effects of hydrothermal processes on cereal starch. Therefore this study tries to give a picture on this issues in order to develop an understanding of the effects of these processes on ruminal starch degradation and fermentation.

Starch in cereal grain and its importance in dairy diets

Carbohydrates represent the most important source of energy for dairy cows. They may be classified on the basis of their nutritional importance for dairy cows: this involves distinguishing between fibrous and non-fibrous carbohydrates. Fibrous carbohydrates are only slowly digested by cows, while non-fibrous carbohydrates such as sugars, starch and pectin are rapidly fermented and digested. Carbohydrates are quantitatively the most important substrates for rumen fermentation and intestinal digestion thereby providing the dairy cows with substantial quantities of metabolites to support milk component synthesis. The supply of nutrients from the polysaccharides, in starch and cell wall constituents, depends on enzymatic hydrolysis to release their component monosaccharides.

Starch is composed of the two major molecules amylose and amylopectin. Amylose is a linear polymer of α 1-4 D-glucose units while amylopectin is a branched polymer with

linear chains of D-glucose that has a branch point every 20 to 25 glucose units (French, 1973). Ruminants digest starch in the small intestine through the action of endogenous enzymes. Starch is degraded to glucose which, after absorption, is used metabolically. In contrast, the hydrolysis of structural polysaccharides is dependent on the enzymes of the microbes in the digestive tract, primarily in the forestomachs. Ruminal degradation of these carbohydrates depends on other nutrients, such as amino acids and nitrogen, to meet microbial nutrient requirements. In ruminants, part of the starch is hydrolysed by microbial enzymes and after further degradation, becomes available as microbial fermentation products such as microbial protein and volatile fatty acids. The amount of starch escaping rumen fermentation intact depends on its rate of degradation and the rate of passage out of the rumen (Nocek and Tamminga, 1991). Starch passing to the small intestine is digested, and absorbed as glucose to a varying degree. However, higher resistance to microbial enzymatic degradation in the rumen is related to a lower intestinal digestion. In cattle given whole grains there may be fermentation in the large intestine and starch voided in faeces. High quantities of starch entering to the small intestine may exceed the capacity of the small intestine to digest it. Nevertheless, since enzymatic digestion of non-structural carbohydrates in the small intestine yields 11 to 30% more net energy than when it is fermented in the rumen (Leng, 1981), it may be worthwhile to accept some faecal loss of starch to increase the amount digested in the small intestine, vs. that fermented in the rumen.

Ruminal degradation of starch varies from 39 to 94% depending on grain source, differences in processing method and other factors (Nocek and Tamminga, 1991). Rapidly degraded sources of starch are wheat, barley, and oats while resistant sources are maize, milo, rice, sorghum, and starches in legume seeds, such as peas and beans. Starch digestion by ruminants has been reviewed and discussed (Sutton, 1979; Theurer, 1986; Visser *et al.*, 1992; Hale, 1973, Ørskov, 1976; Ørskov 1986; Owens *et al.*, 1986; Campling, 1991; Nocek and Tamminga 1991; Mills *et al.*, 1999), and all emphasise the importance of the site of starch digestion, as well as the influence of the nature of feed starch and technological processing, on the NE values of the feedstuffs.

Differences between different grain starches

Generally the starch content of cereal grains varies (Table 1). This difference in starch values is the primary reason why barley, wheat or oats have a lower energy content than maize or sorghum (Hunt, 1996). However barley starch is degraded rapidly, and almost completely in the rumen whereas often a substantial proportion of maize starch escapes rumen fermentation (Waldo, 1973). Within the endosperm, starch granules are surrounded by a protein matrix (Rooney and Pflugfelder, 1986) which must be digested to allow amylolytic digestion of starch granules. McAllister *et al.* (1993) suggested that variation in the protein-starch matrix could be a factor responsible for differences in ruminal digestion of cereals. In maize, a highly crystalline amylopectin matrix (French, 1973) can be found and the protein matrix surrounding the starch granules is strong (Rooney and Pflugfelder, 1986) and extremely resistant to invasion.

Table 1 Starch content and physico-chemical characteristics of starch granules for a range of cereal grains

Grain	Starch % DM ¹	Granule size (mm) ²	Amylose % ²	Gelatinization range °C ²
Barley	55-74	20	22	59-64
Maize	65-76	15	26	62-72
			(waxy 1, high 80)	
Sorghum	68-80	20	26	68-75
Wheat	68-82	25	25	62-75
Oats	42-69	25	27	-
Milo	68-78	25	-	-

¹ Waldo, 1973; Herrera-Saldana *et al.*, 1990; Nocek and Tamminga, 1991; Huntington, 1997; Prestløkken, 1999.

² Pomeranz, 1984; Kent and Evers, 1994.

Rumen fungi appear to be the only ruminal microorganism capable of penetrating this structure (McAllister *et al.*, 1990). The protein matrix in barley is readily digested by a variety of proteolytic bacterial enzymes and digestion in the rumen generally proceeds

rapidly (McAllister *et al.*, 1990) resulting in the production of large quantities of the glucogenic precursor propionic acid, as well as the lipogenic precursor acetic acid.

Starch granules of maize and milo are reported to be similar in size, shape and composition, although the protein surrounding the starch granules differs in amount and composition (Wall and Paulis, 1978). Wheat starch is more available to ruminal microorganisms (Aimone and Wagner, 1977) because it has a less dense starch structure, a higher amylose content and a higher reducing-sugar content than does maize or milo (Banks and Greenwood, 1975). Readily degradable prolamines (gliadins) in the native wheat are the cause of the relatively high degradability of cereal crude protein in the rumen (68%) whereas zein in maize, a protein rich in disulphide bonds, gives rise to only 50 % degradation (Sommer *et al.*, 1994). There are important differences between grains in *in situ* parameters characterising the rumen degradation of starch (Table 2). The water soluble fraction (W), which is assumed to be instantaneously and completely available is very high in oats, and more than 60% in wheat and barley, therefore the starch degradation in the rumen of this grains are faster than that in maize, milo or sorghum.

Heat treatments

It is clear that chemical composition and physical structure of starch are primary factors influencing the ruminal degradability of starch. Other factors that can influence the extent of rumen degradation are pH, microbial population, feeding frequency through an effect on synchronisation, and physical form of the concentrates if the cereal grains are processed. Processing is generally associated with an increased efficiency of nutrient utilization as it disrupts the protein matrix and allows starch to be more accessible to enzymatic digestion. However, processing may lead to formation of indigestible starch-protein complexes (Thorne *et al.*, 1983) due to the Maillard reaction. Heat treatments tend to increase both the soluble starch fraction and the rate of digestion of the potentially digestible starch fraction, resulting in improvements of the efficiency of nutrient utilisation in the gastrointestinal tract. Chemical treatment of grains has been shown to enhance or retard ruminal degradation of starch, depending on the chemical and concentration used.

Table 2 *In situ* mean degradability characteristics for different starch sources in lactating dairy cows

	W %	D %	k_d %/h	ESD % (only insoluble starch escape)
Barley ¹	62	37	23	92
Maize ¹	25	75	5	58
Sorghum ¹	32	68	3.5	61
Wheat ²	68	32	18	93
Oats ²	96	4	19	98
Milo ²	32	68	3.5	61

W: water soluble fraction, D: insoluble, potentially degradable fraction,

k_d : the fractional rate of degradation of D, ESD: effective starch degradability in the rumen

¹ Philippeau *et al.*, 1999; Cerneau and Michalet-Doreau, 1991; Herrera-Saldana *et al.*, 1990; Tamminga, 1989; Nocek and Tamminga, 1991.

² Nocek and Tamminga, 1991.

Generally heat treatment involves the gelatinization of starch, denaturation of protein, binding of lipids, inactivation of antinutritional factors, and it affects the site of digestion of the starch and protein. Heat treatments can broadly be divided in thermal and nonthermal processes. Nonthermal processes do not involve the addition of external heat, such as roller and hammermill grinding. Thermal processes can be further divided into dry (i.e. roasting, popping, micronizing) and wet (i.e. autoclaving, steam-flaking, steam pelleting, expanding, extruding, toasting) processes.

In practice, many heating techniques and processing systems are applied to cereal grains (Table 3). For each of the hydrothermal processes the efficiency of heat treatment on the nutritional value of cereals depends on a combination of particle size (if ground), process temperature, heating time, initial moisture content and amount of water added during the heat process. Since the mid-1980's high temperature, short time conditioning (HTST) has been described as a process of a wide range of machines capable of feed pasteurisation and as an effective means to kill pathogenic micro organisms. In the feed manufacturing industry these processes spread firstly in monogastric animal nutrition but more recently also in ruminant nutrition.

Table 3 Potential methods for thermal treatments and decisive variables (after Van der Poel, 1990 and Van der Poel *et al.*, 1990)

Process ¹	Temperature (°C)	Time (sec)	Heating source
HTST			
Extrusion	80-200	30-150	Steam
Expander	80-140	5-15	Steam
Micronizing	80-130	40-60	Gas
Steam Plosion	140-210	20-45	Steam/gas
Pressurised Toasting	100-140	60-300	Steam
Roasting	90-190	10-120	Gas/elect.
Infrared radiation	80-130	40-60	Natural gas
MTMT			
Autoclaving	110-130	600-1000	Steam
LTLT			
Conventional toasting	90-105	1800-2700	Steam
LTMT			
Flaking	90-95	600-1200	Steam
Pelleting (long conditioning)	60-95	70-250	Steam
LTST			
Pelleting (short conditioning)	60-90	25-35	Steam

¹ H/M/L T= high/medium/low temperature, S/M/L T = short/medium/long time

Effect of wet (hydro-) thermal treatment on reduction of salmonella

The hygienic quality of feeds is very important. Hygienic quality involves the control of microbiological contamination of feeds based on levels of enterobacteria and Salmonella (Israelsen *et al.*, 1996; Sreenivas, 1998; McCapes *et al.*, 1989). There are over 2300 types of salmonella, many of which are shared by humans and animals. In cattle, salmonella cause diarrhea, decreased milk production, abortions and sometimes death. Some salmonella such as *Salmonella dublin* affect primarily calves while others like *Salmonella*

typhimurium attack adult animals. To completely get rid of enterobacteriaceae such as salmonella and other potentially pathogenic bacteria in feed is extremely difficult.

Conventional steam pelleting reduces salmonella contamination of feed with a varying degree of success. The salmonella reduction from pelleting is influenced by a low moisture content and by the temperature of the pellets, although changes in retention time have little effect. Expanding, pressure conditioning or HTST conditioning is a technique which offers the possibility of reducing salmonella more efficiently than pelleting alone because higher temperatures and moisture contents can be achieved. In several recent experiments with the expander treatment the salmonella content has been estimated indirectly by using other enterobacteria that are less pathogenic than salmonella and simpler in measuring quantitatively (Heidenreich and Löwe, 1994; König, 1995). Naturally infected feed is more resistant to heat treatment than artificially infected material (König, 1995). The reason maybe that heat resistance increases with the age of bacteria cell. A typical level of salmonella in infected raw material is around 1 bacteria per 100 g, if this typical level reduced to 100000 times by expanding and pelleting, only 1 bacteria will remain per 10 tons feed (Israelsen *et al.*, 1996). Very little salmonella is detected in feed that is expanded and pelleted. Salmonella bacteria is typically reduced to about 0.001% of the level in the raw material. In feed that was not pelleted, no salmonella was found at die temperatures of 112 °C. A similar level of contamination could be found at low die temperatures of 88 °C, due to lower steam conditions (Figure 1). The reason for this pattern of increased contamination maybe the shorter retention period during which the feed is held at the processing temperature. When processing with the expander alone, a somewhat higher temperature is required to reduce salmonella than when expansion is combined with pelleting.

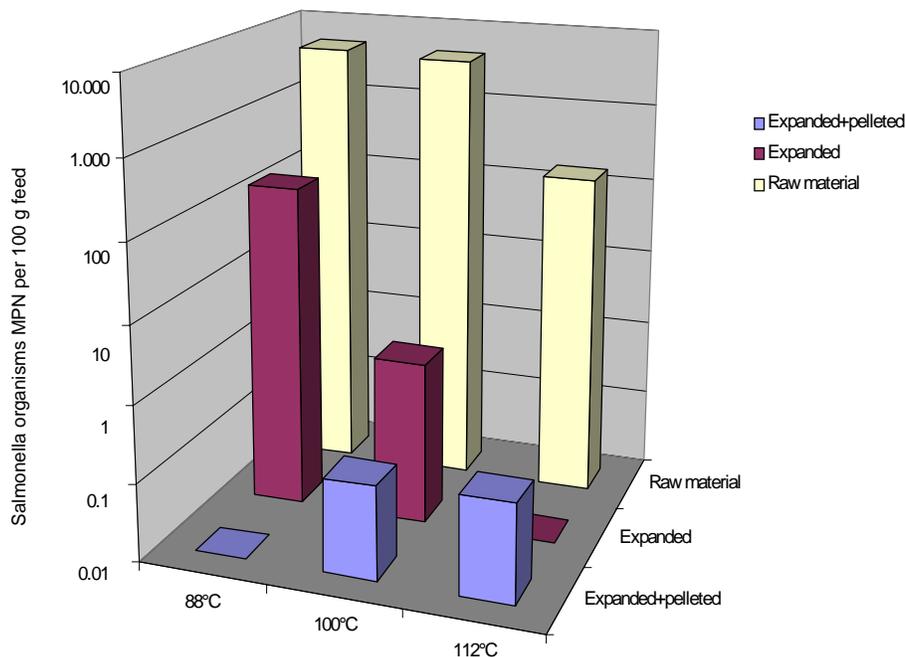
Steam pelleting

Pelleting was defined by Falk (1985) as the agglomeration of small particles into larger pellets by means of a mechanical process using a combination of moisture, heat, and pressure. When the product is mixed and after a low pressure steam addition of 2 to 5

bars, it is conveyed into a rotating die. This die contains hundreds of holes through which the product passes, pushed by rotating rolls situated inside the die.

The production parameters of this process have been extensively explained in the literature (Putier, 1993). Heating and moisture added during conditioning activate the natural binding in many ingredients primarily between starch and protein. The heating and moisture addition act on the particle surface to gelatinise the starch. This gelatinised starch then becomes the liquid bridge between particles that actually form the pellet or agglomerate. It is not clear whether the agglomeration of feed particles that occurs during pelleting will negate the benefits of reducing particle size although pelleting generally reduces the resistance of starch to ruminal degradation by about 15% (Tamminga and Goelema, 1995).

Figure 1 Number of salmonella in untreated, expanded and expanded + pelleted feed at low, medium and high expander die temperatures (Sreenivas, 1998)



Expanding (Expander pelleting)

Expander processing is used in the animal compound feed industry (Veenendaal, 1990; Pipa and Frank, 1989). There are a number of types of pressurised screw-type conditioners called expanders or, by its full name, annular gap expanders, in use but the principles of this construction and operation are the same. This pressurised high-temperature-short time (HTST) conditioners were designed to be positioned after the mixer and before the pelleter. Steam conditioned meal is fed into a compression screw into which more steam is injected, and the mass is then subjected to increasing pressure and shear action and then forced through a variable exit gap. The compressed product after reduction in particle size, is fed into a standard pelleting press.

Most expanders work under 25 to 40 bars of pressure and temperatures between 90 to 130 °C, with treatment times between 5 and 20 seconds. The temperature/time patterns of these pre-pelleting processes differ. A temperature of 80 °C can be reached in the conditioner and this is increased, and peaks during pelleting, due to frictional heat in the die. The total time of approximately 15 min shows how long it takes for the pellets to be brought back to ambient temperature cooling. The maximum temperature reached is increased by double pelleting, but the total time to return to ambient temperature will be much the same. In order to improve liquid absorption, and achieve a better elimination of bacteria, some feed mills may hold the meal at higher temperatures (i.e. above 130 °C) for several minutes. Because of the longer time at higher temperature and high moisture levels (i.e. 20 to 35%) involved, the loss or destruction of heat sensitive nutrients such as vitamins, amino acids and enzymes will be increased (Pickford, 1992).

Because of the heat and pressure involved in expander conditioning, control of undesirable microbes is relatively easily obtained. Several studies have shown substantial decreases in all forms of bacteria and fungi present in typical feed ingredients. Israelsen *et al.* (1996) reported that expanding, in combination with pelleting, is an effective means of reducing the content of Salmonella in a compound feed containing severely infected ingredients with high moisture content of 14 to 15% before steam addition. In the study of Prestlokken *et al.* (1999), the expander treatment reduced ruminal degradation of protein to a much higher extent than it reduced the degradation of dry matter (DM). This

means that the treatment had a specific effect on the ruminal degradation of protein. Nielsen (1994) showed that expander processing reduced the effective protein degradability of raw materials by average 8 % units. These affects may lead to differences in milk production and composition in the animals fed the processed feeds.

Pressure toasting (athmospheric and pressurized steaming), autoclaving and steam flaking

Autoclaving for 30 min at 120 °C is mainly used for legume seeds (Aguilera *et al.*, 1992). Steam flaking, which is a combination of toasting for 15 to 30 min under 100 to 105 °C followed by flaking the heated grains is the technique used for maize and sorghum. It was generally concluded by several authors (Hale, 1973; Ørskov, 1976; Theurer, 1986; Nocek and Tamminga, 1991) that steam flaking increases the amount of starch fermented in the rumen and increases its intestinal digestibility (Owens *et al.*, 1986). Toasting can be carried out at atmospheric pressure or in pressurised vessels such as an autoclave. In the latter case there is a positive correlation between steam pressure and temperature in the autoclave (Van der Poel *et al.*, 1990) Processing times can be varied, although during autoclaving very short treatment times are difficult to achieve because pressure has to be built up after closing the autoclave. Steam heating at 100 °C, a process generally termed toasting, is commonly applied as a heat treatment for legumes and some oilseeds. In the oil extraction industry it is normally used in conventional vertical, so called cascade-type, toasters.

A laboratory scale toaster for batch type or continuous steaming was developed at the Wageningen Agricultural University (Van der Poel, 1990) and this pressurised toaster permits use of short processing times. To be able to control processing time and temperature, special equipment with higher precision was developed (Van der Poel *et al.*, 1990), which enables control of processing temperatures and times. Toasting is generally applied to inactivate antinutritional factors in legume seeds, but toasting may also result in a shift of the degradation of starch in the rumen to digestion in the small intestine.

Effect of wet (hydro-) thermal treatment on carbohydrate and protein synchronization

Rumen microorganisms require protein and carbohydrates to synthesise microbial protein and VFA. The concept of carbohydrate fractionation also applies to protein. The goal should be to balance carbohydrate and protein availability, such that one or the other is not limiting at any time. Thermal processing of grains facilitates the achievement of an optimal ruminal balance in the availability of energy and protein for microbial synthesis. Tamminga *et al.* (1990) reported that when degradation of carbohydrates and proteins are synchronized and take place in a ratio of approximately 5:1, microbial protein synthesis will occur most efficiently and with minimal N losses from the rumen. For example, if a diet has high levels of soluble protein (fresh grass), adequate quantities of readily fermentable carbohydrates (starch) should be included in the diet to avoid ammonia loss. For an optimal synchronisation of energy supply and microbial growth, supplements should have a rate of carbohydrate fermentation close to that of fresh grass crude protein (Van Vuuren, 1993). Grains meet the requirement of a low protein and high carbohydrate content, but their rate of degradation may not always match with that of fresh grass. A variety of feed processing methods can be applied to alter the degradation characteristics of grains and make them more effective as a supplement to fresh grass. Thus, to optimise rumen fermentation, degradative behaviour of the rumen should be controlled. Manipulation through processing is a useful tool for optimising lactating dairy cow production. Another tool is to shift the site of digestion of protein and starch from the rumen to the small intestine.

Effect of wet (hydro-) thermal treatment on ruminal and intestinal starch digestion

Studies of effects of processing on starch degradability were mainly set up to increase rumen degradability of maize and sorghum (e.g. Theurer, 1986; Owens *et al.*, 1986). Heat treatment like extrusion increased (Walhain *et al.*, 1992; Focant *et al.*, 1990), while pressure toasting (Goelema, 1999) reduced, the *in situ* degradability of legume seed starch in ruminants. Steam flaking of cereals results in an increased digestibility of the

starch in ruminants, and also of dry matter (DM) because starch is its major component (Theurer, 1986). In sorghum, steam flaking has been found to increase ruminal starch digestion, compared with ground (McNeill *et al.*, 1971), dry rolled or ground (Hinman and Johnson, 1974) and whole or rolled (Aguirre *et al.*, 1984) grain. Consistent increases in ruminal starch digestion have also been observed for steam-flaked maize compared with whole (Lee *et al.*, 1982), ground (Beever *et al.*, 1970), or dry rolled maize (Galyean *et al.*, 1976; Zinn, 1990). Zinn (1993) reported increased ruminal starch digestion with steam rolled barley compared to dry rolled barley. Cone *et al.* (1989) showed that steam rolling of barley decreased the nylon bag degradation of DM and starch compared with dry rolling. In contrast, Malcolm and Kiesling (1993) reported that steam flaking barley tended to increase rumen DM degradation *in sacco*. These experiments have generally shown that steam flaking increases the amount of starch fermented in the rumen and increases the intestinal digestibility of starch that escapes the rumen.

Pressure toasting has been shown to reduce rumen availability and *in situ* rumen degradability of legume seeds protein (Goelema, 1999; Aguilera *et al.*, 1992; Sommer *et al.*, 1994, Singh *et al.*, 1995) and starch (Goelema, 1999) by reducing the size of the water soluble fraction and the fractional rate of degradation of it. Goelema (1999) reported a concomitant shift of protein digestion from the rumen to the intestines. There are no studies on the effect of pressure toasting on cereal grains.

Expander treatments may improve the energy values for ruminants (Nielsen, 1994; Prestløkken, 1994) by increasing the size of the water soluble fraction and fractional rate of degradations of starch, although effects on degradability of protein and starch are not always consistent (Arieli *et al.*, 1995; Goelema *et al.*, 1999). Arieli (1995) reported a decreased *in situ* rumen starch degradability of several cereals, while Goelema (1999) showed increased starch degradability after expander treatment with legumes, due to a higher washable fraction and rate of degradation of starch.

In steam treatment the steaming time plays an important role. Zinn (1990) showed that altering steaming time during processing of maize grain could affect rumen digestibility of starch. Steam treatment of milo for either 10 or 20 min appeared to reduce rate of starch disappearance *in sacco* compared with either no treatment or only a 2 min treatment (Thomas *et al.*, 1988) as shown in Table 4.

Table 4 Disappearance of starch from dacron bags (*in situ*) (Thomas *et al.*, 1988)

Starch source	120 °C autoclaving (min)			
	0	2	10	20
Maize grain	75.0	77.6	75.5	72.3
Milo grain	74.5	75.7	73.2	66.1

Effect of wet (hydro-) thermal treatment on rumen fermentation and dry matter intake (DMI)

During wet (hydro-) thermal treatments, particle size reduction increases the surface of grains and therefore facilitates microbial enzymatic digestion. Particle size reduction results in increased rate of starch digestion in the rumen. This is reflected in the experiment of Joy *et al.* (1997). These researchers measured higher total VFA production and increased percentage of propionate when fed steam flaked maize instead of dry rolled maize. On the other hand, particle size reduction caused a depression in rumen pH close to higher VFA concentrations (Visser *et al.*, 1992). The lower rumen pH can reduce the activity of cellulolytic bacteria resulting in a lower rate of degradation for NDF (Cameron *et al.*, 1991; Gasa *et al.*, 1991).

A depression in NDF digestion may also occur because of the addition of flaked maize starch (Hoover, 1986). In early lactation cows, feeding steam rolled barley compared to ground maize increased ruminal digestion of starch, decreased DMI and decreased NDF digestibility in the rumen and total digestive tract (Overton *et al.*, 1995; McCarthy *et al.*, 1989). In other studies (Poore *et al.*, 1993; Chen *et al.*, 1994) steam processing of maize starch increased rumen fiber digestion and DM intake. It seems that the intake response to starch supplementation of dairy cows rations is effected by the method of feeding employed, the level and degradability of dietary protein, the type of forage and the type and amount of starch fed (Reynolds *et al.*, 1997). The effects of thermal processing on total DMI seem inconsistent. Chen *et al.* (1994) reported an elevated intake with steam flaked sorghum compared with dry rolled sorghum but with the same treatment Oliviera *et al.* (1993) showed decreased DMI and Simas *et al.* (1997) found no effect. Simas *et al.*

(1997) suggested that the animal factors (stage of lactation, body score) or environmental factors could influence the DMI response.

Effect of wet (hydro-) thermal treatment on milk production

The potential to change milk components through ration formulation depends upon the component (Bequette *et al.*, 1998; Kenelley *et al.*, 1999; Sutton, 1989). An example of how starch fermentability can affect milk yield and components is illustrated in a series of experiments on steam-flaking maize or sorghum grain (Theurer *et al.*, 1999). Compared to dry-rolling, steam-flaking maize or sorghum increased starch digestion in the rumen and intestines (Table 5).

Table 5 Effect of steam-flaking versus dry-rolling maize on starch digestion and microbial protein flow to the small intestine (Theurer *et al.*, 1999)

Measurement	Processing		P<
	Dry-rolled maize	Steam-flaked maize	
Starch digestibility			
Ruminal (% intake)	35	52	0.03
Intestinal (% entry)	61	93	0.05
Microbial protein (kg/d)	1.04	1.23	0.08

Because carbohydrate fermentability is the main determinant of growth of ruminal bacteria (Chalupa and Sniffen, 1996), steam-flaking maize increased the flow of microbial protein to the small intestine. An increased mammary uptake of amino acids in cows fed steam-flaked grains was associated with an increased yield of milk protein. Milk fat yield was not increased by feeding steam-flaked grains but neither were mammary uptakes of the milk fat precursors acetate and butyrate. Steam-flaking increased mammary uptake of glucose that was accompanied by an increased milk yield. Kronfeld (1976) estimated that 1 kg of milk is produced for every 72 g of glucose uptake. Data in Table 6 shows that mammary uptake of glucose was increased 117 g/d when

cows were fed steam-flaked maize or sorghum versus steam-rolled maize or dry rolled sorghum. This equates to 1.6 kg/d of milk. Milk yield of cows fed steam-flaked maize was 2.2 kg/d greater than cows fed steam-rolled maize. When cows were fed steam-flaked grains, protein yield increased more than milk yield so concentration of protein in milk increased (Table 7). On the other hand, feeding steam-flaked grains did not increase fat yield so concentration of fat in milk decreased.

Table 6 Effect of steam-flaking (SF) maize or sorghum versus steam-rolling (SR) maize or dry-rolling (DR) sorghum on mammary uptake of substrates (Theurer *et al.*, 1999)

	Processing		P<
	SR maize and DR sorghum	SF maize and SF sorghum	
Net mammary uptake			
Amino acid N (g/d)	61	84	0.01
Glucose (g/d)	1609	1726	0.16
L-Lactate (g/d)	68	94	0.19
Acetate (mol/d)	11	12	0.58
Propionate (mol/d)	0.21	0.27	0.09
n-Butyrate (mol/d)	0.05	0.05	0.91
B-Hydroxybutyrate (mol/d)	2.20	2.70	0.03

The energetic efficiency of starch utilisation is the highest if not more than 1.5 kg starch per day enters the small intestine (Flachowsky and Lebzien, 1997). Results of 11 feeding experiments (In Table 8 dataset based on 424 Holstein-Friesian cows, 10 kg/day sorghum intake) confirm this recommendation. Huber *et al.* (1994) stated that an elevated amount of bypass starch resulted in a decreased milk yield. In the case of a higher starch flow to the duodenum starch should be treated to increase ruminal degradation. Under practical conditions the digestion of starch is mainly related to the physical characteristics of starch and not to animal factors (Van Vuuren *et al.*, 1997).

Table 7 Effect of steam-flaking (SF) versus steam-rolling (SR) maize on milk yield and composition (Theurer *et al.*, 1999)

Measurement	Processing			P<
	SR maize	SF maize	Response	
Milk (kg/d)	35.80	38	+2.20	0.02
Protein (kg/d)	1.07	1.16	+0.09	0.01
Protein (%)	2.99	3.06	+0.07	0.11
Fat (kg/d)	1.12	1.13	+0.01	0.44
Fat (%)	3.11	2.98	-0.13	0.02

Table 8 Effect of steam-flaking on milk yield and composition (Huber *et al.*, 1994)

Measurement	Processing	
	Ground sorghum	Steam flaked sorghum
Ruminal degradation (%)	51	76
Bypass starch (kg, estimated value)	3.3	1.5
DMI (kg/d)	26.3	25.3
Milk production (kg/d)	35.0	37.4
Milk protein (%)	2.9	3.0
Milk fat (%)	3.24	3.1

Conclusions

Wet (hydro-) thermal processing causes gelatinization of grain starch, denaturation of protein, bonding of lipids, inactivation of anti-nutritional factors, pasteurisation of processed material. Using wet (hydro-) thermal processing, a technique which effectively controls Salmonella in feed, increases ruminal starch degradability of cereals, affects the site of digestion of the starch and of the protein, simultaneously improving intestinal rate of degradation. Steam processing and flaking grains renders the starch fraction more available to rumen microorganisms and enzyme attack. Starch degraded in the rumen

supports synthesis of microbial protein and VFA but rapid or excess degradation may induce ruminal acidosis, increased secretion of insulin and decreased fat in milk. Conversely too much undegraded starch exiting the rumen may exceed the digestive or absorptive capacity of the small intestine or both.

Steam processing may not proportionally improve barley and wheat as much as it does sorghum grain since barley has most of its starch digested in the rumen even without steam processing. Therefore processing methods developed for maize and sorghum cannot be applied directly to barley. In the maize endosperm, starch is tightly packed within a protein matrix. The objective of maize processing is the release of starch from the matrix so that digestion is increased. Because barley is encased in a hull, the objective of processing barley is breaking the hull so that starch can be digested. Barley starch is more fermentable than maize starch.

Because steam processing grains increases ruminal fermentation of starch, application of steam processing methods to barley may excessively increase ruminal fermentation of starch to cause low ruminal pH, acidosis and metabolic problems. On the other hand, under-processing can lead to reduced ruminal and intestinal digestibility. On the economical side we have to note that hydrothermal treatments are approximately 2-3 times more expensive than dry rolling.

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CHAPTER 3

Effect of Expander Processing on Fractional Rate of Maize and Barley Starch Degradation in the Rumen of Dairy Cows Estimated Using Rumen Evacuation and *In Situ* Techniques

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Abstract

Effects of heat treatment (expanding) on ruminal and intestinal digestibility of starch in barley and maize grains were studied in an extended 4×4 Latin square experiment. Four lactating Danish Holstein Friesian cows fitted with ruminal, duodenal and ileal cannulae were offered grass-clover silage and grass-clover hay based diets supplemented with soybean meal and either untreated barley, expanded barley (105 °C), untreated maize or expanded maize (95 °C). Ruminal degradation characteristics of starch for untreated and expanded grains were determined *in situ* using nylon bags with two pore sizes (15 or 36 µm). Rate of starch degradation in the rumen was also determined based on series of total rumen evacuations. *In vivo* digestibility was estimated by sampling duodenal and ileal contents and faeces. Ruminal degradation of starch determined *in situ* was higher for barley than maize regardless of heat treatment. *In situ* studies (36 µm) showed expanding increased effective maize starch degradability in the rumen from 0.60 to 0.72, mainly due to an increased soluble fraction. Effective barley starch degradability, however, was unchanged at 0.96 as an increase in the soluble fraction was counterbalanced by a decrease in rate of degradation from 0.63 to 0.36 h⁻¹ with 36 µm nylon bags. *In vivo* degradation characteristics based on rumen evacuations showed that heat treatment increased fractional rate of degradation of starch for both barley and maize, resulting in a slight increase in the effective degradability of barley starch from 0.81 to 0.85 and an increase in rumen effective degradability of maize starch from 0.71 to 0.78, when rumen starch pools were corrected for rumen starch outflow. *In situ* studies seemed to overestimate ruminal degradation of rapidly fermentable starch in barley, and underestimate degradation of slowly fermentable starch in maize, although *in situ* results were highly dependant on assumptions made on digestion and passage of the soluble fraction. Total tract digestibility of barley starch was not affected by heat treatment (0.99), whereas total digestibility of starch in maize increased from 0.84 to 0.96. Apparent rumen digestibility of starch was higher for barley than maize. Duodenal flow of starch in barley was highest 4 h post feeding, whereas a much larger peak was found for maize at 10–12 h post feeding, which indicates a passage time lag for undegraded maize starch in the rumen, or possibly the abomasum. Fractional rate of passage of starch

from the rumen was not constant, indicating that passage of starch does not follow first order kinetics.

Keywords: *In vivo*; Digestibility; Degradability; Heat treatment; Fractional outflow rate; Corn

1. Introduction

The optimal division of starch digestion between the forestomachs and intestines has not been determined (Reynolds *et al.*, 2001). The major site of starch digestion in dairy cows is generally the rumen. However, when slowly rumen degradable starches such as maize, sorghum or rice are fed, substantial quantities of dietary starch may escape rumen fermentation to become available for digestion and absorption in the small intestine, or for microbial fermentation in the hind gut, or lost in faeces. Insufficient ruminal degradation of starch may reduce total tract starch digestibility and impair rumen microbial protein production. However, excessive ruminal degradation of starch may reduce ruminal and total digestibility of fibre and reduce absorption of glucose in the small intestine (Owens *et al.*, 1986; Nocek and Tamminga, 1991; Stensig *et al.*, 1998).

Fractional rate of ruminal fermentation of starch, and the magnitude of the processing effect on starch utilisation, varies extensively among grains and methods of processing (Theurer, 1986). *In situ* incubation studies by Tamminga *et al.* (1989, 1990) revealed differences among and within starch sources in rumen degradation characteristics due to differences in their amylose and amylopectin content, crystallinity, particle size and the processing technique used (Tamminga, 1997). However, McAllister *et al.* (1993) demonstrated that differences in digestion between isolated maize and barley starch granules were small, and structural carbohydrates and proteins in the cell matrix limits microbial starch degradation and may be more important than the chemical and physical properties of the starch (Kotarski *et al.*, 1992).

Subjecting grain to moisture, pressure and heat makes the starch granules more accessible for bacterial attachment (Huntington, 1997) and so for ruminal fermentation and enzymatic digestion in the intestine (Nocek and Tamminga, 1991). Expanders are

screw presses where pressure and temperature are raised by friction, steam and shear, and controlled by an adjustable die. Cereal starches begin to swell at 50–60 °C, and the swelling continues as the mash moves through the expander, and is considerably accelerated by the rapid rise in temperature (Peisker, 1992 a, b). The final gelatinisation occurs directly before the outlet. Here, the sudden drop in pressure results in bursting of the swollen starch granules, and the expanded product assumes a dough-like consistency.

The *in situ* method has been used in numerous studies to evaluate fractional rates of digestion and rumen digestibility of starch, although validation of the method for starch kinetics is limited. The objective was to compare starch degradation characteristics obtained using *in situ* methods to values obtained *in vivo* (i.e. rumen evacuation and digestibility measurements) and to study the effect of expander processing on starch metabolism in the rumen of dairy cows.

2. Materials and methods

2.1. Animals and management

The present experiments complied with the guidelines of the Danish Ministry of Justice (Act no. 726, 1993) with respect to animal experimentation and care of animals under study. Four multiparous lactating Holstein Friesian dairy cows varying in weight from 674 to 740 kg were used. Three cows were in their second lactation, one cow was in its fifth lactation, and the cows averaged 264 days post partum at the beginning of the experiment. The cows were surgically fitted with a ruminal cannula and simple T-shaped cannulae in the duodenum and ileum. The duodenal cannula was placed approximately 50 cm caudal to the pylorus, and the ileal cannula was placed approximately 20 cm cranial to the caecum. Methods to relieve pain at surgery and procedures to monitor cannulated animals were conducted as described by Misciattelli (2001). The cows were tethered in tie stalls and milked twice daily at 6:00 h and 17:00 h.

2.2. *Experimental design*

The experiment was originally based on a 4×4 Latin square design with four cows, four treatments and four periods, but an additional period with three cows was added, due to variations in intake of concentrate in the previous periods. Each experimental period consisted of 29 days. Days 1–14 were used for adaptation, days 15–17 for *in situ* measurements and days 18–20 for digestibility measurements.

From day 21, concentrate was fed once a day (pulse dose) to measure ruminal starch degradation kinetics *in vivo*. Day 22 and days 25–29 were used for rumen evacuations and day 24 to measure patterns in appearance of undegraded starch at the duodenum subsequent to pulse dosing the concentrate.

2.3. *Treatments*

The four treatments were ground barley grain, expander processed (105 °C) and subsequently pelleted barley grain, ground maize grain and expander processed (95 °C) and subsequently pelleted maize grain. A Matador M6 pellet press (Sprout-Matador, Esbjerg, Denmark) and a Kahl expander model OEE 23.1 (Amandus Kahl, Reinbek bei Hamburg, Germany) were used. On a DM basis, the initial diet consisted of 7 kg per day of grass-clover silage, 1 kg per day of soybean meal, 1 kg per day of grass-clover hay, 0.15 kg per day of vitamins and minerals and 5 kg per day of one of the test grains as the starch source. The chemical composition of the ingredients is in Table 1, where chemical composition of grass-clover silage is the average of samples taken after each of the five periods, and where chemical composition of the other feeds is based on a pooled sample from subsamples from each period. Grass-clover silage and grass-clover hay were first cut grass-clover mixtures (i.e. perennial ryegrasses, white clover and red clover). Due to reduced voluntary feed intake during the experiment, feed offered was reduced accordingly in order to keep the ratio of concentrate to forage constant and minimise residues and selection.

Table 1 Chemical composition of the feedstuffs (g/kg DM)

	Barley grain		Maize grain		Other diet ingredients		
	Untreated ^a	Expanded ^a	Untreated ^a	Expanded ^a	Soybean meal ^a	Grass-clover silage ^b	Grass-clover hay ^a
Ash	21	24	18	17	83	113	69
Crude protein	135	144	104	103	503	206	114
Crude fat	30	32	38	42	29	35	12
NDF	154	148	100	89	128	481	631
Starch	567	542	743	725	10	3	2

^a Value represent duplicate assays of pooled samples from subsamples from each period.

^b Value represent duplicate assays of five samples.

2.4. Feeds and feeding

Grass-clover hay and grass-clover silage was offered twice daily at 10:15 and 17:30 h close to, but below, ad libitum. Refusals were removed and recorded daily. Throughout the first 20 days in each period, concentrate was offered twice daily at 10:15 and 17:30 h in equal meals. After day 20 the daily offer of concentrate was fed only once a day at 10:15 h, and concentrate not voluntarily consumed within 30 min was manually put into the rumen via the cannula.

2.5. Estimation of fractional rate of degradation for starch *in situ*

In situ incubations in the rumen started on day 15 in each experimental period. Polyamide cloths with a pore size of 36 and 15 µm were used, as earlier studies in our laboratory have shown a substantial loss of particulate starch from the ordinarily used nylon bags with pore size 36 µm (Mupeta, 1999). The bags measured 7.5 cm x 11 cm, and bags with a pore size of 36 µm were sewn with double lines and curved corners, whereas bags with a pore size of 15 µm were heat sealed. Untreated barley, expanded barley, untreated maize and expanded maize were ground through a 1.5 mm screen, and

the sample weights were 0.5 g for 0, 2 and 4 h incubations and 1.0 g for 8, 24 and 48 h incubations in order to secure starch residues within detection ranges, as starch in residues was analysed quantitatively. Incubated feeds matched the type of concentrate the cows were fed in each period. One bag for each of the two pore sizes was fixed on a rubber stopper, and one stopper for each incubation time was fixed to a plastic stick. The stick was attached to the rumen cannula by two 40 cm long nylon strings and the position maintained by a 250 g sinker. All bags were inserted at 8:00 h on day 15. At the end of each incubation time, bags were washed under running tap water and stored at -18 °C. After all bags had been removed, they were thawed and washed in a washing machine for 15 min. Residue was transferred to AGF 607–90 mm filter paper (Frisenette, Ebeltoft, Denmark) of known dry weight and dried (24 h at 100 °C) to determine DM loss, after which the starch residue was determined quantitatively.

Water solubility measured on filter paper, and extent of initial loss of small particles from the nylon bags, were determined at the end of the experiment as described by Hvelplund and Weisbjerg (2000). Water solubility was the mean of four measurements, and the loss of small particles was estimated as the difference between the loss from the nylon bags when they were only washed in the washing machine and the average solubility measured on filter paper. Tabulated values for degradation characteristics are not corrected for initial loss of small particles.

2.6. Digestibility measurements and rumen fluid sampling

Ten grams Cr_2O_3 and 40 g polyethylene glycol (PEG) were administered as digestibility markers before each feeding via the rumen cannula. Over a 64 h period on days 18–20, 12 samples of rumen fluid (100 ml) were taken in duplicate, 12 samples of duodenal (600 ml) and ileal (300 ml) content and 12 grab samples of faeces (250 ml) were collected. Each sample type was pooled within cow and period. The collection procedure was arranged to give representative samples of the diurnal flow (i.e. every second hour of the 24 h day). Samples from the duodenum and ileum were collected in tubeformed plastic bags mounted to the cannulae with plastic knees. Pooled samples of duodenal and ileal digesta, and faeces were frozen, and at the end of each period,

subsamples were freeze dried and analysed for DM, starch, Cr₂O₃ and PEG. Ruminant fluid was obtained by suction from the ventral rumen compartment using a rumen spear and pH was immediately determined. The 12 samples for ammonia and short chain fatty acid (SCFA) analysis, respectively, were pooled and kept frozen during the sampling period. Feed samples from three consecutive days (i.e. days 18–20) were pooled within period, freeze dried and analysed for DM, N, crude fat, NDF, ash and starch. Refusals on days 19–21 were pooled within period and cow, freeze dried and analysed for DM, N, NDF and starch.

2.7. Duodenal starch flow after feeding

The flow pattern of starch to the duodenum after pulse dosing concentrate once a day was examined by continuous infusion of solvent PEG into the rumen and hourly sampling from the duodenum. From days 21–24, 80 g PEG dissolved in 5 l per day of water was infused continuously into the rumen. Duodenal sampling started on day 24 and during 13 h post feeding, 14 separate 400 ml samples of duodenal fluid were collected and frozen. Samples were subsequently freeze dried and analysed for PEG and starch.

2.8. Rumen evacuation

On days 21–29, concentrate was fed once daily and the decline in the rumen starch pool was measured by a series of rumen evacuations. The total weight of the rumen content was measured by manually emptying the rumen of each animal 0.5 h before feeding concentrate or 1.5, 2.5, 4.5 or 7.5 h post feeding. A maximum of one rumen evacuation was performed per cow per day. Rumen evacuations were according to the procedure described by Børsting and Weisbjerg (1989), and rumen contents were collected into a sieve basket hanging in an iron stand in a circular tub. The basket allowed bailable liquid to pass to the tub, leaving the mat fraction in the basket. At each evacuation time a sample was composited from subsamples of rumen liquid and mat proportional to the weight of each fraction. Samples were dried at 80 °C for 20 h for DM determination and subsequent starch analysis.

2.9. Analytical procedures

Ash was analysed according to AOAC method 923.03, with the modification that combustion was performed at 525 °C for 6 h. N was analysed by a Kjeldahl method using an automated Kjel–Foss apparatus (Foss Electric, Denmark), AOAC method 978.02 (AOAC, 1990; Hansen and Sørensen, 1996). Crude fat was determined in a Soxhlet-apparatus with petroleum ether extraction after 3M HCl hydrolysis (Stoldt, 1952). Ash-free NDF was determined using a Fiber-Tec system, according to Van Soest *et al.* (1991). An overnight pre-treatment with α -amylase (A6380, Sigma, St. Louis, USA) at 38 °C according to Ferreira *et al.*, (1983), was followed by addition of sodium sulfite and a heat stable α -amylase (Termamyl, Novo Nordisk, Bagsværd, Denmark) during neutral detergent boiling. Starch was determined based on gelatinisation of starch and simultaneous partial hydrolysis at 100 °C using a thermostable α -amylase (Termamyl, Novo Nordisk, Bagsværd, Denmark), followed by complete hydrolysis at 60 °C using amyloglucosidase and determination of released glucose spectrophotometrically as described by Åman and Hesselman (1984). Starch content was corrected for the content of free glucose in the original sample by incubation without enzymes. Cr_2O_3 was determined colourimetrically after oxidation to chromate (Schürch *et al.*, 1950) and PEG was determined by its turbidity according to Hydén (1955). The SCFA were determined by gas chromatography according to Richard and McCalley (1987) and ammonia concentrations in ruminal fluid were determined by the colorimetric assay of Crooke and Simpson (1971).

2.10. Calculations

In situ degradation of starch was determined as described by Ørskov and McDonald (1979). Data were fitted to the exponential equation $Y(t) = a + b(1 - e^{-ct})$, where $Y(t)$ is degraded proportion at time t , a the water soluble and momentary degradable fraction, b not water soluble, but potentially rumen degradable fraction, and c is (h^{-1}) the fractional rate of degradation of fraction b .

$a + b$ was constrained to <1 . Effective ruminal degradation (D1) was calculated as $D1 (\%) = 100\% \times (a+bc/(c+k))$, assuming the fractional rate of passage (k) to be 0.05 h^{-1} as used in several protein evaluation systems (e.g. Madsen *et al.*, 1995; Hvelplund and Weisbjerg, 2000). Based on true water solubility of starch, the a and the b fractions can be corrected for initial particulate loss of starch, and corrected effective starch degradation (D2) can be calculated (Hvelplund and Weisbjerg, 2000).

Effective ruminal starch degradation (D3) was also calculated by arbitrarily assuming that 10% of the soluble starch escapes rumen degradation and passes from the rumen (Nocek and Tamminga, 1991), and with the average fractional rate of passage of starch assumed to be 0.03 h^{-1} . This fractional rate of passage of starch was calculated using an equation for fractional rate of passage of concentrates, based on average DM intake, body weight, forage % in DM and eNDF%, where eNDF% is NDF that is effective in meeting fibre requirements in % of DM (Sniffen *et al.*, 1992; Alderman *et al.*, 2001).

The a -fraction in the Ørskov and McDonald (1979) equation is assumed to be immediately degraded in the rumen. If this is not true, the a -fraction, like the b -fraction, has to be weighted based on its fractional rate of degradation and its fractional rate of passage in order to calculate effective starch degradation (D4; Hvelplund and Weisbjerg, 2000). Average fractional rate of passage of the b -fraction was assumed to be 0.03 h^{-1} (Sniffen *et al.*, 1992). Fractional passage rate of the a -fraction was assumed to be similar to the average fractional rate of passage for fluids, assumed to be 0.08 h^{-1} , based on average DM intake and body weight (Sniffen *et al.*, 1992) and average fractional rate of degradation of the a -fraction was arbitrarily assumed to be 1.00 h^{-1} .

Flow of duodenal and ileal digesta, and faecal output, were assumed to be the average DM flow calculated from the markers Cr_2O_3 and PEG, and apparent total digestibility and apparent digestibility in rumen, small intestine and hind gut could subsequently be calculated based on intake and flow of nutrients. Patterns in duodenal flow of undegraded starch were calculated based on the continuous PEG infusion and the starch:PEG ratio in each of the duodenal samples. Duodenal flow of starch (g/h) was calculated as rate of infusion of PEG (g/h) multiplied by the starch:PEG ratio in the individual duodenal samples. Starch degradation based on rumen evacuations was calculated based on rumen pools at different times after feeding (i.e. 1.5, 2.5, 4.5, 7.5 or 23.5 h) either relative to

starch intake or relative to the rumen pool of starch 1.5 h after feeding, in order to overcome variations in feed intake patterns. Starch degradation characteristics were estimated using the above described model of Ørskov and McDonald (1979), with the modification that only one starch pool (b) was used. In order to obtain the decline in the rumen starch pool resulting only from microbial degradation, ruminal starch pools were subsequently corrected by adding outflow of undegraded starch, based on the above described duodenal flow pattern of starch. The cumulative starch outflow was corrected for degradation, assuming that starch leaving the rumen would have been degraded in a similar way as starch left in the rumen. Effective rumen degradability (D5) was calculated as $D5 (\%) = 100\% \times (bc/(c + k))$, where b is the pool of potentially degradable starch, c fractional rate of degradation (h^{-1}) and k is the assumed fractional rate of passage of 0.05 h^{-1} . Effective rumen degradability (D6) was calculated similarly, assuming a fractional rate of passage of 0.03 h^{-1} . Fractional rates of passage at 1.5, 2.5, 4.5, 7.5 and 23.5 h after feeding starch were calculated as starch flow at the duodenum (g/h) divided by uncorrected rumen starch pools (g). Starch flow at the individual times were the average of the flows just before and just after the rumen evacuation times (e.g. the starch outflow 4.5 h after feeding was the average of the flows 4 and 5 h after feeding).

2.11. Statistical analysis

Data were analysed using PROC GLM of SAS (1995). Cow, period, and treatment (i.e. heat, grain and the interaction between heat and grain) were the class variables in the model, and the statistical analysis was based on 19 observations. For rumen evacuation kinetics, data and one set due to obvious outliers. Results are reported as least squares means and standard error of least squares means. P values were according to the SS2 procedure of PROC GLM in SAS 6.12 (SAS, 1995). Treatment effect within feed type was judged using PDIF in SAS 6.12 (SAS, 1995). Parameters for ruminal degradability (i.e. *in situ* and rumen evacuation data) of starch were calculated for each cow and period using PROC NLIN in SAS 6.12 (SAS, 1995). Significance was declared when $P < 0.05$. Water solubility and initial loss of small particles are average values based on four replicates.

3. Results

3.1. Diets and feed intake

The chemical composition of experimental feeds is in Table 1 and the average daily feed intake is in Table 2. Differences in DM intake were minor and due to a slightly reduced feeding level during the experiment. Mean milk yield decreased from 14.3 kg per day in the first period to 7.3 kg per day in the fifth period.

Table 2 Intake of nutrients (kg per day)

	Barley grain		Maize grain		S.E.M. ^a	P		
	Untreated	Expanded	Untreated	Expanded		Grain ^b	Heat ^c	G x H ^d
DM	12.4	13.0	12.5	12.2	0.2	0.2	0.4	0.06
Ash	0.89	0.95	0.91	0.89	0.02	0.5	0.4	0.06
Crude protein	2.41	2.57	2.33	2.25	0.04	0.002	0.3	0.02
Crude fat	0.38	0.41	0.42	0.43	<0.01	0.002	0.02	0.2
NDF	4.18	4.39	4.10	3.97	0.09	0.04	0.6	0.1
Starch	2.76	2.77	3.42	3.29	0.06	<0.001	0.4	0.3

^a Standard error of least squares treatment means.

^b Main effect of grain type.

^c Main effect of heat treatment.

^d Effect of interaction between grain type and heat treatment

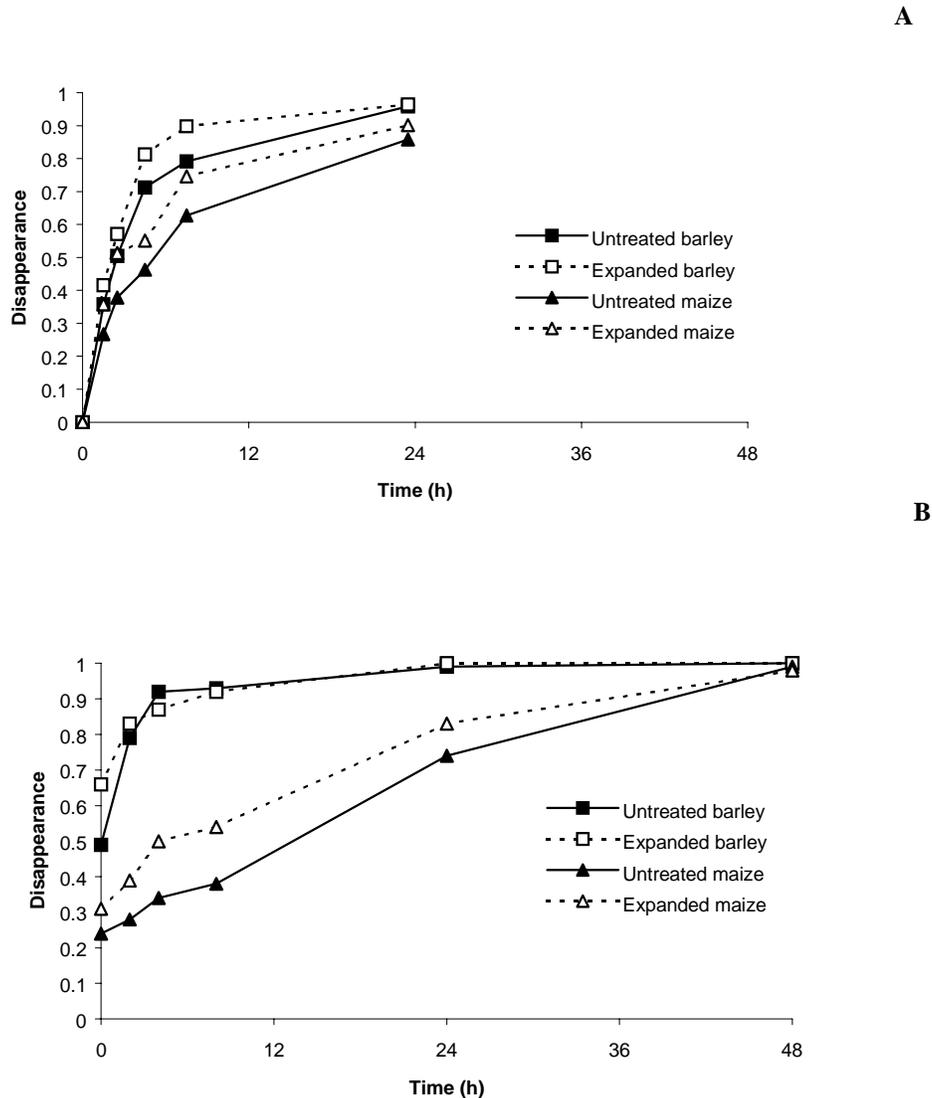
3.2. *In situ* experiments

Degradation characteristics of starch is in Table 3 and the disappearance curves obtained using bags with a pore size of 15 µm are illustrated in Fig. 1B. Heat treatment increased initial particle loss from nylon bags irrespective of pore size used and type of grain. Water solubility determined on filter paper decreased due to heat treatment. The *in situ* a-fraction was higher than water solubility determined using filter paper. The a-fraction varied from 0.21 for untreated maize grain, determined using bags with a pore size of 15

μm to 0.75 for expanded barley grain, determined using bags with a pore size of 36 μm , and the fraction was lower ($P < 0.001$) for maize grain than for barley grain. The a-fraction increased ($P < 0.001$) due to heat treatment irrespective of pore size or type of grain, and was higher when a pore size of 36 μm was used instead of 15 μm . The increase in the a-fraction when grains were expanded was accompanied by a similar decrease in the non-soluble, but potentially degradable fraction (b). Fractional rate of degradation (c) varied from 0.04 h^{-1} for expanded maize grain, determined using bags with a pore size of 15 μm , to 0.63 h^{-1} for untreated barley grain determined using bags with a pore size of 36 μm . Fractional rate of degradation for untreated barley was approximately 10 times higher than the average fractional rate of degradation for maize, and although fractional rate of degradation decreased (15 μm : $P < 0.001$; 36 μm : $P = 0.01$) due to heat treatment of barley grain, fractional rate of degradation for expanded barley was still almost six times higher than the average fractional rate of degradation for maize. Due to the opposite effects on effective degradability of the increase in the a-fraction, and the decrease in fractional rate of degradation, effective degradability (D1) of starch for barley was not affected by heat treatment. Although heat treatment increased ($P < 0.001$) effective degradability of starch from maize from 60 to 72% (36 μm), effective degradability from expanded maize grain was still about 25% points lower than average effective degradability for barley grain. Nylon bag pore size seemed only to have minor effect on effective degradability, despite that the a-fraction and the fractional rate of degradation was numerically higher when a pore size of 36 μm was used compared to 15 μm .

Correction for initial particle loss decreased effective degradability (D2, Table 7), especially for expanded grains due to a high particulate loss (Table 3). For expanded barley, the correction for initial particle loss decreased effective degradability from 94 to 81% and from 96 to 86%, respectively, when 15 or 36 μm bags were used. D1 was significantly higher for expanded maize than for untreated maize and D1 was similar for untreated and expanded barley (Table 7). However, when corrected, effective degradability of starch (D2) was similar for untreated and expanded maize and a numerical decrease in effective degradability (D2) occurred when barley was expanded. The correction thus changed the ranking of feeds in effective degradability of starch.

Figure 1 Disappearance curves for starch based on either *in vivo* data from rumen pools corrected for passage of undegraded starch to the intestine (A) or based on *in situ* data using nylon bags with a pore size of 15 μm (B).



3.3. Starch degradation based on rumen evacuations

Table 4 shows the ruminal starch disappearance characteristics based on rumen evacuations after feeding concentrate once a day, and the corresponding degradation curves are shown in Fig. 1A. To overcome variations in feed intake patterns, degradation parameters were also calculated based on the rumen pool of starch 1.5 h after feeding starch. To calculate true microbial degradation of starch, the pool at each evacuation time

Table 3 Water solubility of starch determined on filter paper, initial loss of small particles and *in situ* ruminal starch disappearance calculated according to Ørskov and McDonald (1979).

	Barley grain		Maize grain		S.E.M.	P		
	Untreated	Expanded	Untreated	Expanded		Grain	Heat	G x H
Water solubility ^a	0.057 (0.005)	0.031 (0.002)	0.167 (0.002)	0.069 (0.003)	-	-	-	-
Pore size 15 µm								
Particle loss ^b	0.43 (0.02)	0.63 (0.03)	0.08 (0.02)	0.26 (0.03)	-	-	-	-
a ^{c,d}	0.49	0.67 ^h	0.21	0.32 ^h	0.02	<0.001	<0.001	0.08
b ^{d,e}	0.49	0.32 ^h	0.79	0.68 ^h	0.02	<0.001	<0.001	0.1
c ^f	0.48	0.24 ^h	0.05	0.04	0.03	<0.001	0.002	0.004
D ₁ ^{d,g}	93	94	60	69 ^h	1	<0.001	0.005	0.007
Pore size 36 µm								
Particle loss ^b	0.49 (0.03)	0.72 (0.02)	0.10 (0.02)	0.35 (0.02)	-	-	-	-
a ^{c,d}	0.55	0.75 ^h	0.23	0.40 ^h	0.02	<0.001	<0.001	0.4
b ^{d,e}	0.45	0.24 ^h	0.77	0.60 ^h	0.02	<0.001	<0.001	0.3
c ^f	0.63	0.36 ^h	0.05	0.07	0.06	<0.001	0.05	0.04
D ₁ ^{d,g}	96	96	60	72 ^h	1	<0.001	<0.001	<0.001

^a Average of four determinations, S.E.M. in brackets.

^b Particulate loss is calculated as residual starch after washing minus average water solubility, S.E.M. in brackets.

^c Water soluble starch.

^d Degradation characteristics are not corrected for particulate loss.

^e Non-soluble but potentially degradable starch

^f Fractional rate of starch degradation (h⁻¹).

^g Effective degradability (%), assuming a fractional rate of passage of 0.05 h⁻¹.

^h Within feedstuff, significant effect of heat treatment (P<0.05)

was corrected for previous outflow of starch, under the assumption that rumen escape starch would have been degraded similar to starch left in the rumen. Potential degradable starch (b) in contrary to the model used for *in situ* data also includes water soluble starch. When kinetics for disappearance by both degradation and passage was calculated based on disappearance of the 0 or 1.5 h pool, the potential disappearance (b) was very close to 1.00, and no effect of type of grain or heat treatment occurred. When kinetics of disappearance by digestion alone was calculated based on corrected rumen pools, the potentially degradable fraction was low, especially when degradation was calculated based on the 1.5 h pool and especially for untreated maize. No effects of type of grain or heat treatment were found. When rumen pools were corrected for duodenal flow of starch, and rumen pools were related to starch intake, fractional rate of degradation (c) varied from 0.19 for untreated maize grain to 0.40 for expanded barley grain. Fractional rate of degradation increased ($P=0.04$) with expanding, and was higher ($P=0.007$) for barley grain than for maize grain. When degradation was calculated based on 1.5 h pools, expanding had no effect on fractional rate of degradation. Fractional rate of degradation averaged 0.36 h^{-1} for barley grain and 0.15 h^{-1} for maize grain, and was higher ($P=0.009$) for barley than for maize. Calculation of degradation based on intake or 1.5 h pool and correction for previous duodenal flow had only minor effects on fractional rates of degradation of untreated and expanded barley, whereas fractional rate of degradation for maize seemed to be lower when disappearance was based on 1.5 h pools compared to intake. Effective degradability (D5) was higher (0 h: $P=0.003$; 1.5 h: $P=0.001$) for barley than for maize, and expanding increased ($P=0.03$) effective degradability when disappearance was related to intake, and a tendency ($P=0.06$) to an increase was found when related to the 1.5 h pool. Calculation of degradation based on disappearance of the 1.5 h pool, instead of intake, decreased effective degradability and, for untreated and expanded maize, the effective degradability was 10% points lower than when based on intake.

Table 4 Degradation characteristics of starch based on rumen evacuations

	Barley grain		Maize grain		S.E.M.	P		
	Untreated	Expanded	Untreated	Expanded		Grain	Heat	G x H
Relative to 0 hour values (intake) ^a								
b ^b	0.98	0.98	0.98	1.00	0.01	0.4	0.5	0.6
c ^c	0.31	0.39 ^g	0.18	0.24 ^g	0.02	<0.001	0.006	0.7
Relative to 1.5 hour values ^b								
b ^b	0.95	0.96	0.96	0.98	0.02	0.4	0.5	0.7
c ^c	0.30	0.41	0.12	0.17	0.05	0.004	0.1	0.5
Relative to 0 hour values (intake), corrected for duodenal flow of starch ^{a,c}								
b ^b	0.95	0.96	0.90	0.93	0.02	0.2	0.6	0.6
c ^c	0.31	0.40 ^h	0.19	0.27	0.03	0.007	0.04	1
D ₅ ^f	81	85	71	78 ^g	2	0.003	0.03	0.4
Relative to 1.5 hour values, corrected for duodenal flow of starch ^{d,e}								
b ^b	0.92	0.91	0.80	0.89	0.05	0.2	0.5	0.3
c ^c	0.30	0.42	0.13	0.17	0.06	0.009	0.2	0.5
D ₅ ^f	77	81	61	68	3	0.001	0.06	0.6

^a Residual starch pool is related to starch intake.

^b Potential degradable fraction.

^c Fractional rate of degradation (h⁻¹).

^d Residual starch pool is related to starch pool after 1.5 h.

^e Measured rumen pools are corrected for bypassed starch as described in 2.10.

^f Effective starch degradability (%), assuming a fractional rate of passage of 0.05 h⁻¹.

^g Within feedstuff significant effect of heat treatment (P<0.05).

^h Within feedstuff tendency to a significant effect of heat treatment (0.05<P<0.1).

3.4. Flow and digestibility of starch

Although, large numerical differences were found in digestibility coefficients of starch from untreated and expanded barley entering the small intestine and the hind gut, the actual amounts digested in the small intestine were similar and the post duodenal flows and amounts digested in the hind gut were negligible (Table 5). No effects of heat treatment of barley was found on digestibility in any part of the gastro intestinal tract, and rumen digestibility and total digestibility was 91 and 99%, respectively. In general, heat treatment of maize influenced digestion of starch more than for barley, and rumen digestibility of maize was lower (P=0.01) than for barley. Expanding maize grain increased (P<0.001) total digestibility from 84 to 96%, and total digestibility of expanded maize was almost as high as for barley. Decision of digestion between the rumen and small intestine was made difficult by a lower measured duodenal flow (0.85 kg per day) than ileal flow (0.99 kg per day) for untreated maize, resulting in a negative small

intestinal digestibility. If duodenal flow of starch for untreated maize is assumed to be no less than the ileal flow, a rumen digestibility of 71% can be estimated. The amount of starch entering the small intestine was about three times higher for maize than barley and the amount entering the hind gut was approximately 10 times higher. For untreated maize grain 15% of ingested starch was lost in faeces, equal to 0.54 kg per day, whereas 0.02 kg per day for barley and 0.12 kg per day for expanded maize was lost.

Table 5 Flow and digestibility of starch in different sections of the digestive tract

	Barley grain		Maize grain		S.E.M.	P		
	Untreated	Expanded	Untreated	Expanded		Grain	Heat	G x H
Flow (kg starch per day)								
Intake	2.76	2.77	3.42	3.29	0.06	<0.001	0.4	0.3
Duodenal	0.25	0.25	0.85	0.65	0.08	<0.001	0.3	0.3
Ileal	0.08	0.05	0.99	0.48 ^c	0.07	<0.001	0.01	0.02
Faecal	0.02	0.02	0.54	0.12 ^c	0.04	<0.001	0.002	0.001
Digestibility (%)								
Rumen	91	91	75 (71 ^b)	79	3	0.001	0.5	0.5
Small intestine ^a	70	83	-27 (0 ^b)	19	17	0.002	0.1	0.4
Hindgut ^a	80	64	45	73	7	0.08	0.5	0.02
Feed-ileum ^a	97	98	72	86 ^c	2	<0.001	0.008	0.01
Total	99	99	84	96 ^c	1	<0.001	<0.001	<0.001

^a Percentage of the amount entering the section of the gut.

^b Calculated using a duodenal flow no less than the ileal flow (0.99 kg), i.e. a small intestinal digestibility ≥ 0 .

^c Within feedstuff significant effect of heat treatment ($P < 0.05$).

3.5. Ruminal fermentation

Ruminal pH was measured when cows were fed concentrate twice daily, and ruminal mean pH, minimum pH and average concentrations of SCFA was similar for all four treatments (Table 6). Ruminal concentration of $\text{NH}_3\text{-N}$ varied from 16 mg/100 ml for expanded maize to 22 mg/100 ml for untreated barley, and both for maize and for barley, heat treatment decreased (barley: $P=0.002$; maize: $P<0.001$) ruminal concentration of $\text{NH}_3\text{-N}$.

Table 6 pH, SCFA and ammonia concentration in ruminal fluid.

	Barley grain		Maize grain		S.E.M.	P		
	Untreated	Expanded	Untreated	Expanded		Grain	Heat	G x H
pH ^a	6.35	6.36	6.37	6.32	0.04	0.8	0.6	0.5
pH _{MIN.} ^b	5.60	5.75	5.82	5.72	0.08	0.2	0.7	0.2
SCFA (mmol/l)	101	105	104	101	3	1	0.8	0.3
$\text{NH}_3\text{-N}$ (mg/100 ml)	21.6	18.8 ^c	21.0	16.0 ^c	0.4	0.006	<0.001	0.03

^a Mean of 12 values, measured every 2 h during the day.

^b Lowest measured pH.

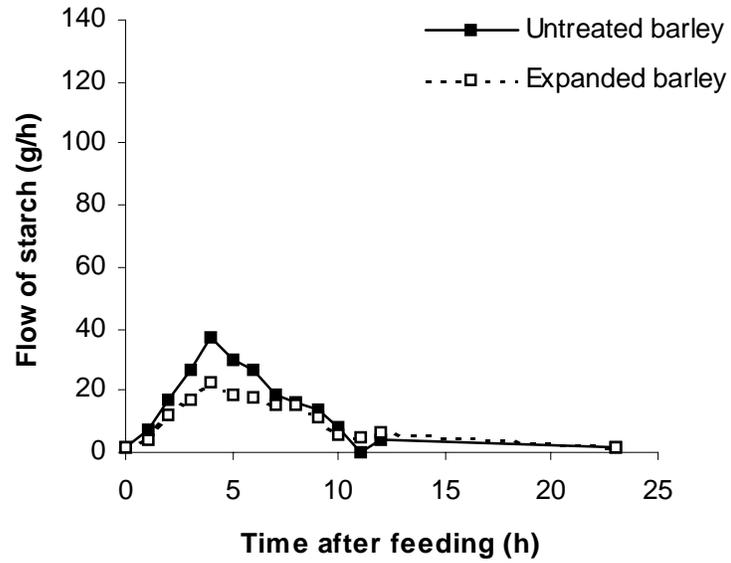
^c Within feedstuff, significant effect of heat treatment ($P<0.05$).

3.6. Starch flow to duodenum

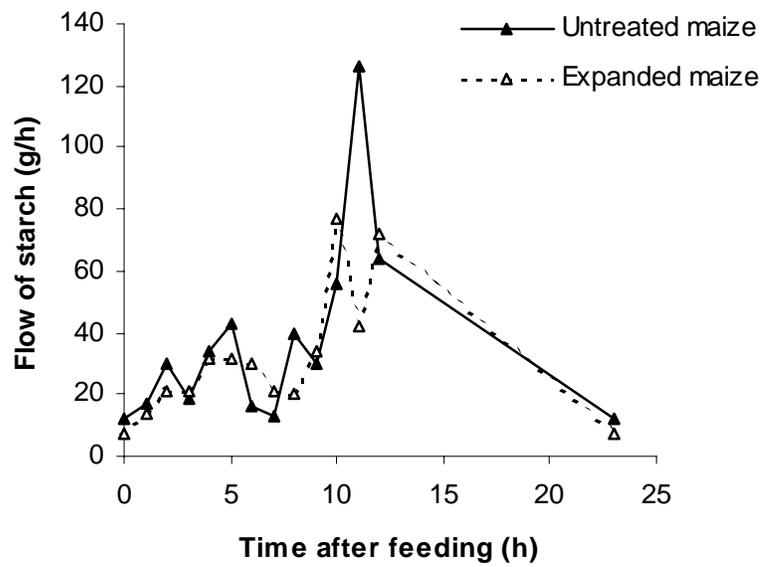
Starch flow to the duodenum (g/h) subsequent to feeding concentrate once a day is in Fig. 2. The highest duodenal flow of starch was found after 4 h (37 g/h) for untreated barley, 4 h (23 g/h) for expanded barley, 11 h (126 g/h) for untreated maize and 10 h (77 g/h) for expanded maize. Heat treatment did not affect duodenal flow of starch from barley at any time, and for maize a difference ($P=0.007$) was only found 11 h post feeding, where duodenal flow was 126 and 42 g/h for untreated and expanded maize, respectively. A peak in starch flow 4–6 h post feeding was followed by a decrease in duodenal flow of starch from barley, whereas an additional larger peak in starch flow was observed about 11 h post feeding for maize.

Figure 2 Duodenal flow (g/h) of ruminal undegraded starch after feeding barley (A) or maize (B) once a day at time 0.

A



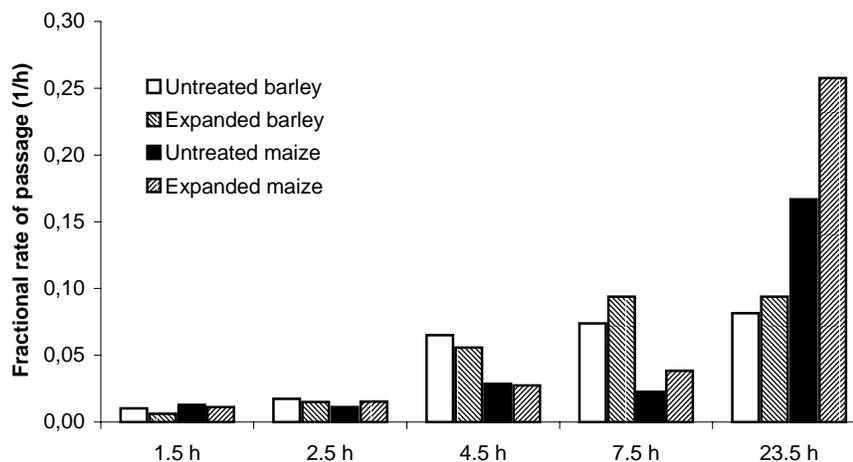
B



Similar patterns were found when hourly duodenal flows were corrected for variation in starch intake (data not shown). Fractional rates of passage calculated based on rumen pool size and concurrent duodenal flow of undegraded starch are in Fig. 3. Fractional rate of passage was not constant and varied from 0.006 h⁻¹ for expanded barley 1.5 h after feeding starch to 0.258 h⁻¹ for expanded maize 23.5 h after feeding starch. No effects of heat treatment or type of grain was found on fractional rate of passage 1.5 and 2.5 h after feeding. Between type of grain and heat treatment, fractional rate of passage was on average 0.008 and 0.011 h⁻¹, 1.5 and 2.5 h after feeding, respectively.

Fractional rate of passage was higher for barley compared to maize 4.5 (P=0.05) and 7.5 h (P=0.02) after feeding. For barley, the average fractional rate of passage after 4.5 and 7.5 h was 0.06 and 0.08 h⁻¹, whereas for maize, the average fractional rate of passage at both times was 0.03 h⁻¹. Although large numerical differences were found 23.5 h after feeding, no effect was found of heat treatment or type of grain, and fractional rate of passage averaged 0.146 h⁻¹ between heat treatment and type of grain, 23.5 h after feeding starch.

Figure 3 Fractional rate of passage of starch from the rumen (h⁻¹), calculated at different times after pulse dose of concentrate, based on concurrent measurements of duodenal flow of starch and rumen starch pool.



4. Discussion

4.1. *In vivo* digestibility and rumen fermentation

In vivo rumen digestibility of starch from untreated barley (91%) and untreated maize (71%) was higher than the mean digestibility of 83 and 47%, respectively, reported by Mills *et al.* (1999). However, in a review by Owens *et al.* (1986), a rumen digestibility of 78% for starch from untreated maize was reported. These inconsistencies among experiments are probably due to differences between varieties, feeding levels, starch intake and proportion of starch in the ration as rumen digestibility of starch decreases as starch intake is increased (Mills *et al.*, 1999). In the present experiment, starch intake was low compared to reviewed data from Mills *et al.*, (1999) and this may explain the higher rumen digestibility of starch especially from maize. However, total digestibility of starch from maize was low compared to literature values (Owens *et al.*, 1986; Mills *et al.*, 1999). Other results with lactating cows confirm the decrease in rumen digestibility of starch when barley was replaced by maize (McCarthy *et al.*, 1989; Overton *et al.*, 1995; Yang *et al.*, 1997). Application of various combinations of heat, moisture, time, and mechanical action increase digestibility of starch by providing opportunities for bacterial attachment (Huntington, 1997). However, heat treatment had no effect on digestibility of starch from barley in any part of the gastrointestinal tract, and similar results were reported by Prestløkken and Harstad (2001), although they found that expander treatment numerically increased ruminal starch digestion. Due to the high ruminal digestibility of starch from untreated barley, the potential for increased rumen digestibility is limited, whereas processing can increase digestibility for maize. Heat treatment of maize tended to increase starch digestibility in agreement with Crocker *et al.* (1998) and Theurer *et al.* (1999), although the increase was limited, probably due to the relatively low processing temperature (95 °C). Size of starch particles in the ruminal outflow was observed to be much larger and to have a higher specific gravity in untreated versus expanded maize, which might have resulted in unrepresentative duodenal samples low in starch content, leading to negative small intestinal digestibility of starch for untreated maize. Similarly, rumen digestibility of starch was overestimated for untreated maize and this explains the

minor effect of expanding on *in vivo* rumen digestibility compared to results obtained *in situ* and using rumen evacuations. Starch entering the duodenum is important as a major source of glucose from nutrients absorbed from the digestive tract. The amount of starch entering the duodenum was almost three times higher on the maize diets than on the barley diets. However, the efficiency of starch digestion in the small intestine has been observed to vary between starch sources, as ruminally and intestinally starch digestibility are positively correlated (Nocek and Tamminga, 1991). Barley starch was highly digestible in the small intestine, in contrast to maize starch, resulting in a higher small intestinal absorption in gram per kilogram starch ingested for barley compared to maize. Lack of differences in rumen pH between barley and maize were unexpected, and may be due to the relatively high rumen digestibility of the maize in the present study compared to literature values (Mills *et al.*, 1999). Prestløkken and Harstad (2001) reported a decrease in rumen pH from 6.1 to 5.9 when pelleted barley was replaced in the diet by expanded barley and, in the present experiment, pH was lower for cows fed expanded grains compared to those fed native grains in the first hours after the morning feeding, whereas no differences were found after the evening feeding. The reason for this discrepancy between feeding times was probably a much larger interval between the evening feeding and the morning feeding, and therefore a smaller ruminal pool before the morning feeding. A high intake of readily degradable starch in expanded cereals would therefore have a more pronounced effect on pH after the morning feeding. Rumen concentrations of SCFA were similar for untreated and expanded maize, despite the finding that heat treatment increased rumen digestibility of starch. A similar absence of effect on concentration of SCFA was found by Overton *et al.* (1995) and Crocker *et al.* (1998), whereas Prestløkken and Harstad (2001) found an increase in rumen SCFA concentration when barley was expanded. In the present experiment, concentrations of ammonia in ruminal fluid were decreased by heat treatment, probably due to a larger incorporation in microbial protein, and similar to results reported by Overton *et al.* (1995), Crocker *et al.* (1998) and Prestløkken and Harstad (2001). However, the lower effective protein degradation of barley due to expanding (Lund *et al.*, 1999) would also decrease ruminal concentrations of ammonia.

4.2. *In situ*

Although, fractional rates of degradation varying from 0.15 to 0.57 h⁻¹ and a-fractions varying from 0.27 to 0.82 have been reported, effective starch degradability for untreated barley in the present experiment of 93–96% was at the same level as reported by Herrera-Saldana *et al.* (1990), Cerneau and Michalet-Doreau (1991), Nocek and Tamminga (1991) and Yang *et al.* (1997), but higher than the 81% reported by Batajoo and Shaver (1998). Variation between experiments in digestion kinetics for untreated maize was much smaller than for barley. Fractional rate of degradation was generally between 0.04 and 0.06 h⁻¹ and the a-fraction was generally between 0.2 and 0.3, resulting in effective degradabilities of untreated maize of 55–60% (Herrera-Saldana *et al.*, 1990; Michalet-Doreau *et al.*, 1997). These values are similar to values reported in the present experiment, but much lower than the fractional rate of degradation of 0.09 h⁻¹ and effective degradability of maize of 90% found by Arieli *et al.* (1995). The lower effective starch degradability for maize compared to barley is consistent with Ørskov (1986), Nocek and Tamminga (1991) and Yang *et al.* (1997).

The lower a-fraction for untreated barley grain compared to expanded barley grain is compensated for by a higher initial fractional rate of degradation for untreated barley within the first 2–6 h. The higher fractional rate of degradation of untreated barley compared to expanded barley was only due to the initial difference in fractional rate. For maize grain, the degradation profiles were similar for untreated and expanded maize, and the only difference was an apparent vertical parallel displacement within the first 24 h. Overall only minor differences were found on fractional rate of degradation due to expanding for maize compared to barley. As the fractional rate of degradation of barley starch decreased due to expanding, relatively easily degradable starch in the b-fraction in untreated barley was probably made soluble by heat treatment, leaving the more slowly degradable starch in the b-fraction. Similar considerations for maize indicated that the b-fraction in maize had a more homogenous composition compared to barley starch.

In the present experiment, expanding barley increased the a-fraction, decreased fractional rate of degradation and did not affect effective degradability, and similar results for protein free DM have been reported by Prestløkken (1993). Although, Arieli *et al.*

(1995) also found a decrease in fractional rate of degradation, a concurrent increase in the estimated indigestible fraction ($100-a-b$) from 2 to 28% resulted in a marked decrease in effective degradability of barley, and similar to Engstrom *et al.* (1992), who reported that *in situ* disappearance was reduced in steam-rolled barley versus dry-rolled barley. The increase in effective degradability due to heat treatment of maize was not found by Arieli *et al.* (1995), who found a decrease in degradability due to an increase in the indigestible fraction. This indicates that severe heat treatment in some cases can result in a protection of the starch granules from degradation, probably due to physical protection of the starch granules by the heat treated protein matrix surrounding the starch granules (Kotarski *et al.*, 1992). Variable results obtained by different groups underline the need for caution when comparing digestibility data between studies, from different processing methods (Mills *et al.*, 1999).

In situ pore size needs to be such that influx of rumen digesta and efflux of microbial products is possible and efflux of undegraded particles is limited. Increasing pore size from 15 to 36 μm increased, in general, the a-fraction by up to 10% and fractional rate of degradation by up to 50%, probably due to a higher loss of particles from the nylon bags and a higher availability of residual starch in the nylon bags, respectively. However, size and ranking of effective degradability was similar, illustrating the stability of the *in situ* method. Interpretation of *in situ* results is dependant upon the assumptions used, and this results in assumption dependent conclusions. Therefore, the usefulness of the *in situ* method for estimation of starch digestion in the rumen cannot be evaluated without an evaluation of the assumptions. As a result of grinding, small particles are produced that can pass through the pores in the nylon bag without any further degradation and, during degradation, more small particles may be produced, which have the potential to leave the nylon bag. These losses are usually regarded as being soluble and immediately degraded in the rumen, but should probably be treated as are the particles remaining in the bag. Correction of the degradation profile for initial loss of small particles, as described by Hvelplund and Weisbjerg (2000), is an option and, using this approach (D2) heat treatment, in contrast to the original method (D1), decreased effective degradability for barley and did not have an effect of effective degradability of maize (Table 7). Assuming that 10% of the soluble starch escapes rumen degradation (Nocek and Tamminga, 1991)

but changing the fractional rate of passage from 0.05 to 0.03 h⁻¹, an effect of heat treatment on effective degradability (D3), was only found when 36 µm bags were used. These revised assumptions decreased effective degradability for barley and increased effective degradability for maize, compared to D1, although the ranking of feeds was similar. Similar ranking and similar degradabilities occurred when it was assumed that the soluble fraction is available for passage with an assumed fractional rate of degradation and passage of the soluble fraction similar to liquid (D4). Using this approach, heat treatment increased D4 for maize, irrespectively of nylon bag pore size.

4.3. Rumen evacuation

A series of rumen evacuations has not before been used to determine rumen kinetics of starch digestion. Disappearance of starch calculated using rumen evacuations is the combined effect of microbial degradation and outflow of undegraded starch. In order to calculate the true microbial degradation of starch, pools at each evacuation time were corrected for outflow of starch, under the assumption that rumen escape starch would have been degraded similar to starch left in the rumen.

The values for the b-fraction for barley and expanded maize were around 0.9 when based on disappearance of the corrected 1.5 h pools, whereas the b-fraction for untreated maize was only 0.8. A major reason for the low b-values compared to the asymptote (a + b) obtained in nylon bag studies was probably that the outflow of starch was overestimated, as starch flow was only measured for the first 13 h after grain feeding, and then again 23 h after grain feeding, and a linear decrease in duodenal starch flow in the mean time was assumed. This probably resulted in an overestimation of duodenal flow, and so an overestimation of rumen pool sizes corrected for outflow, resulting in underestimation of b-values and overestimation of c-values. Further, the use of 1.5 h values as basis is problematic, when b is less than 100%, due to an upscaling of the undegradable fraction and thereby reduction in the size of the b-fraction. The higher outflow of maize starch compared to barley starch can explain why maize parameters were more affected by correcting for duodenal starch flow.

Table 7 Comparison of ruminal starch digestibility calculated based on intake and duodenal flow of starch (D), nylon bag incubations (D₁, D₂, D₃, D₄) and rumen evacuations (D₅, D₆)

Grain	Heat	<u>In vivo</u> ^a	<u>In situ</u> ^b		<u>In situ</u> ^c		<u>In situ</u> ^d		<u>In situ</u> ^e		Rumen evacuation ^f		Rumen evacuation ^g	
		D	D1 (15 µm)	D1 (36 µm)	D2 (15 µm)	D2 (36 µm)	D3 (15 µm)	D3 (36 µm)	D4 (15 µm)	D4 (36 µm)	D ₅ ^h	D ₅ ⁱ	D ₆ ^h	D ₆ ⁱ
Barley	Untreated	91	93	96	88	92	90	92	91	93	81	77	86	82
	Expanded	91	94	96	81	86	89	90	91	92	85	81	89	85
Maize	Untreated	71 ^j	60	60	57	55	68	68	69	68	71	61	77	67
	Expanded	79	69 ^k	72 ^k	57	57	74	76 ^k	75 ^k	77 ^k	78 ^k	68	83	75

^a Apparent ruminal digestibility, from Table 5.

^b D₁ from Table 3, fractional rate of passage: 0.05 h⁻¹.

^c D₂, corrected for initial loss of particulate starch, fractional rate of passage: 0.05 h⁻¹.

^d D₃, assuming 10 % of the a-fraction pass out of the rumen undigested and fractional rate of passage (b-fraction): 0.03 h⁻¹.

^e D₄, assuming that the a-fraction is available for passage. Fractional rate of degradation (a-fraction): 1.00 h⁻¹, fractional rate of passage (a-fraction) = 0.08 h⁻¹ and fractional rate of passage (b-fraction): 0.03 h⁻¹.

^f D₅ from Table 4, fractional rate of passage: 0.05 h⁻¹.

^g D₆, fractional rate of passage: 0.03 h⁻¹.

^h Relative to 0 hour values, corrected for duodenal flow of starch.

ⁱ Relative to 1.5 hour values, corrected for duodenal flow of starch.

^j When calculated using a duodenal flow no less than the ileal flow (0.986 g), i.e. a small intestinal starch digestibility ≥ 0.

^k Within feedstuff significant effect of heat treatment (P<0.05).

The reason for the large effect on maize degradation parameters due to change in initial starch pool was probably that soluble maize starch was degraded faster than non-soluble starch. The evacuation technique does not discriminate between a soluble starch a-fraction with a fast/infinite fractional rate of degradation and a non-soluble starch b-fraction with a more slowly degradation as in the *in situ* procedure. Therefore, it is possible that after 1.5 h only the slowly degradable and non-soluble maize starch remains, which the lower values for both fractional rate of degradation and potentially degradable fraction indicate, when calculated relative to 1.5 h values and not to intake. The reason why similar effects were not seen for barley was probably that the difference in fractional rate of degradation for soluble and non-soluble barley starch was minor compared to maize.

4.4. Fractional rate of degradation

Fractional rate of degradation of starch was expected to be higher when calculations were based on corrected rumen evacuation data relative to intake compared to *in situ* values, because the potentially degradable b-fraction calculated using rumen evacuations included the immediately soluble starch. In accordance with model calculations by Ewing and Johnson (1987), fractional rate of degradation of starch in maize was markedly higher when measured using rumen evacuations relative to 0 h values (0.19–0.27 h⁻¹) than *in situ* (0.04–0.07 h⁻¹). For barley, results were less conclusive, although fractional rate of degradation of untreated barley seemed to be overestimated *in situ*. Rates of degradation based on rumen evacuation pools relative to 1.5 h values should be more comparable with *in situ* results, as most of the soluble fraction probably was fermented within the first 1.5 h, but once again the fractional rates for maize measured using the rumen evacuation technique (0.13–0.17 h⁻¹) were higher than *in situ* results, whereas results for barley again were less conclusive. In contrast to results from *in situ* studies, where fractional rate of degradation decreased due to heat treatment of barley, fractional rate of starch degradation in barley calculated from rumen evacuation data increased due to expanding when determined based on rumen evacuation data. Similarly for maize, the *in situ* studies predicted that heat treatment had no effect on fractional rate of degradation of maize,

although the rumen evacuation data indicated an increase in fractional rate of degradation. Use of *in situ* method therefore, seems to be a problem for accurate prediction of both the fractional rate of degradation of starch and the effect of heat treatment on fractional rate of degradation.

4.5. Rumen digestibility of starch

In situ estimates of the effective ruminal degradability (D1) of starch in barley were unaffected by heat treatment, whereas heat treatment increased effective ruminal degradability of starch in maize. However, comparing results of uncorrected *in situ* studies of starch degradation with corresponding values of starch digestibility obtained *in vivo* (Table 7), indicates that the uncorrected *in situ* method substantially underestimates the digestibility of the slowly degraded maize, but only slightly overestimates the digestibility of the rapidly degraded barley. Therefore, results question the use of the uncorrected *in situ* method, in respect to absolute values for ruminal starch digestibility. Results are consistent with results tabulated by Nocek and Tamminga (1991, their Table 2) where *in situ* studies underestimated rumen digestibility of slowly degradable starch in maize and sorghum, and overestimated digestibility of rapidly degraded starch in barley and oats. Nocek and Tamminga (1991) predicted that *in vivo* digestibility and *in situ* degradability were equal when the *in vivo* digestibility was 0.89. Above this value, *in situ* methods overestimate digestibility and below it, *in situ* methods underestimate digestibility. However, deviation from *in vivo* digestibility increased with decreased degradability, and since only few feeds have very high starch degradabilities (i.e. above 0.89), the uncorrected *in situ* method in general underestimate rumen digestibility of starch and particularly when the digestibility is low. The lower starch degradability *in situ* than the actual *in vivo* digestibility could be due to a lower bacterial numbers within the bag than in the surrounding rumen content (Meyer and Mackie, 1986) or lower microbial activity (Nozière and Michalet-Doreau, 1996). Introducing a correction for initial loss of particulate starch decreased degradability, especially in expanded feeds, but was not a solution to overcome the underestimation of rumen digestibility of slowly degradable starch in maize. Decreasing fractional rate of passage to 0.03 h^{-1} in combination with

assumed passage of part of the a fraction (D3), or assuming that the a-fraction was available for passage (D4), resulted in degradability in agreement with *in vivo* digestibility. Additionally nylon bag pore size seemed to not affect the ranking or the absolute values when corrections for initial particle loss were not made. The *in situ* method could therefore be used to predict both ranking and absolute values for rumen digestibility of starch, when a suitable fractional rate of passage is chosen and appropriate assumptions on passage of the soluble a-fraction are made. When *in vivo* digestibility of starch is compared with ruminal starch degradability calculated based on rumen evacuations relative to 0 h, using a fractional rate of passage of 0.05 h^{-1} (Table 7), rumen evacuation results underestimated starch digestibility in the rumen for barley, but a good agreement was found for maize.

When a fractional rate of passage of 0.03 h^{-1} was used, agreement improved for barley, whereas for maize the rumen evacuation method then overestimated digestibility. This indicates that the fractional rate of passage is not similar for all feeds, and that a fixed fractional passage rate for maize should be around 0.05 h^{-1} whereas a fixed fractional passage rate for barley should be 0.03 h^{-1} . Alternatively, the one compartment model with first order kinetics may have been too simple to describe rumen metabolism of starch as fractional passage rates *in vivo* are not constant (Fig. 3).

4.6. Determination of fractional rate of passage *in vivo*

Diurnal variation in rumen outflow of starch has not been estimated previously. Passage of undegraded starch out of the rumen was assumed to follow first order kinetics in the models for calculating rumen degradation kinetics based on *in situ* and rumen evacuation data. However, rate of passage did not follow an exponential decline. For barley, duodenal flow of starch subsequent to the peak at 4–6 h post feeding seemed to be limited in agreement with the high rumen digestibility of starch from barley. Time series of duodenal flow of rumen undegraded starch from barley therefore, indicates a passage time lag of newly ingested starch prior to passage. Outflow of starch from maize seemed to follow the same pattern as barley, at least for the first 8 h post feeding, but instead of an exponential declining flow, an additional unexpected and very large peak appeared

around 11 h after feeding. The pattern in duodenal flow of starch differed among cows. For expanded barley and untreated maize, pattern and amplitude was similar for all cows, whereas for untreated barley and for expanded maize the amplitude of the peak differed among cows. This indicates a possible interaction between cow and feed in the pattern of passage, and underlines that passage kinetics are a combination of both the physical and physiological status of the cow and intrinsic feed characteristics. The large peak in starch flow for expanded maize, and especially for untreated maize, occurred after the evening feeding of forage (7.5 h after feeding concentrate), indicating a passage of starch to the duodenum, possibly due to additional intake of forage, of either undigested starch in the rumen or possibly of starch that had accumulated in the abomasum. If the peak in starch flow for untreated and expanded maize after the evening feeding of forage was due to flow of starch accumulated in the abomasum, and not flow from the rumen, starch flow out of the rumen after the evening feeding was overestimated when rumen outflow was based on duodenal flow of starch. Starch flow out of the rumen prior to the evening feeding would then be underestimated, if the observed starch at duodenum after the evening feeding already previously had left the rumen. When ruminal starch pools subsequently are corrected for outflow of rumen escape starch, based on duodenal flow of starch, starch pool 7.5 h after feeding would be overestimated, whereas starch pools prior to the evening feeding would be underestimated, resulting in an underestimation of the b-value, as seen for untreated and expanded maize and discussed previously. At each rumen evacuation time, a fractional rate of passage of undegraded starch from the rumen was calculated as duodenal flow of undegraded starch divided by the corresponding ruminal starch pool assuming first order kinetics (Fig. 3). Fractional outflow rate calculated in this way was apparently not constant for the different evacuation times, and starch passage clearly does not follow simple first order kinetics. Using a fixed fractional rate of passage for calculation of rumen degradation of starch is therefore not supported, since newly ingested starch may be retained cranial to the duodenum.

5. Conclusions

Kinetics of degradation, and especially of rumen outflow of starch, is complex and difficult to describe quantitatively. When compared to fractional rates of degradation

obtained from a series of rumen evacuations, the *in situ* method seems to be inadequate for determination of fractional rate of degradation of starch but, if proper precautions are taken, the method seems reasonable for prediction of rumen starch digestibility. The rumen evacuation technique, combined with duodenal flow of starch, has the potential to predict fractional degradation and passage rates, but suffers from being laborious and dependant upon a biologically sound model. Heat treatment had only a minor effect on starch digestion for barley, whereas rumen digestion of starch from maize increased, primarily due to an increased rate of degradation.

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CHAPTER 4

Effect of Feed Processing on Grass Intake, Rumen Pool Sizes, Ruminal Kinetics and the Performance of Grazing Lactating Dairy Cows

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Abstract

Five multiparous lactating Holstein-Friesian dairy cows fitted with a large rumen cannula were allowed to graze ryegrass (*Lolium perenne*) swards. Next to a control treatment of grazing only, pelleted barley (PB), pelleted maize (PM), toasted and subsequently pelleted barley (TPB), and toasted and subsequently pelleted maize (TPM) were fed as a supplement in two equal portions of 3 kg each in the milking parlour during the morning and evening milking. The grass intake, rumen pool sizes, ruminal kinetics and the performance of the grazed and supplemented dairy cows were studied. Before and after 3 hours of grazing the rumen content was evacuated, weighed, sampled and returned to the animals. Then the cows were kept inside the barn and starved for 6 hours, after which rumen evacuations were repeated. Feeding of heat treated grains numerically, but not significantly reduced dry matter intake (DMI) of grass in the first grazing bout. Compared to unsupplemented animals the apparent ruminal clearance of nitrogen was significantly ($P < 0.001$) reduced. The estimated clearance rates of starch significantly differ between grain types, but the effect of toasting more pronounced on barley. Supplementation with processed grains significantly ($P < 0.001$) decreased milk fat percentage and milk urea content. Milk production and protein percentage increased for PB, TPB, PM and TPM with 2.7, 2.4, 2.3 and 1.8 kg/d and 0.27, 0.16, 0.21 and 0.21 percentage units respectively, compared to the performance of the animals that had not received supplementation. It is concluded that supplementing grass based diets with high-energy low protein feeds, such as grains substantially improves the N utilisation and reduces the urea output in milk.

Keywords: Dairy; Rumen; Grazing; Supplementation; Barley; Corn

1. Introduction

In high-producing Holstein Friesian dairy cows, even an optimal rumen fermentation results in insufficient microbial protein to maintain milk productions of over 25 kg/cow/day (Beever and Siddons, 1986). Crude protein content of highly fertilized fresh

grass is frequently high and this protein is easily degradable and to a large extent fermented in the reticulorumen (Van Vuuren *et al.*, 1986; 1995). As a result recovery of grass N in milk is low, and N losses in urine are high (Van Vuuren and Meijs, 1987; Korevaar, 1992; Tamminga, 1992; Hof *et al.*, 1994). Hence, dairy cow nutrition based on grazed pasture, gives little scope to change milk production and composition in order to achieve maximum expression of the genetic potential of modern dairy cows. Consequently, when dairy cow nutrition is based on fresh grass, supplementation is needed. Partial replacement of grass by low protein, high carbohydrate concentrates maybe an adequate method to improve protein utilization by grazing cows and to reduce N excretion (Van Vuuren *et al.*, 1987). For an optimal synchronization of energy supply and microbial growth, supplements should have a rate of carbohydrate fermentation close to that of fresh grass crude protein (Van Vuuren *et al.*, 1993). Cereal grains meet the requirement of a low protein and high carbohydrate content, but their rate of degradation may not always match with that of fresh grass. A variety of feed processing methods can be applied to alter the degradation characteristics of cereal grains and make them more effective as a supplement to fresh grass.

Under grazing conditions, periods of herbage eating and fasting alternate. For lactating dairy cows, two major grazing bouts have been observed, one in the morning, the other one in the afternoon (Rook *et al.*, 1994; Gibb *et al.*, 1997). The experiments described in this paper focused on the morning grazing. This space of time is characterized by a grazing bout between 1 and 3 hours. During this period intake rate is high and intake varies between 1 and 5 kg of dry matter during the first hour (Chilibroste, 1999). In some experiments it was shown that feeding easily fermentable carbohydrates resulted in a reduced roughage intake (Meijs, 1986; Thomas *et al.*, 1986; Phipps *et al.*, 1987) but not in others (Castle *et al.*, 1981; Sloan *et al.*, 1987; De Visser *et al.*, 1990). The present study was designed to compare the effect of two different treatments (pelleting and pressure toasting followed by pelleting) of two cereal grains differing in their starch content and starch structure (maize and barley) on the dry matter intake (DMI), rumen fill and ruminal kinetics of nutrients in dairy cows. A second objective of this experiment was to investigate the effect of processed grains on the performance of grazing high yielding Holstein-Friesian dairy cows.

2. Materials and methods

2.1. *Animals and management*

The experiment was carried at the experimental farm 'De Ossekampen' of Wageningen University, The Netherlands. Five multiparous lactating Holstein-Friesian dairy cows fitted with a large rumen cannula (10 cm id., Bar-Diamond Inc., Parma, Idaho, USA) were used. Two cows were in their 7th and the others were in 2nd, 4th and 6th lactation, respectively. At the beginning of the experiment the cows produced 28.6 ± 4.6 kg/day milk and averaged 173 days post partum. The animals were milked twice daily at 6:30 h and 17:00 h.

2.2. *Experimental design*

The experiment was based on a 5 x 5 Latin square design with five cows, five treatments and five periods. Each experimental period consisted of 14 days. Days 1-9 were used for adaptation and days 10-14 for sample collection.

2.3. *Treatments and feed processing*

The five treatments were control (grass with no supplement, NS), grass supplemented with pelleted barley (PB), grass supplemented with pelleted maize (PM), grass supplemented with toasted and subsequently pelleted barley (TPB), grass supplemented with toasted and subsequently pelleted maize (TPM).

Grain processing was carried out at the Wageningen Feed Processing Centre (WFPC). A laboratory scale pressurised toaster was used for pressure toasting the grains for 1.5 minutes at 135 °C.

After toasting, the grains were dried in a forced air oven for 16 h at 35 °C, and subsequently pelleted. Pelleting (80 °C, 10 s) was carried out with a 5 x 65 mm (bore x hole) die, using a V2-30 pelleting press (Robinson milling systems B.V., Boxtel, The Netherlands).

2.4. Sward management, measurements and grass analysis

An experimental pasture divided in five experimental plots (5 x 880 m²) of predominantly perennial ryegrass (*Lolium perenne*) was available during the experiments. The grass in the plots was mowed with a cutter bar every second week (from 25 May to 21 July). Before the 3rd period manure was applied to the experimental pasture as fertilizer (approximately 7 m³ manure per ha). On day 10 and day 14 of each collection period 5 measuring points were selected in the whole experimental pasture (138 m x 32 m). At each point the sward height was measured with a plate meter (weight 9.47 g, diameter 0.1 m) and the grass mass was cut (50 x 25 cm square) at 2.5 cm with a garden shears at 13:00 h.

After being weighed fresh, the samples were oven dried (80 °C, 48 hours) and weighed again, and the dry matter content of the grass was calculated. On each experimental day the sward height was measured with the plate meter in each experimental plot (27.6 m x 32 m) before grazing (31 measurements/day) and for the whole experimental pasture before (76 measurements) and after grazing (76 measurements). Grass samples from the plots were taken each experimental day at around 10:00 h (when the grass was not dewy) and analysed for dry matter (DM), ash, nitrogen (N), acid detergent lignin (ADL) and neutral detergent fibre (NDF).

2.5. Concentrate feeding and rumen evacuations

Next to a control treatment of grazing only, the four forms of processed grains were fed as a supplement in two equal portions of 3 kg each in the milking parlour during the morning and evening milking. In the 10 days long adaptation period the cows were allowed to graze freely with the herd in a pasture of perennial ryegrass. On days 11 and 13 (also days 12 and 14), rumen evacuations were conducted after milking for 3 (the other days 2) cows, in the same sequence with a time interval of 30 minutes. After rumen evacuation, at 8:00 h, cows were allowed to graze individually, tethered within a circular plot of a fixed area with a radius of six meters. This method was used earlier by different researchers (Forbes, 1988; Dougherty *et al.*, 1992) and the procedure was further

developed by Chilibröste (1997). After 3 hours of grazing each cow was removed from the plot and brought to the barn and rumen evacuations were repeated at 11.00 h. Then the animals were kept inside the barn and starved until 17:00 h, at which time rumen evacuations were repeated in order to determine the clearance rate of the rumen contents.

After the last rumen evacuations the cows were allowed to graze freely in experimental pasture of perennial ryegrass until the next morning. During the starvation period the animals had free access to water and mineral blocks (KNZ Liksteen). Manual emptying of the rumen contents was performed according to the procedure described by Borsting and Weisbjerg (1989), but the mat fraction and the rumen liquid was not separated. Solid rumen contents were removed through the rumen cannulae by hand into plastic containers. Liquid not removable by hand was collected with a plastic beaker, sampled and returned to the rumen immediately. The evacuated solid material was weighed and thoroughly mixed by hand and then samples (400 g) were taken and kept at -20 °C until analysis.

2.6. Chemical analysis

Processed cereal grains, grass and rumen content samples were analysed for dry matter (DM), starch (except the grass samples), nitrogen (N), neutral detergent fibre (NDF) and acid detergent lignin (ADL) and ash. DM was determined by drying at 103 °C to a constant weight according to ISO-standard 6496, Ash by combustion at 550 °C following ISO-standard 5984. Nitrogen was determined with the Kjeldahl method with CuSO_4 as the catalyst, according to ISO standard 5983. ADL was analysed by the method of Goering and Van Soest (1970). NDF was determined by the VVR/protocol NSP analyses. This method is similar to the method of Van Soest *et al.* (1991), but includes an incubation step with 1 ml heat stable amylase (Sigma 6814, 1350 U/ml) and 0.25 ml protease (Alcalase, 2.4 L NOVO, 2.4 AU/g) in 60 ml phosphate buffer (pH 7). This incubation is carried out for 15 min at 40 °C after boiling and removal of the ND. Starch was analyzed according to the NIKO method (Brunt, 1992). Total starch was analysed by extracting soluble sugars with a 40% ethanol solution, followed by autoclaving for 3 hour at 130 °C followed by enzymatic breakdown for 1 hour (at 60 °C, pH 5) to glucose, using an enzyme cocktail containing amyloglucosidase, alpha-amylase and pullulanase. After

cooling in ice-water 2 ml Carrez 1 (potassium- hexacyanoferrate (II) 0.25 M: 10.6 g: 21.95 g. $K_4Fe(Cn)_6 \cdot 3H_2O$ p.a. per 100 ml.) and 2 ml Carrez 2 (Zinkacetate solution 1 M in 0.5 M acetic acid: 21.95g $Zn(C_2H_3O_2)_2 \cdot 2H_2O$ p.a. and 3.0 g acetic acid per 100 ml) were added. Glucose was converted to glucose-6-phosphate (G6P) by the enzyme hexokinase and ATP, then G6P was oxidized by $NADP^+$ in the presence of G6P-DH. The amount of NADPH formed was proportional to the concentration of G6P in the samples and measured spectrophotometrically at 340 nm.

2.7. Performance measurements

During the experimental periods cows were milked twice daily. Milk yield was measured and recorded using milk meters. Milk samples, taken during two consecutive morning and evening milkings in each experimental period, were analysed for fat, protein, lactose and urea using an automated infrared milk analyzer (Melkcontrole Station West-Veluwe, Ede, The Netherlands). The milk samples were frozen until analysis.

2.8. Calculations

Grass dry matter intake (DMI_R) was estimated according to Chilbroste *et al.* (1997) from changes in the DM rumen pools before and after the grazing period and clearance of DM during the grazing sessions. The calculations for DMI were based on NDF analysis of rumen content, because NDF in the feed has been suggested as the best predictor of rumen fill (Van Soest *et al.*, 1991; Mertens, 1994). NDF clearance rate was estimated for the starvation period assuming first order kinetics (Robinson *et al.*, 1986).

Dry matter intake was calculated as follows:

$$DMI_R = (RP_{AG} - RP_{BG}) + RP_{BG} (1 - \exp^{-k_{cl} \cdot GT}) + CNGI$$

$$CNGI = (RP_{AG} - RP_{BG}) + RP_{BG} (1 - \exp^{-k_{cl} \cdot GT}) * (1 - \exp^{-k_{cl} \cdot 2.5})$$

where RP_{AG} is the size of the estimated DM rumen pool after grazing (kg), RP_{BG} is the size of the estimated DM rumen pool before grazing, corrected by clearance of DM

rumen content in the time elapsed between emptying the rumen and the start of grazing, k_{cl} is DM clearance rate (h^{-1}) during starvation, GT is grazing time (h) and CNGI is clearance of the newly ingested grass (kg). To calculate CNGI the following assumptions were made: a uniform pattern of ingestion through the grazing bout and a mean residence time of the particles ingested (2.5 h).

2.9. Grass characterization

For the available grass characterisation before and after grazing a regression line calculated from the relationship between sward mass and sward height, and based on the measuring points of day 10 and day 14 was used.

The regression line calculated and used was the following:

$$y = 14.43 (4.02) + 1.089 (0.26) x$$

where,

y= grass sample weight ($g/1250cm^2$),

x= sward height (mm), standard deviation in brackets.

2.10. Statistical analysis

Experimental data were analysed using the PROC GLM procedure of SAS (1995). Cow, period and treatment (i.e. supplementation, grain, heat and the interaction between heat and grain) were the class variables in the model. Results are reported as least squares means and standard error of least square means. P values were according to the SS2 procedure of PROC GLM in SAS 6.12 (SAS, 1995). Treatment effect within feed type was judged using PDIF in SAS 6.12 (SAS, 1995). Significance was declared when $P < 0.05$.

3. Results and discussion

3.1. Chemical composition of supplements

The chemical composition of the processed grains is shown in Table 1. The values show good agreement with tabular values (CVB, 1994). As expected, maize contained the highest amount of starch and barley had the highest crude protein and neutral detergent fibre content. Barley is usually higher in fibre than other cereal grains (maize, sorghum, wheat). Variation in fibre content may indicate separation of the grain kernel into its components, particularly toasted grains containing greater quantities of pericarp. Also, artefact lignin may be formed when grains are processed at temperatures above 65 °C. Heat treatments increase lignin-like components that can analytically be determined as insoluble fibre (Van Soest and Mason, 1991). Total starch content of barley and maize increased after toasting. These results agree with those obtained by Malcom and Kiesling (1992), but are in contrast with what Goelema (1999) found in legume seeds, where total starch content in peas and faba beans decreased after toasting. Maybe the effect of steam treatment on the protein matrix embedding the starch (Holm *et al.*, 1995) have rendered the starch accessible for hydrolysing enzymes during analysis.

Table 1 Chemical composition of processed grains¹

	Barley		Maize	
	PB	TPB	PM	TPM
Dry Matter (g/kg)	972.2	972.9	970.9	971.8
In dry matter (g/kg DM)				
Organic matter	977.7	978.9	986.6	986.1
Crude protein	114.9	113.7	92.0	92.9
Starch	571.9	596.7	682.9	710.1
NDF	139.0	134.0	80.1	84.0
ADL	7.8	11.3	4.3	8.5

¹ PB: pelleted barley, TPB: toasted and pelleted barley, PM: pelleted maize, TPM: toasted and pelleted maize

3.2. Weather conditions, sward measurements, characterization

Weather conditions during the experiment are presented in Table 2. The information was collected from a meteorological station (Haarweg Station Wageningen, Meteorology and Air Quality Group) located approximately 400 m from the experimental pasture. In the first three hours of the experimental days (grazing period) the weather conditions were stable. The air conditions (temperature, relative humidity, windspeed) were higher than in former years and the low rainfall which is also quite unusual in this season may have effected the grazing behaviour of the experimental animals.

Table 2 Weather conditions during the experiments

Per.	Date (Year: 1997)	Air	Rel.	Wind	Rainfall		Grazing time (8:00 h to 11:00 h)		
		temp. °C	humidity %	ms ⁻¹	mm	min.	Air temp. °C	Rain, mm	Rain, min.
1	02 to 06 June	18.6	46.6	3.6	0	0	20.1	0	0
2	16 to 20 June	14.2	61.4	2.9	0.4	36	20.8	0	0
3	30 June to 04 July	15.0	77.8	3.5	0.5	36	20.8	0	0
4	14 to 18 July	17.3	81.2	2.4	1.8	96	20.8	0.4	11
5	28 July to 01 August	17.3	75.0	3.1	1.3	84	20.8	0.1	4

(Data source: Haarweg Station Wageningen, Meteorology and Air Quality Group)

The sward characteristics before and after grazing are given in Table 3. For the available grass characterisation we used the calibration line on day 10 and day 14 to calibrate the relationship between sward mass and sward height. A comparison of the slopes of day 10 and day 14 revealed no differences from which it was concluded that between day 14 and day 10 the condition of the offered grass remained the same in the five days period. The DM herbage mass and the sward height (mm) before grazing corresponded well with the values of Chilibroste *et al.* (1997). Growing conditions like radiation, rainfall and temperature affected the chemical composition and nutrient content of the grass, as can be seen in Table 4. Results showed a high but somewhat variable N content throughout the grazing season. Pasture N content was highest in Period 3, which may have resulted from N fertilisation with manure application immediately before Period 3 started. An increase in crude protein content after Period 1 may have resulted from an increase of the

proportion of leaves, then the dry July resulted in an increase in the proportion of stem which has lower crude protein content than leaves. NDF increased during the warm summer months, but no significant differences were observed between the periods.

Table 3 Mean sward height and sward mass before and after grazing in each experimental period

Variable	Period					<i>average</i>
	1	2	3	4	5	
	02 to 06 June	16 to 20 June	30 June to 04 July	14 to 18 July	28 July to 01 August	
Sward height (mm)						
Before grazing ¹	193.5	130.8	103.7	153.4	126.3	141.5
After grazing ²	111.9	83.5	70.5	98.3	94.1	93.1
Sward mass (kg DM per ha)						
Before grazing ¹	2841	2294	2058	2490	2255	2387
After grazing ²	2446	1882	1769	2111	1974	2036

¹ before grazing: measurements of the first experimental day

² after grazing: measurement of the last (after the fifth) experimental day

The residual fraction (OM-NDF-CP) was high in Period 1 compared with the other periods, which is assumed to be the result of a high content of soluble sugars. In fresh perennial ryegrass 70% of the water soluble carbohydrates are normally present as fructosans (McGrath, 1988), the rest is other mono-, di-, oligo- and polysaccharides. Free sugars usually occur at low concentrations. With increasing maturity fructosans increase until a peak, and after this peak they are either translocated or synthesized into structural carbohydrates (Blaster, 1964).

The OM intake of fresh grass is limited. For cows from 550-700 kg liveweight, maximum daily grass intake varies between 15 and 20 kg OM (Meijs, 1982). In this experiment the calculated OM intake based on pasture measurements was approximately 13.1 kg for NS animals, this means that the grass in the pasture was sufficient for the cows to reach maximum OM intake.

Table 4 Chemical composition of the grass from different experimental periods

	Period				
	1	2	3	4	5
Sampling time	02 to 06 June	16 to 20 June	30 June to 04 July	14 to 8 July	28 to 31 July
Dry matter (g/kg)	238.2	228.2	170.4	163.9	217.8
In dry matter (g/kg DM)					
Organic matter	912.2	908.2	894.3	899.8	902.4
Nitrogen	25.04	33.71	39.66	37.89	32.66
Crude protein	156.5	210.7	247.9	236.8	204.1
NDF	360.9	382.9	371.0	382.0	378.6
ADL	21.7	21.5	18.8	20.2	22.6
Residue ¹	394.8	314.6	275.4	281.0	319.7
Ash	87.8	91.8	105.7	100.2	97.6
ADL/NDF (g/kg)	60	56	50	53	60

¹Residue=1000-(Ash+CP+NDF)

3.3. DM intake, rumen pool kinetics and rumen pool sizes

DM intakes and evacuated rumen contents of the experimental animals are shown in Table 5. The total rumen DM content before grazing was significantly higher in the supplemented animals, than in the control animals, because of the supplementation. The weight of total rumen content was not significantly ($P>0.05$) affected by supplementation after grazing and after starvation. Cows consuming only grass tended to have greater weights and volumes of rumen contents after grazing, but lower rumen DM content than cows offered a processed cereal grain supplement.

The estimations of DMI_R derived from rumen pool sizes before and after grazing shows, that there are neither significant differences in DM intake between the unsupplemented control and the supplemented animals nor between the treatments. Numerically as could be expected, the intake of grazing only animals was higher, than that of the supplemented cows. The variation of estimated values is high, which would affect the reliability of

calculations of DMI from rumen pool sizes. Under grazing conditions daily feed intake would probably vary substantially more between days than under the more controlled feeding conditions. Higher level of supplementation, more animals and longer observation period than five days may be required to average out the effects of daily fluctuations in grass intake.

Table 5 Dry matter intake (DMI_R, kg) during 3 h grazing, total and dry matter (DM) rumen pools of cows grazing grass pasture¹, and supplemented with processed cereal grains²

Item	NS	Barley grain		Maize grain		SEM	P ³		
		PB	TPB	PM	TPM		G	H	GxH
DMI _R	3.41	2.79	3.17	2.00	2.54	0.61	0.2	0.3	0.9
Rumen pools size, before grazing									
Total, kg	83.8	84.0	83.4	81.0	81.6	2.81	0.5	0.9	0.7
DM, kg	10.1 ^S	12.5	11.9	11.6	11.6	0.43	0.2	0.5	0.5
Rumen pools size, after grazing									
Total, kg	89.5	86.7	88.1	83.3	85.4	4.22	0.5	0.7	0.9
DM, kg	10.2	10.5	11.4	10.5	10.5	0.50	0.3	0.5	0.1
Rumen pools size, after starvation									
Total, kg	60.5	58.3	61.8	60.5	63.4	2.62	0.5	0.2	0.9
DM, kg	6.66	5.95	6.31	6.02	6.42	0.35	0.8	0.3	0.9

¹ NS: no supplement addition.

² PB: pelleted barley, TPB: toasted and pelleted barley,
PM: pelleted maize, TPM: toasted and pelleted maize.

³ G: effect of grain type, H: effect of type of heat treatment, GxH: effect of grain type and heat interaction.

^S figure with different superscript is significantly (P<0.05) different.

According to Hodgson (1985) the dry matter intake under grazing is the product of grazing time, rate of biting and weight of pasture per bite. The allowed grazing time in this experiment was 3 hours for the animals, and the grazing sessions were followed by a long period of starvation. In the first hour of grazing we observed an active eating,

probably because of the effect of feeling hunger after starvation during milking and rumen evacuations. But in our observation the effective grazing bout was only about 1.5 hour. We normally observed that the cows laid down and started rumination, instead of actively biting. A reduction in grazing time has been reported when cows have been exposed to short swards, and this sward condition is not preferred by cattle (Le Du *et al.*, 1979; Ungar *et al.*, 1991; Rook *et al.*, 1994). But based on measurements of sward height this was not the reason to stop grazing.

Temporal pattern of grazing may be altered due to poor weather conditions, mainly high temperatures (relatively hot summer days) and some rainy mornings (Faverdin *et al.*, 1995; Chilibroste *et al.*, 1999). In this experiment maybe because of the warm air conditions in grazing hours and because of the continuously high daily temperature, active grazing was predominantly observed in the early hours before milking in the morning (from 5:00 h to 6:00 h). The resulting high rumen fill could have limited grazing time and also the intake of the experimental animals in the grazing session after milking.

Rumen clearance by degradation and by outflow is considered to follow first order kinetics (Robinson *et al.*, 1986) which means that per unit of time a rather constant fraction of what is present is cleared from the rumen of dairy cows by degradation and passage to the lower gut. Therefore we estimated clearance rate (k_{cl}) for NDF, ADL, N and starch from the changes in rumen pool sizes after grazing, during the six hours of starvation. The results are presented in Table 6.

The estimated clearance rates of starch show significant ($P < 0.001$) differences between grain types. The combination of pressure toasting and pelleting resulted in a 15% units per hour slower clearance rate for barley and a numerical but not significantly elevated clearance rate of maize as compared to pelleting alone. The N clearances were also influenced by grain type, but there was no statistically significant difference between the heat treatments.

The supplementation caused a significant reduction in the apparent clearance rate of N, most likely resulting from more N being captured by the rumen microbes. In 3 of the 4 supplemented diets the clearance rate of lignin was significantly reduced ($P < 0.05$) as compared to no supplementation.

Table 6 Rumen pool kinetics of starch, nitrogen (N), neutral detergent fibre (NDF) and acid detergent lignin (ADL) of cows grazing grass pastures¹, and supplemented with processed cereal grains², values calculated from rumen evacuation data (rates in % h⁻¹).

Item	NS	Barley grain		Maize grain		SEM	P ³		
		PB	TPB	PM	TPM		G	H	GxH
Starch rates									
k _{cl}	-	67.4 ^a	52.4 ^b	32.1 ^c	35.9 ^c	0.51	<0.001	0.3	0.07
N rates									
k _{cl}	7.77 ^S	3.94	4.17	4.49	4.18	0.40	0.5	0.9	0.6
NDF rates									
k _{cl}	7.38	6.27	6.73	6.26	6.22	0.67	0.2	0.6	0.2
ADL rates									
k _{cl}	5.39 ^S	3.81	3.34	4.60	3.42	0.48	0.4	0.1	0.4

¹ NS: no supplement addition.

² PB: pelleted barley, TPB: toasted and pelleted barley,

PM: pelleted maize, TPM: toasted and pelleted maize.

³ G: effect of grain type, H: effect of type of heat treatment, GxH: effect of grain type and heat interaction.

^{a,b,c} figures with different superscript are significantly different

^S figure with superscript is significantly (P<0.05) different from others.

This could indicate a slowing down of the degradation of organic matter, resulting in an increased retention time in the rumen, but this was not clearly reflected in a reduced clearance rate of NDF. Table 7 shows the results of the rumen pools analysis. The interaction between grain type and heat treatment was not significant for any of the measured variables. Starch pools before grazing (BG) were significantly higher because of the supplementation of starchy grains. After grazing (AG) the changes, that is the disappearance of starch for PB, TPB, TM and TPM were 83.2, 75.8, 59.7, and 64.6 %, respectively. After the starvation period 97.4 and 96.4 % of starch in PB and TPB had disappeared, while after treatments PM and TPM 85.8 and 92.5% had disappeared. The highest rumen starch pools with maize supplementation (PM and TPM) can be explained by the lower k_{cl} of maize starch as compared to starch originating from barley, which agrees with other observations by Waldo (1973), Visser (1993) and reviewed by Nocek and Tamminga (1991) comparing the digestion of starches in the gastrointestinal tract of

Table 7 Rumen pool sizes of organic matter (OM), nitrogen (N), starch, neutral detergent fibre (NDF), acid detergent lignin (ADL) and ash of cows grazing grass pasture¹, and supplemented with processed cereal grains², before and after grazing and after the starvation period³

Item	NS	Barley		Maize		SEM	P ⁴		
		PB	TPB	PM	TPM		G	H	G x H
OM pool, kg									
BG	8.95 ^S	11.41	10.85	10.60	10.62	0.40	0.2	0.5	0.4
AG	9.11	9.54	10.36	9.52	9.36	0.47	0.3	0.5	0.3
AS	5.87	5.26	5.56	5.31	5.70	0.32	0.7	0.3	0.9
N pool, kg									
BG	0.37	0.37	0.38	0.37	0.36	0.01	0.6	0.3	0.5
AG	0.39	0.34	0.39	0.37	0.35	0.01	0.9	0.3	0.3
AS	0.25	0.22	0.25	0.25	0.25	0.01	0.3	0.3	0.2
starch pool, kg									
BG	0.27 ^S	1.13 ^a	1.20 ^{ab}	1.34 ^{ab}	1.47 ^b	0.09	0.01	0.3	0.7
AG	0.27 ^M	0.19 ^a	0.29 ^a	0.54 ^b	0.52 ^b	0.06	0.001	0.5	0.3
AS	0.07 ^M	0.03 ^a	0.04 ^a	0.19 ^b	0.11 ^b	0.04	0.007	0.7	0.2
NDF pool, kg									
BG	4.54 ^{PB}	5.76 ^a	5.13 ^{ab}	4.74 ^b	4.78 ^b	0.23	0.07	0.2	0.1
AG	4.51 ^B	5.19 ^{ab}	5.42 ^a	4.77 ^{ab}	4.58 ^b	0.22	0.01	0.9	0.3
AS	3.04 ^{TPB}	3.58	3.60	3.27	3.44	0.19	0.2	0.6	0.7
ADL pool, kg									
BG	0.49 ^{PM}	0.49 ^{ac}	0.52 ^a	0.43 ^b	0.47 ^{bc}	0.01	0.03	0.08	0.8
AG	0.50 ^M	0.47 ^{ab}	0.53 ^a	0.44 ^b	0.45 ^b	0.02	0.02	0.2	0.2
AS	0.38	0.35 ^a	0.40 ^b	0.33 ^a	0.36 ^{ab}	0.02	0.10	0.02	0.6
Ash pool, kg									
BG	1.11	1.07	1.03	1.00	0.98	0.04	0.2	0.5	0.8
AG	1.15	0.99	1.08	0.99	0.97	0.04	0.2	0.4	0.2
AS	0.79	0.70	0.76	0.71	0.73	0.03	0.8	0.2	0.5

¹ NS: no supplement addition.

² PB: pelleted barley, TPB: toasted and pelleted barley,
PM: pelleted maize, TPM: toasted and pelleted maize.

³ BG: before grazing (8 h), AG: after grazing (11 h), AS: after starvation (17 h).

⁴ G: effect of grain type, H: effect of type of heat treatment, GxH: effect of grain type and heat interaction.

^{a,b,c} figures with different superscript are significantly different.

^{S,B,PB,TPB,M,PM} figure with superscript is significantly different (P<0.05), from all other treatments (^S), from all maize supplementation (^M), from all barley supplementation (^B), from PM, PB, or TPB supplementation (^{PM,PB,TPB}).

of dairy cows. Pressure toasting decreased the starch degradation of barley starch and elevated the degradation of maize starch. A small starch pool was also observed in the control treatment, only grazing animals.

Because the starch content of grasses in a vegetative status is low, being usually less than 10 g/kg DM (Smith, 1973), this was presumably starch of microbial origin. This starch of microbial origin was taken into account when we calculated the starch pool of supplemented animals. The NDF pool measured in supplemented animals was below the threshold level of 1.1-1.2 % of body weight described by Mertens (1994) as the average NDF holding capacity of dairy cows on a daily basis. Therefore the NDF pool was not considered the main signal received by the cows to stop the grazing session, rather the environmental factors and rumen fill. The numerically decreased ruminal turnover of NDF on supplemented animals compared with only grazing cows is in agreement with results of Robinson *et al.* (1987), who observed a linear decrease in turnover of NDF when the starch content in the diet increased.

3.4. Performance measurements

Feeding heat treated grains increased the average daily milk yield for PB, TPB, PM and TPM with 2.7, 2.4, 2.3 and 1.8 kg/d, respectively, compared to the performance of the animals that had not received supplementation (Table 8). This may have resulted from an increased energy supply, because supplementation did not result in a significantly decreased grass intake. The increase in milk yield with supplementation was usually accompanied by an increase in the milk protein content. Delaby *et al.* (2001) showed that the increase in milk protein content averaged 0.2 g/kg per kg of concentrate DM and was linear up to 6 kg of concentrate. In this experiment milk protein output was increased by on average 114 g/day, which may be the result of an improved protein supply, probably because of the synthesis of more microbial protein. Rooke *et al.* (1987) showed an increase in microbial protein synthesis in the rumen, when the rate of degradation of the protein and the carbohydrates was in balance.

Under these more balanced conditions the supply of aminogenic nutrients to the mammary gland may have been improved, and have resulted in a higher protein yield.

The milk fat concentration for PB, TPB, PM and TPM decreased with 0.44, 0.62, 0.71 and 0.75 percentage units respectively, whereas milk protein content for PB, TPB, PM and TPM, increased with 0.27, 0.16, 0.21 and 0.21 percentage units, respectively. This was probably because of the supposedly increased supply of glycogenic nutrients with intestinal digestible starch, propionic acid and aminogenic nutrients with (microbial) protein. It seems that pressure toasting had an appreciable effect on decreasing milk fat yield but this could statistically not be proven.

Table 8 Milk production and milk composition of cows grazing grass pasture¹ and supplemented with processed cereal grains²

Item	NS	Barley grain		Maize grain		SEM	P ³		
		PB	TPB	PM	TPM		G	H	GxH
Milk (kg/d)	22.65 ^T	25.37 ^a	25.10 ^b	24.95 ^b	24.44 ^{ab}	0.50	0.4	0.2	0.03
Protein (%)	3.41 [*]	3.68	3.57	3.62	3.62	0.07	0.9	0.5	0.5
Fat (%)	3.99 ^S	3.55	3.37	3.28	3.24	0.12	0.2	0.5	0.7
Lactose (%)	4.33	4.35	4.45	4.32	4.44	0.07	0.8	0.2	0.9
MUN ⁴	18.64 ^S	12.57 ^{ab}	13.57 ^a	10.83 ^b	10.77 ^b	0.80	0.03	0.6	0.5

¹ NS: no supplement addition.

² PB: pelleted barley, TPB: toasted and pelleted barley,
PM: pelleted maize, TPM: toasted and pelleted maize.

³ G: effect of grain type, H: effect of type of heat treatment, GxH: effect of grain type and heat interaction.

⁴ MUN: milk urea nitrogen, mg per 100 ml milk.

^{a,b} figures with different superscript are significantly different.

^S figure with superscript is significantly (P<0.001) different from others.

^T figure with superscript is significantly (P<0.05) different from others.

^{*} figure with superscript is tend to significantly different from others (0.05<P<0.1).

The decreased fat concentration of on average 85 g/day can partly be explained by a dilution effect, which is related to the limited amount of available lipogenic energy for milk synthesis. In the study of Delaby *et al.* (2001), the reduction in milk fat content was consistent and averaged -0.6 g/kg per kg DM of concentrate between 0 and 5.4 kg of concentrate DM. This also might be a consequence of the ruminal fermentation pattern because the acetic to propionic ratio in the rumen might have decreased in supplemented cows (Delagarde *et al.*, 1999). The lactose content of milk was not influenced by

nutritional factors. Consequently this observed production response with feeding heat-treated grains is probably related to optimization of the fermentation process in the rumen, as well as to the increased supply of intestinal digestible starch and protein.

Milk urea nitrogen (MUN) concentration decreased when the animals received heat treated grains as a supplement, if we compare it to the grazing only status. Supplementation of maize resulted in a stronger decrease than barley did, because maize lower in crude protein and have lower rumen degradability. But we found no differences between the heat treatments. Generally milk urea concentration in milk is a valuable tool to monitor the rumen degraded protein balance in the ration and the N losses (Hof *et al.*, 1997; Schepers and Meijer, 1998). Milk urea nitrogen concentrations of cows grazing ryegrass pastures is around 18 mg MUN per 100 ml milk which can vary due the maturity and protein content of the grass and the selection by the grazing cows (Trevaskis and Fulkerson, 1999; Merwe *et al.*, 2001). Visser *et al.* (1997) showed that the concentration of ammonia in rumen fluid, ammonia release in portal-drained viscera, urea synthesis in the liver, urea release from the liver and milk urea highly correlate. Therefore in this experiments the higher MUN values found in unsupplemented than in supplemented animals indicates that the protein and carbohydrates were not properly combined which resulted in an excess of ruminal ammonia.

4. Conclusions

Supplementation of grazing dairy cows with 3 kg pelleted and pressure toasted grains probably not influence the DMI of grass in the first grazing bout in the morning after milking, but it did affect the ruminal clearance of nitrogen. The estimated clearance rates of starch significantly differ between grain types, but the effect of toasting more pronounced on barley. Pelleting as well as toasting followed by pelleting did affect production responses in dairy cows, by elevating milk protein and decreasing milk fat production, but no significant differences between these two treatments were found in this experiment.

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CHAPTER 5

Effect of Feed Processing on In Situ Parameters of Cereal Starch and the In Situ Degradation of the Available Grass in Grazing Dairy Cows

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To be submitted

Abstract

Three rumen-cannulated, lactating Holstein Friesian cows grazed in a controlled experimental pasture and supplemented daily with a mixture of differently processed concentrates were used to measure effects of different heat treatment of cereal grains (pelleted barley, PB; toasted and pelleted barley, TPB; pelleted maize, PM; toasted and pelleted maize TPM) on *in situ* degradability of protein and starch and to measure *in situ* degradability of fresh perennial ryegrass (*Lolium perenne*). Pelleting and pressure toasting increased the undegradable fraction of dry matter and organic matter and decreased *in situ* protein degradability of the cereal grains. Undegraded intake protein (%UIP) increased after toasting from 36.4 to 52.5% for barley and from 50.3 to 58.2% for maize, respectively. Undegraded intake starch (%UIS) increased from 14.9 to 16.7% after toasting barley and decreased from 36.1 to 31.3% for toasted maize, respectively. After pressure toasting compared to pelleting alone, washable fractions (W) of each cereal grain decreased for both constituents, the fractional rate of degradation (k_d) of protein decreased, while the k_d of starch increased for maize, but decreased for barley. The chemical composition and *in situ* degradation characteristics of grass samples (in summer months, from June 2 to July 31) did not result in significant differences between sampling times but with maturation the rate of degradation of potentially degradable, but insoluble organic matter and crude protein tended to decrease. It is concluded that the crude protein of fresh grass is highly degradable in the rumen therefore supplementation with processed cereals gives more balanced ruminal protein and energy availability, but the differences are more effective between grain types than between the heat treatments applied in this study.

Keywords: Dairy; Rumen; Grazing; Supplementation; Concentrates

1. Introduction

Fresh grass from intensively fertilized swards contains high concentrations of crude protein, of which 70-90% is present as true protein (Tamminga, 1986). It is not only

rapidly degraded in the rumen, but its extent of degradation is high and often in asynchrony with that of energy yielding substrates. Hence, only part is captured by rumen microbes. It is estimated that 50% of the crude protein ingested with such grass may be lost. This not only means a severe loss of protein, but it may also have a detrimental impact on the environment (Tamminga, 1992). Ways to reduce the imbalance between the degradation of protein and energy in the rumen are firstly to reduce the N input. A possible way to reduce N consumption is to replace part of the N-rich herbage by a low-N roughage like maize silage. Other recommendations are to supplement fresh grass with concentrates, low in protein but rich in either rapidly degradable structural or non-structural carbohydrates (Valk and Hobbelink, 1992; Van Vuuren *et al.*, 1993c).

The availability of protein and energy may not only be imbalanced on a daily basis, but also within a day because of differences in rate of degradation. Maize and barley are sources of readily available energy, but their starch degradabilities are different, because large differences exist in the surface of the endosperm and pericarp of the grains (Rooney and Plugfelder, 1986). Starch in barley is more easily degraded in the rumen than that in maize, and as a result, energy made available to the rumen microbes may not be in synchrony with the availability of rumen degradable grass protein ingested during grazing. Heat treatments of grains affect rumen protein degradability by altering the three-dimensional structure and create protease resistant products between protein and carbohydrate through Maillard reactions (Satter, 1986) depending on the processing temperature, the processing time and added moisture content (Stern *et al.*, 1985). The application of heat, moisture, time, pressure and shear causes the disruption of intermolecular H bonds, resulting in gelatinisation (Lund, 1984; Zobel, 1984) which also affects degradability of cereal starch (Hale, 1973; McAllister *et al.*, 1990; Zinn, 1990; Nocek and Tamminga, 1991; Sauvant *et al.*, 1994). After heat processing resulting in a change in the structure of starch, the impact of ruminal microbial attack can alter (Campling, 1991; Treurer, 1986). But it is also possible for gelatinized and structural changed starch to form complexes with protein that reduce digestion of both starch and protein (Thorne *et al.*, 1983).

Goelema (1999) studied the effect of heat treatment on the protein value of legume seeds, like peas and faba beans and found that pressure toasting (136 °C, 3 min) reduced

the *in situ* rumen starch and protein degradability by decreasing the size of washable fraction. This author also reported that pelleting (80 °C, 10 s) increased the rumen *in situ* degradability of protein and starch in legume seeds as a result of an increased washable fraction and rate of degradation, particle size reduction and the more porous and more accessible structure of the pelleted product (Goelma, 1999). It is hypothesized that when the underlying mechanisms are the same for other starchy feedstuffs, the results of toasting and pelleting on *in situ* starch and protein degradability may depend on the starch to protein ratio but not exclusively due to specific characteristics of legume seeds.

Therefore the objective of this experiment was to compare the effects of pressure toasted and subsequently pelleted and pelleted only grains on rumen *in situ* degradability of protein and starch. This could result in a range of supplements differing in rumen degradation rates for energy and protein and therefore tailor-made for grasses differing in quality. The *in situ* ruminal degradation characteristics of 5 qualities of fresh perennial ryegrass (*Lolium perenne*) were also determined.

2. Materials and methods

2.1. Animals

The *in situ* trial was carried at the experimental farm ‘De Ossekampen’ of the Wageningen University, The Netherlands. Three multiparous lactating Holstein-Friesian dairy cows fitted with a ruminal cannula (10 cm id., Bar-Diamond Inc., Parma, Idaho, USA) were used. The cows were in their 7th, 4th and 6th lactation, respectively. At the beginning of the experiment the cows produced 31.0 ± 4.3 kg/day milk. The experimental animals were grazed in a controlled experimental pasture and were supplemented daily with two equal proportions of 3 kg of a mixture of the 4 processed concentrates (Table 1).

2.2. Samples and treatments

Processing were carried out at the Wageningen Feed Processing Centre (WFPC). Barley and maize grain were pelleted or pressure toasted for 15 minutes at 135 °C. After toasting, the grains were dried in a forced air oven for 16 h at 35 °C, and part of the grains were subsequently pelleted for 10 s at 80 °C through a 5 x 65 mm (bore x hole) die.

Perennial ryegrass samples were collected from the experimental pasture (5 x 880 m²) where the experimental animals were kept. The grass in the pasture was mowed with a cutter bar every second week (from 25 May to 21 July, 1997). Before the 3rd period manure was applied to the experimental plots as fertilizer (approximately 7 m³ manure per ha). The ryegrass was grown for 8 days, then on five consecutive days samples were taken at around 10:00 a.m (when the grass had dried from the dew) from different plots of the pasture using the hand-plucking technique. Hand plucked grass samples (approx. 500 g) were collected in plastic bags and stored in a freezer at -20 °C until incubation.

2.3. Rumen incubations

Rumen incubations were carried out according to Dutch standard methods (CVB, 1996) and nylon bags with a pore size of 40 µm (PA 40/30, Nybolt, Switzerland) were used to prepare bags with an inner size of 10 x 19 cm. The processed grain samples were ground through a 3.0 mm sieve (Retsch ZM1 centrifugal mill). The coded nylon bags were filled with the sample weight of 5.0 g for incubation times of 0, 2, 4, 8, 24 and 48 h respectively.

Before incubation grass samples originating from experimental pasture were taken out of the freezer and kept at room temperature for 24 h and then chopped with a sharpened common paper guillotine to ca. 5.0 mm long pieces. The sample weight was from 35.0 to 40.0 g chopped material, equivalent to c. 5 g DM and incubation times were for 0, 2, 4, 8, 24, and 48 h. After filling the bags were tied and stored at -18 °C until rumen incubation. After incubation the nylon bags were immediately placed in cold water and rinsed with tap water to stop fermentation. The bags were subsequently washed in a domestic washing machine for 50 minutes with 70 l of cold water, without centrifugation. After this procedure the bags were freeze dried, air equilibrated and weighed. Residues from the bags were pooled over cows. Rumen incubations of the samples were repeated once, in the same cows.

2.4. Chemical analysis

All the samples were analyzed for DM, inorganic matter (ash), crude protein (6.25 x N), neutral detergent fiber (NDF), and starch (except the grass samples). Grass samples were also analyzed for acid detergent lignin (ADL). Prior to analyses, the samples were ground through a 1 mm sieve. Pooled rumen incubation residues were analyzed for DM, crude protein (CP), NDF and starch (except the grass samples). All chemical analyses were carried out following procedures as described by Tóthi *et al.* (2002).

2.5. Calculation of degradability

Crude protein and starch degradation characteristics of processed grains were classified in three fractions as described by Tamminga *et al.* (1990) and Sauvant *et al.* (1994).

- 1) Washable fraction (W): readily available measured as the fraction disappearing after washing only (0 h incubation).
- 2) Undegradable fraction (U): which will not be degraded, measured as the asymptote of the degradation curve at infinite incubation time.
- 3) Potentially degradable fraction (D): $D = 100 - W - U$.

The fractional rate of degradation of the D fraction (k_d in %/h) was calculated using a first order degradation model, without a lag time, as described by Robinson *et al.* (1986).

The fraction of effectively rumen undegraded intake starch (%UIS) and rumen undegraded intake protein (%UIP) were also calculated based on the following equations (Tamminga *et al.*, 1994). For starch it was assumed that 10% of W escapes rumen fermentation and U is 0 (Tamminga *et al.*, 1994).

$$\%UIS = 0.1 \times W + D \times k_p / (k_d + k_p),$$

$$\%UIP = U + D \times k_p / (k_d + k_p), \text{ where } k_p \text{ is a Dutch standard rumen passage rate of } 6\%/h.$$

For grass the instantly degradable, washable fractions (W) of OM, DM and CP were estimated as fractions disappearing from the bags during washing (zero incubation time). Residues in the bags after different times of incubation were fitted by a first order degradation model (Robinson *et al.*, 1986), including D, k_d , and U fractions (assumed passage rate for grass was 4.5%/h).

2.6. Statistical analysis

Experimental data were analysed using the PROC GLM procedure of SAS (1995) with the following models:

Processed grains: $Y_{ijk} = \mu + R_i + G_j + H_k + (G \times H)_{jk} + e_{ijk}$

where Y_{ijk} is the dependent variable under examination (U and k_p), μ is the overall mean, R_i is the replicate effect ($i = 1,2$), G_j is the grain effect ($j = 1,2$), H_k is the heat processing effect ($k = 1,2$) and $(G \times H)_{jk}$ is the interaction of grain and heat processing, and e_{ijk} is the residual error term.

Grass: $Y_{ij} = \mu + P_i + R_j + e_{ij}$

where Y_{ij} is the dependent variable under estimation (U and k_d), μ is the overall mean, P_i is the period effect ($i = 1-5$), R_j is the replicate effect ($j = 1,2$) and e_{ij} is the residual error term.

Results are reported as least squares means and standard error of least square means. P values were according to the SS2 procedure of PROC GLM in SAS 6.12 (SAS, 1995). Treatment effect within feed type was judged using PDIF in SAS 6.12 (SAS, 1995). Significance was declared when $P < 0.05$.

3. Results and discussion

3.1. Dry matter, organic matter and NDF degradability of processed grains

Chemical composition and rumen degradation characteristics of the processed grains are presented in Table 1. Dry matter disappearance (DMD) was increased among all grains as incubation time increased (Figure 1). No differences were observed as result of processing, but a difference was observed between grain types. Barley had greater DMD than maize throughout the complete incubation period. This is believed to be the result of differences in physical characteristics among grains because of which the fractional rate of degradation of barley is significantly higher than the fractional rate of degradation of maize.

Between cereal grains, there appears to be a difference in the size of the water soluble fractions (Table 1) or fractions available to enzymatic attack at different times. When the grains were pressure toasted as extra processing treatment, the washable fraction (W)

significantly decreased in barley by 7.2% units and in maize by 6.8% units. The size of the undegradable fraction (U) was significantly elevated by toasting, and the effect was greater in maize than in barley, 3.5% and 1.4% units, respectively. Pressure toasting decreased the rate of degradation (k_d) in barley from 18.0 %/h to 15.5 %/h and elevated this in maize from 4.9 %/h to 6.0 %/h. Significant differences ($P < 0.05$) as observed for DM also appeared for the U fraction of OM and D and the size of the U fractions were also elevated as the W fraction decreased after pressure toasting. Pressure toasting compared to pelleting alone elevated the D fraction of NDF while W fraction and U fraction decreased in both cereal grains. The effects of pressure toasting on the fiber components of barley or maize were not monitored and no information is available in the literature. However, the soluble fibers in barley have been reported to increase after pelleting (Graham *et al.*, 1989) and extrusion (Vrantjes and Wenk, 1995).

Figure 1 Disappearance curves for dry matter (DM) of the processed cereal grains. (PB: pelleted barley, TPB: toasted and pelleted barley, PM: pelleted maize, TPM: toasted and pelleted maize)

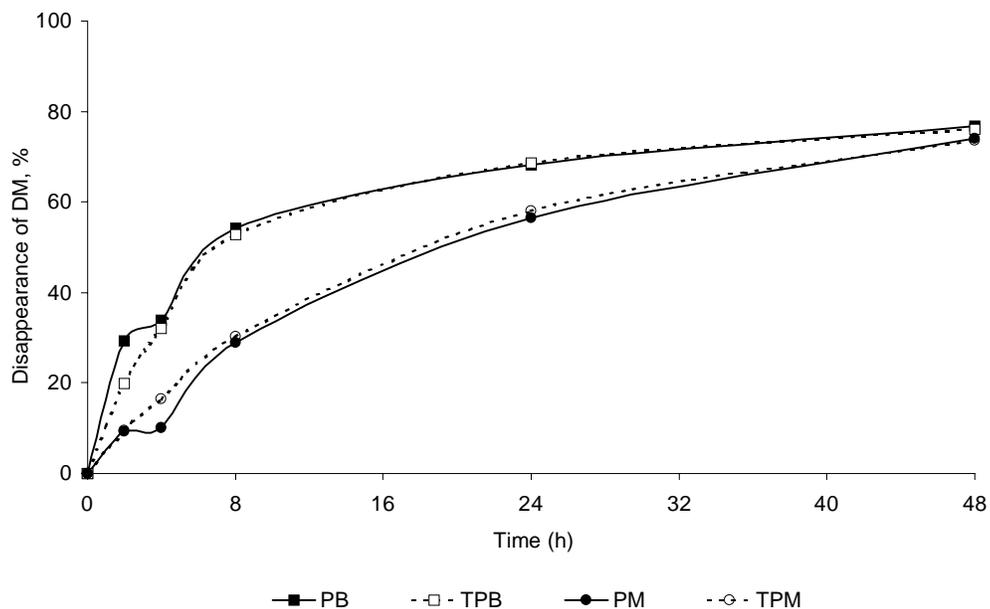


Table 1 Chemical composition of the processed grains¹ and effects of heat processing of cereals on *in situ* rumen degradation characteristics² of dry matter (DM), organic matter (OM), crude protein (CP), starch and neutral detergent fiber (NDF)

	Barley grain		Maize grain		SEM	P ³		
	PB	TPB	PM	TPM		G	H	GxH
DM, %	97.2	97.3	97.1	97.2				
W, %	45.9 ^a	38.7 ^b	36.6 ^c	29.8 ^d	0.41	0.0001	0.0001	NS
D, %	40.8 ^a	46.6 ^a	52.9 ^b	56.4 ^c	0.56	0.0003	0.004	NS
U, %	13.3 ^a	14.7 ^a	10.5 ^b	13.8 ^a	0.56	0.04	0.025	0.04
k _d , %/h	18.0 ^a	15.3 ^a	4.9 ^b	6.0 ^b	2.14	0.014	NS	0.049
Eff. D, %	76.5 ^a	72.3 ^a	60.5 ^b	58.0 ^b	2.75	0.02	NS	NS
OM, % of DM	97.8	97.9	98.7	98.6				
W, %	51.1	44.3	42.3	38.1	0.35	NS	NS	NS
D, %	35.8 ^a	41.2 ^b	47.4 ^c	48.3 ^c	0.59	0.0005	0.013	0.03
U, %	13.1 ^a	14.5 ^a	10.3 ^b	13.6 ^a	0.58	0.049	0.027	0.047
k _d , %/h	17.8 ^a	15.3 ^a	4.8 ^b	5.9 ^b	2.16	0.014	NS	NS
Eff. D, %	77.9 ^a	74.0 ^a	63.4 ^b	62.0 ^b	2.92	0.03	NS	NS
CP, % of DM	11.5	11.4	9.2	9.3				
W, %	35.5	27.5	34.9	32.4	4.12	NS	NS	NS
D, %	59.0	69.2	63.8	64.1	4.24	NS	NS	NS
U, %	5.4	3.3	1.3	3.5	4.2	NS	NS	NS
k _d , %/h	5.5	2.4	1.8	1.0	1.2	NS	NS	NS
% UIP	36.4	52.5	50.3	58.2	3.60	NS	NS	NS
Eff. D, %	63.6	47.5	49.7	41.8	4.32	NS	NS	NS
Starch, % of DM	57.2	59.7	68.3	71.0				
W, %	57.0	49.5	39.6	35.0	1.26	NS	NS	NS
D, %	40.8 ^a	48.9 ^b	57.6 ^c	58.6 ^c	1.33	0.002	0.04	0.07
U, %	2.2	1.6	2.8	6.5	1.34	NS	NS	NS
k _d , %/h	20.6 ^a	19.0 ^a	4.8 ^b	6.6 ^b	2.53	0.012	NS	0.04
% UIS	14.9 ^a	16.7 ^a	36.1 ^b	31.3 ^b	2.12	0.02	NS	NS
Eff. D, %	88.6 ^a	86.7 ^a	65.0 ^b	65.7 ^b	2.27	0.02	NS	NS
NDF, % of DM	13.9	13.4	8.0	8.4				
W, %	15.4	5.3	12.5	10.8	9.86	NS	NS	NS
D, %	18.0	30.0	19.3	24.7	19.35	NS	NS	NS
U, %	66.6	64.7	68.2	64.5	16.34	NS	NS	NS
k _d , %/h	13.2	7.3	8.8	7.9	6.62	NS	NS	NS

¹ PB: pelleted barley, TPB: toasted and pelleted barley, PM: pelleted maize, TPM: toasted and pelleted maize.

² W: washable fraction; U: undegradable fraction; D: not soluble, but potentially degradable fraction; k_d the fractional rate of degradation of D fraction; UIP: rumen undegraded intake protein, calculated for a passage rate of 6%/h., UIS: rumen undegraded intake starch, calculated for a passage rate of 6%/h., Eff. D: effective degradability = $W + D * k_d / (k_d - k_p)$.

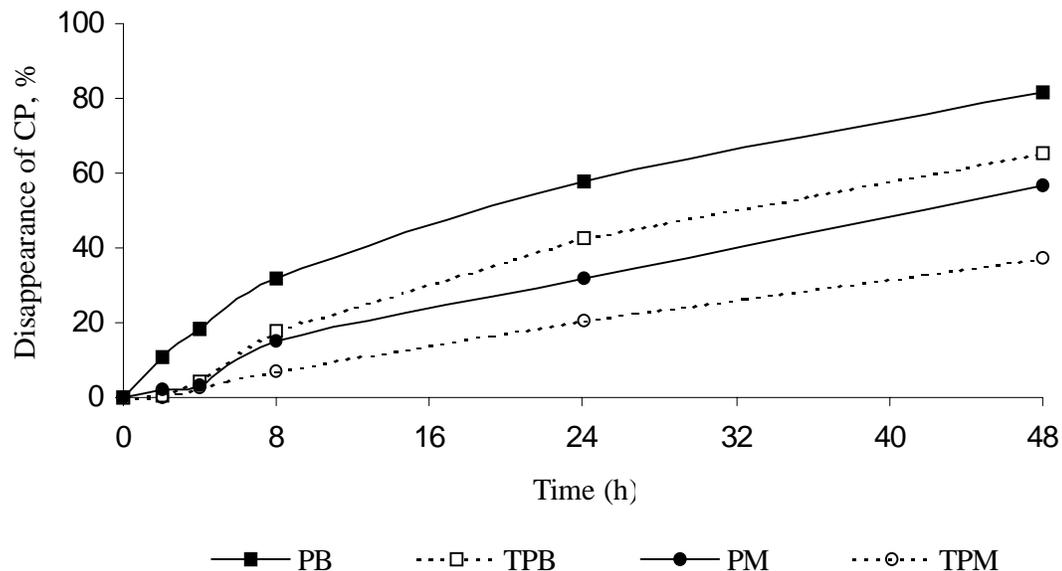
³ G: effect of type of grain, H: effect of type of heat, GxH: effect of grain type and heat interaction.

^{a,b,c,d} figures with different superscript in the same row differ significantly, NS: not significant (P>0.05)

3.2. Protein and starch degradability of processed grains

Protein disappearance from the nylon bags (Figure 2) increased as the incubation time proceeded. The percentage of disappearance after 48 h incubation was greater in pelleted barley (PB) and toasted and pelleted barley (TPB) than in pelleted maize (PM) and toasted and pelleted maize (TPM), 81.8 and 65.3 % vs. 56.9 and 34.8 % respectively. Pressure toasting followed by pelleting decreased the ruminal degradability of barley and maize compared to pelleting alone. Although no studies have been reported on the effects of pressurized toasting on the *in situ* protein or starch degradability of grains, the results are in agreement with numerous other studies where various heat treatments (expanding, flame roasting, steam flaking) showed to be effective in decreasing the rumen degradability of protein (Weisbjerg *et al.*, 1996; McNiven *et al.*, 1994; Prestløkken, 1999).

Figure 2 Disappearance curves for crude protein (CP) of the processed cereal grains. (PB: pelleted barley, TPB: toasted and pelleted barley, PM: pelleted maize, TPM: toasted and pelleted maize)



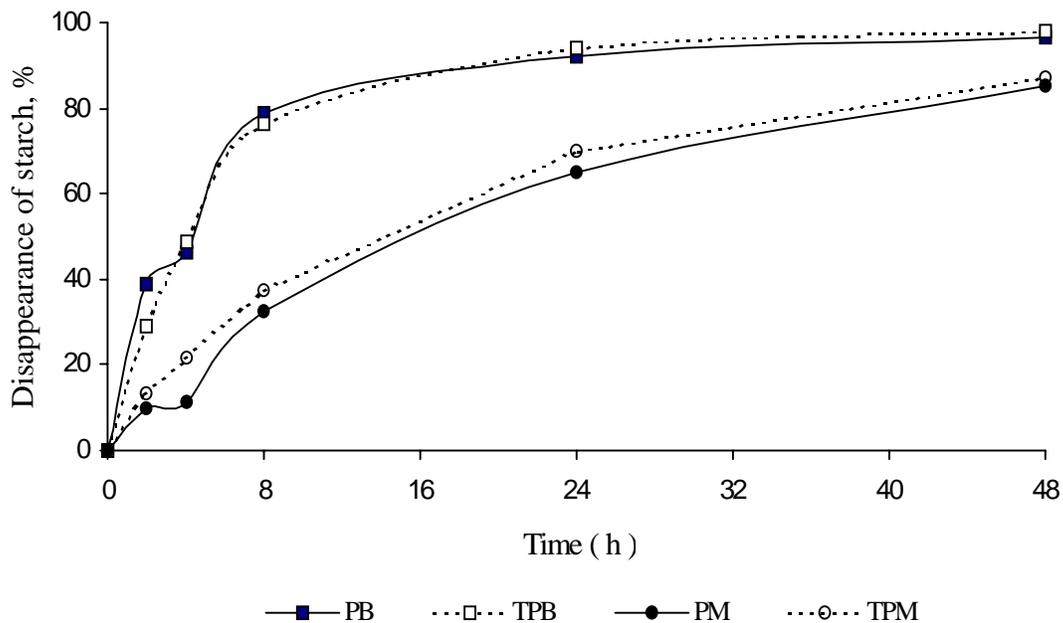
Because of the high SEM no significant differences ($P>0.05$) between the different fractions of the heat treated grain types could be established, but pressure toasting compared to pelleting tended to increase %UIP of both grains by decreasing the washable fraction (W) as well as the rate of degradation of the D fraction. The results are coherent with the results for faba beans and peas (Goelema, 1999), where W consistently decreased when temperature or time of processing were increased. The pressure toasting effect above 100 °C was more effective than pelleting alone and may have resulted in the denaturation of protein and probably transformed the proteins into a more resistant structure (Voragen *et al.*, 1995). In addition Maillard reactions may have happened and cross linkages formed between amino acids and reducing sugars (Hurrell *et al.*, 1976) or between proteins (iso-peptide bonds). These reactions will probably make the cereal grain protein more resistant against degradation in the rumen. McAllister *et al.* (1993) suggested that variation in the protein–starch matrix could be a major factor responsible for differences in ruminal digestion of different cereals. But these authors also concluded that the differences in the properties of the protein matrix among cereal grains did not alter ruminal amylolytic or proteolytic activity.

Differences in the arrangement of the protein-starch matrix might explain the differences in ruminal degradation of protein that was seen between barley and maize. It is well known that the barley kernel is composed of the hull, endosperm, and germ. The hull is a high fiber seed coat accounting for 7 to 17% of the seed weight. The multi-layer endosperm (80 to 90% of the seed weight) contains primarily starch and protein. Starch content is positively related to seed weight and inversely related to protein concentration in the endosperm (Beauchemin and Rode, 1998). In the maize endosperm, starch is tightly packed within a protein matrix and the prolamin fraction zein is more resistant to ruminal degradation than albumins, globulins, and glutelins (Romagnolo *et al.*, 1994). In barley the soluble prolamins and globulins dominate (Hoseney, 1994). These differences in the protein-starch matrix and the content of the different protein fractions may explain why barley seems to respond better to higher treatment intensities than maize (Figure 2).

Disappearance curves of starch are shown in Figure 3. It is easily seen that differences exist between cereal grains but the different heat treatments did not result in different disappearance curves. The pressure toasting followed by pelleting slightly elevated the

disappearance of maize starch compared to pelleting alone. For barley pressure toasting decreased the washable fraction (W) of starch and also slightly the rate of degradation (k_d). For maize the washable fraction (W) tended to be decreased and k_d to be increased (Table 1). As a result, % UIS after pressure toasting increased by 12% units for TPB and decreased by 13% units for TPM. Goelema *et al.* (1999) also found a tendency, that when the processing time and temperature increased, the size of the W fraction of the legume seeds decreased while k_d was increasing. The application of moisture, heat and shear during pressure toasting may induce several processes in the starchy cereal grains, such as swelling and gelatinization (Theurer, 1986; Nocek and Tamminga, 1991).

Figure 3 Disappearance curves for starch of the processed cereal grains. (PB: pelleted barley, TPB: toasted and pelleted barley, PM: pelleted maize, TPM: toasted and pelleted maize)



Swelling results from the exposure of starch to water, combined with gradual heating (Lund, 1984). Swelling is not a likely cause for the decreased W, since pressure toasting decreases swelling power and solubility of starch (Hoover *et al.*, 1993; Eliasson and Gudmundson, 1996). The results for barley are consistent with findings for faba beans and peas (Goelema, 1999) despite the large difference in initial starch degradability and

starch content of this grain. Undegraded intake starch (%UIS) of TPB and TPM was 16.7 and 31.3 %, versus %UIP of 52.5 and 58.2%, respectively (Table1). This indicated that starch was more degradable in the rumen than protein.

The pressure toasting effect on the decreased W and the increased UIS% may have been related to the change in starch distribution over the different particle size classes after pressure toasting. Goelema (1999) observed that drying (oven drying 16 h at 35⁰C) and storage of the toasted legume seeds led to retrogradation of the gelatinized starch. The crystallization of gelatinized starch molecules, i.e. retrogradation has received considerable attention because after retrogradation it is difficult to solubilize and this may decrease W as well. This could be the reason for the decreased W also with the grains, but the degree or extent of retrogradation depends on the botanical source (Kalichevsky *et al.*, 1990; Orford *et al.*, 1987; Silverio *et al.*, 1996). Cereal amylopectin retrograde to a lesser extent than legume seeds amylopectin, which has been attributed to the shorter average chain lengths in the cereal amylopectin (Fredriksson *et al.*, 1998; Kalichevsky *et al.*, 1990).

3.3. Chemical composition of grass

The differences in chemical composition (see Table 2) of the grass are caused by a different growing period (season) and because the samples originated from different plots. Plant organs differ in cell wall content and cell wall digestibility due to the differences in anatomical structure and chemical composition and cell wall thickness (Wilson, 1994). Leaf blades have lower cell wall content and higher cell wall digestibility than leaf sheaths and stem internodes have the highest cell wall content and lowest cell wall digestibility (Hacker and Minson, 1981). The proportions of the different plant organs and their composition and digestibility can vary considerably, depending on stage of plant development and age, environmental factors (light, temperature) and grassland management. Each growing period has its own specific circumstances for example the rainfall, radiation and temperature. The residue fraction (OM-NDF-starch-CP) is high in the grass samples from Period 1 compared with the other four periods and this is most likely caused by a high content of sugars and fructosans. In perennial ryegrass up to 70% of the water-soluble carbohydrates are present as fructosans (McGrath, 1988). Because of

the manure application before the third period, the dry matter (DM) yield decreased in agreement with Willman and Wright (1983) and Valk *et al.* (1996). The residual fraction which contains the sugar also decreased. This maybe the effect of fertilization (McGrath, 1992) and also related to the specific summer weather conditions such as high light intensity and high daily temperature (Smith, 1973). Crude protein content of grass increased after the manure application which is in agreement with Morrison *et al.* (1980) and Van Vuuren *et al.* (1991).

Fiber composition and the ADL to NDF ratio of pasture grass was relatively constant throughout the sampling periods. ADL content of grass followed the NDF change. No important effect of sward maturity was observed on NDF. However a little increase due to season was evident with a slight interaction with maturity. It might be possible that maturity influenced more the NDF content than manure application.

3.4. Rumen degradability of available grass

The *in situ* estimates of parameters for DM, OM, CP and NDF degradation for the grasses sampled in the five periods are shown in Table 2. The disappearance curves of DM (Figure 4), OM (Figure 5), CP (Figure 6), and NDF (Figure 7), are shown in figures. After 48 h of incubation we found an average DMD, OMD, CPD and NDFD of 49.4 ± 4.1 , 50.6 ± 2.5 , 60.6 ± 3.8 and 45.4 ± 3.3 , respectively.

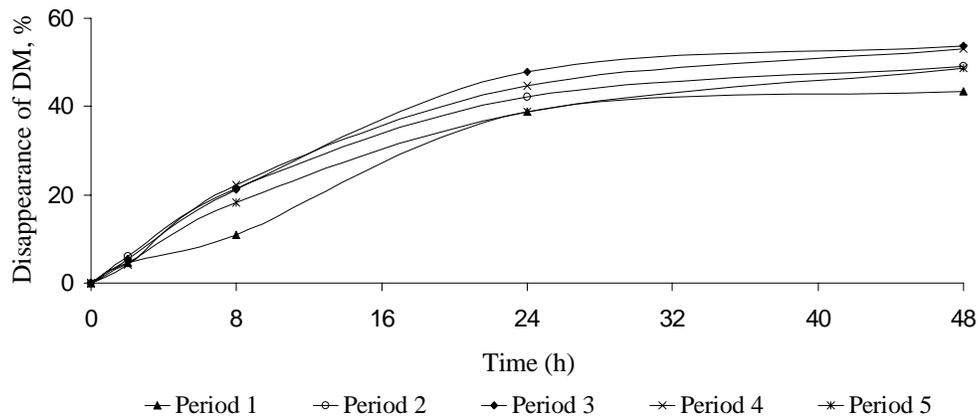


Figure 4 Disappearance curves for dry matter (DM) of perennial ryegrass (*Lorium perenne*) sampled from June 2 to July 31.

Table 2 Chemical composition and the effect of maturation on washable (W) and undegradable fractions (U) and rate of disappearance of the insoluble potentially degradable fraction (k_d ,%/h) of dry matter (DM), organic matter (OM), crude protein (CP), acid detergent lignin (ADL) and neutral detergent fiber (NDF) of perennial ryegrass (*Lorium perenne*) (sampled in June 2 to July 31, 1997).

Date of sampling	Period 1	Period 2	Period 3	Period 4	Period 5
	2-6 June	16-20 June	30 June – 4 July	14-18 July	28-31 July
DM%	23.82	22.82	17.04	16.39	21.78
k_d ,%/h	6.9	6.7	6.4	6.0	5.3
W, %	54.1	46.5	49.9	44.9	44.4
D,%	17.8	27.8	29.1	31.4	30.4
U,%	28.1	25.7	21.0	23.7	25.2
OM,% of DM	91.22	90.82	89.46	89.98	90.24
k_d ,%/h	6.1	6.0	5.8	5.4	4.8
W, %	53.0	45.6	48.0	42.7	42.8
D,%	18.2	28.3	30.8	33.2	32.3
U,%	28.8	26.1	21.2	24.1	24.9
CP, % of DM	15.65	21.07	24.79	23.68	20.41
k_d ,%/h	16.8	7.5	6.5	6.1	6.1
W, %	54.4	55.8	60.5	52.4	49.8
D,%	24.1	29.2	28.8	34.0	34.0
U,%	21.5	15.0	10.7	13.6	16.2
NDF,% of DM	36.09	38.29	37.10	38.2	37.86
k_d ,%/h	2.7	5.1	5.9	5.1	4.3
D,%	59.9	55.9	62.0	59.0	57.2
U,%	40.1	44.1	38.0	41.0	42.8
ADL, % of DM	2.17	2.15	1.88	2.02	2.26
Residue, % of DM	39.48	31.46	27.54	28.1	31.97
ADL/NDF %	6.0	5.6	5.0	5.3	6.0

Figure 5 Disappearance curves for organic matter (OM) of perennial ryegrass (*Lorium perenne*) sampled from June 2 to July 31.

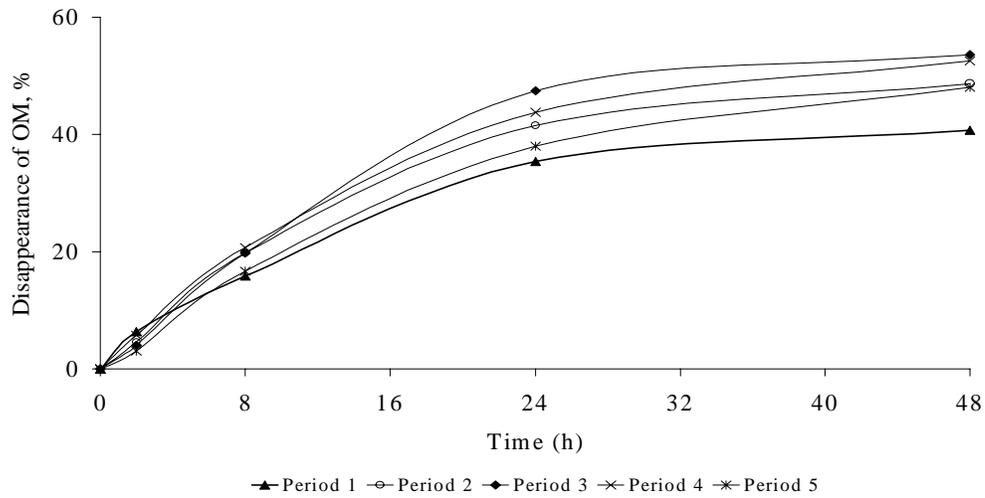


Figure 6 Disappearance curves for crude protein (CP) content of perennial ryegrass (*Lorium perenne*) sampled from June 2 to July 31.

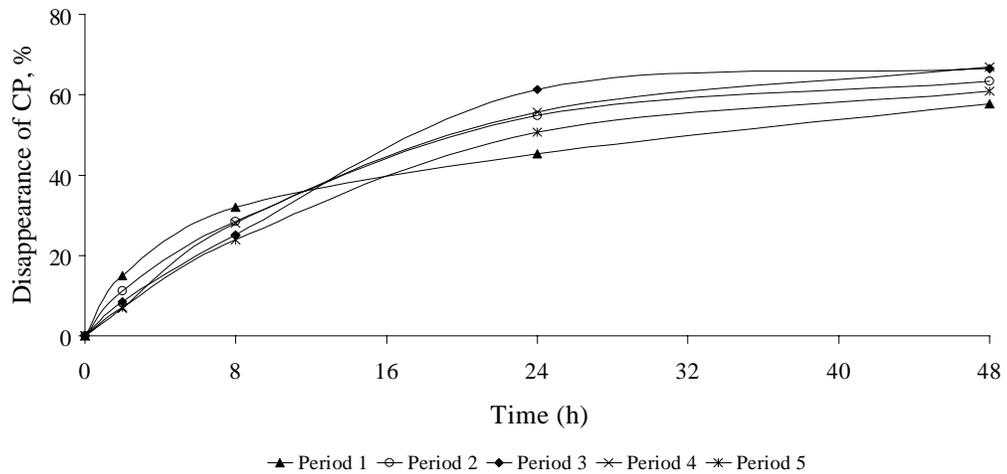
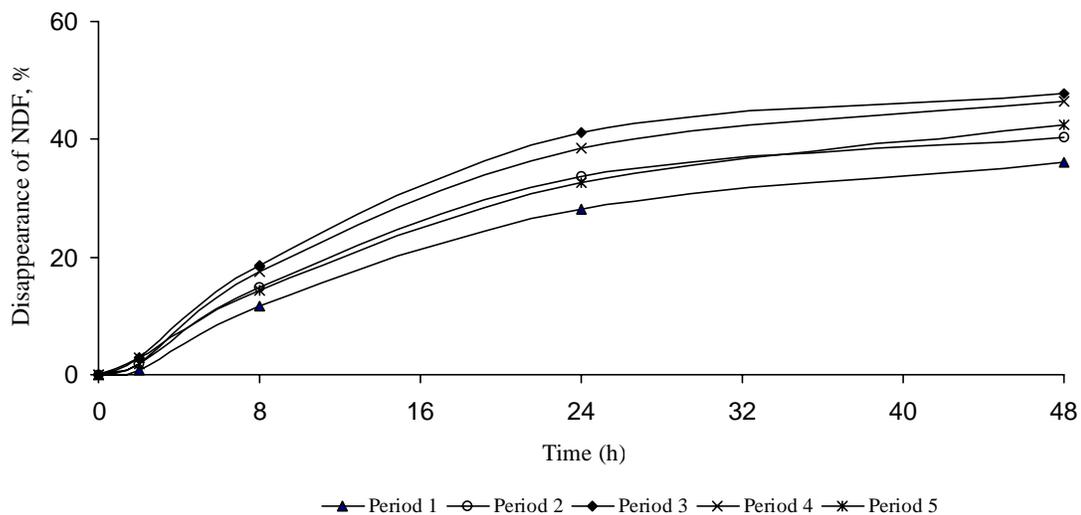


Figure 7 Disappearance curves for neutral detergent fiber (NDF) content of perennial ryegrass (*Lorium perenne*) sampled from June 2 to July 31.



The disappearance of OM and CP from nylon bags suspended in the rumen reflected the pattern in nutritive value as estimated from the chemical composition. The increased stem proportion, and increased structural carbohydrates or a decrease of CP in leaf and stem fractions resulted in this characteristic of OM and CP.

Grass samples from Period 1 had a numerically higher washable fraction (W) and numerically lower insoluble potentially degradable fraction (D) of DM and OM than grass from other periods. The fractional rates of DM degradation of the D fraction are in agreement with another study (Hoffman *et al.*, 1993). Differences in the washable fraction of OM between periods maybe partly explained by the difference in sugar content. The rates of degradation of the D fractions of OM are coherent with the data presented by Van Vuuren *et al.* (1992; 1993). Also the rate of degradation of the D fractions of NDF agree with the results presented by Van Vuuren *et al.* (1992; 1993). Only the data from the first period differ from the mentioned literature date, but the reason for it is probably the dry weather conditions after June 6 in the summer (Tóthi *et al.*, 2002). Application of manure immediately before the 3rd period increased the solubility of CP of grass, which is in agreement with the results of Morrison (1987) and Salette (1982). The size of W of CP is strongly influenced by the choice of the method of

sample preparation and washing procedures, especially because most N in the cell content (partly NPN, partly amino acids) is soluble. The results for W of 50 to 60% is close to the data (40 to 53 %) reported by Van Vuuren *et al.* (1993b, 1993c) when the fresh ryegrass samples were freeze dried and ground prior to *in situ* incubation. Van Vuuren *et al.* (1991) reported lower washable CP fractions of between 5 and 37 % when young grass samples were freshly chopped, filled in the bag than kept frozen until incubation. Steg *et al.* (1994) collected and subsequently chopped the frozen ryegrass samples and used nylon bags with 41 μm pore size, and found 13 to 22% for the washable fraction of CP. Elizade *et al.* (1999) used the same technique as Steg *et al.* (1994) for grass sample preparation but the nylon bags pore size was higher (53 μm) and found 35-50 % for the washable fraction of CP (bromegrass and tall fescue).

In the present study the grass samples were kept after hand plucking in a freezer and before the incubation were chopped, and this may have increased the fraction that disappears from the bags during washing. Van Vuuren (1993a), Boudon and Peyraud (2002) stated that freeze drying and grinding, and also chopping ruptures cell walls, and thus free protein and intact chloroplasts are washed out of the bags.

The rate of degradation of the D fractions of CP was significantly higher in first period (16.8 %/h). The other periods were much lower (from 6.1 to 7.5 %/h) than in the studies of Van Vuuren (1991, 1992, 1993a) where these values ranged from 7.2 to 12.7 %/h. Differences in sample preparation (chopping), pore size of the nylon bags used and washing procedure may be responsible. There are no significant differences between the periods, but it seems that with maturation the k_d of DM, OM and CP slows down.

4. The nitrogen (N) to organic matter (OM) ratio in the OM released from the nylon bags for concentrates and grasses

The calculated ratios for the rate of release of N to OM over a 24 h period using a fractional outflow rate of 0.045 %/h for grass and 0.06 %/h for processed cereal grains are presented in Figure 8 and Figure 9. The change in ratio of N effectively degraded in the rumen to OM effectively degraded in the rumen followed a similar pattern during the day for grass sampled in Period 2-5, but not in the Period 1. *In situ* incubation of

available grass samples resulted in numerically higher washable fractions of OM and DM and a significantly higher rate of degradation of the D fraction of CP in Period 1. This difference between Period 1 and the other periods in that CP content of the grass was lower, resulted in a more unbalanced release of N and OM in the rumen (Figure 8). This difference in release of pasture N and OM could be higher later in the growing seasons.

On the one hand ryegrass from pasture is offered to the cows in The Netherlands from April until October. During this period the environmental changes (rainfall, radiation, air temperatures), nitrogen fertilization (Van Vuuren, 1991) and maturity (Garwood *et al.*, 1980; Sanderson and Wedin, 1989) affect the chemical composition and the *in sacco* degradation parameters of the CP content and the washable fraction of OM of grasses. On the other hand in the grazing situation forages are selected and chemical composition changes with the time of grazing.

From the figures it seems that for supplementation of grass originating from different growing seasons it might be feasible to use differently processed grains that differ in release of OM and N (Figure 9).

Figure 8 Ratio of nitrogen (N) to organic matter (OM) of perennial ryegrass (sampled from June 2 to July 31) degraded in the rumen

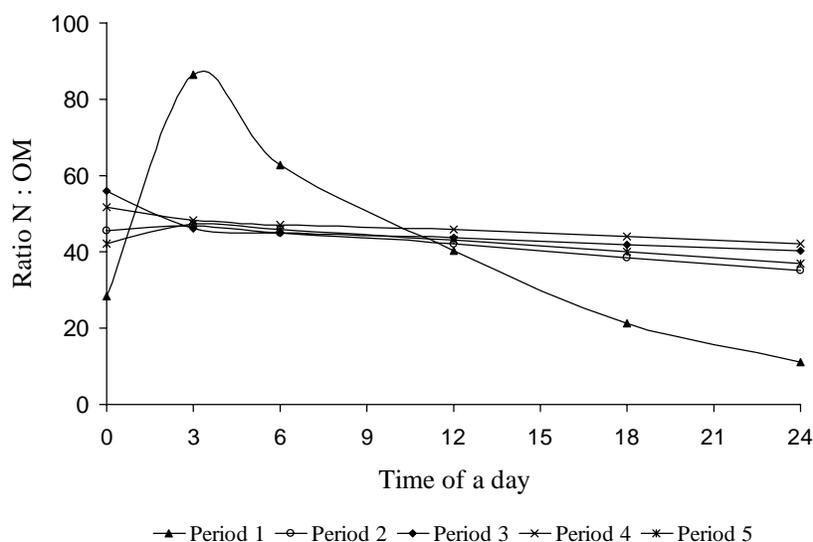
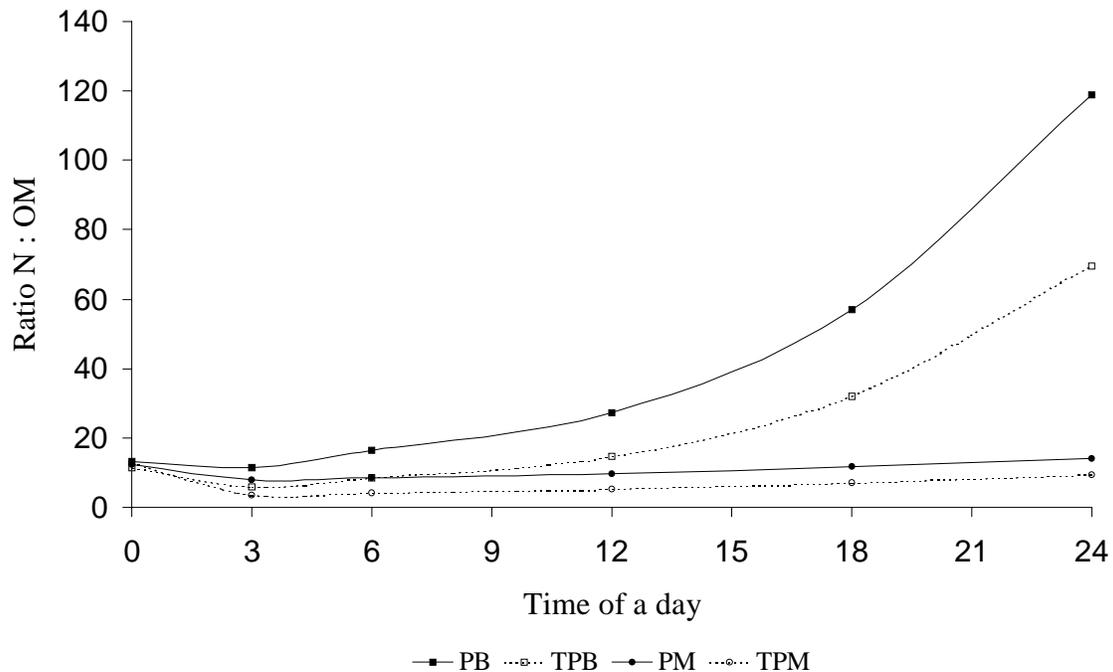


Figure 9 Ratio of nitrogen (N) to organic matter (OM) of processed cereal grains (PB: pelleted barley, TPB: toasted and pelleted barley, PM: pelleted maize, TPM: toasted and pelleted maize) degraded in the rumen



The need of synchrony is specially important with diets based on fresh grass, in which markedly asynchronous rates of release of energy and nitrogen in the rumen occur, therefore using processed grains to generate degrees of ruminal N and OM synchrony to improve N utilization of microbial yield seems feasible, however there appears to be considerable scope for increased research effort.

5. Conclusions

Except in Period 1, limited differences were observed in chemical composition of perennial ryegrass and as a result, *in situ* degradation characteristics were quite similar throughout the experimental season (June to July). The rate of crude protein degradation of ryegrass is relatively high, 0.061 to 0.075 h⁻¹, depending on the state of maturation and environmental factors. Thus for better microbial availability of protein and energy the supplement should have a rate of starch fermentation at least as fast as that of the CP of

the grass. Heat treatments of cereal grains as pelleting or toasting and subsequently pelleting elevate the rate of degradation of their starch content in the rumen. The effect of toasting in this respect is more favorably than pelleting alone, because this heat treatment decreased the rate of degradation of barley starch (from 0.206 to 0.190 h⁻¹) but increased the rate of degradation of maize starch (from 0.048 to 0.066 h⁻¹). Pressure toasting and pelleting increased the undegradable fraction of DM and OM in barley and maize compared to pelleting alone. But it is unclear which effect caused the difference. We found no significant difference between the protein and starch fractions; therefore no clear evidence was found which chemical compounds caused the difference due to processing in the undegradable fraction. If the starch/protein complex caused the difference in the undegradable fraction it is most likely that a protein-starch bound was formed. But if the rest compound caused the difference in the undegradable fraction the occurrence of Maillard-reactions are more likely to be responsible. Therefore further research will have to concentrate on the question which chemical compound(s) give a reaction when grains are pressure toasted.

In conclusion it seems that using different types of grain that differ in rate and extent of ruminal degradation might have more effect than using one of the two processing methods to improve the balance between energy and protein available for rumen microbial growth in fresh grass, but the degree of synchronization needs further research.

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CHAPTER 6

Effect on Ruminal Fermentation of Supplementing Grazing, Lactating Dairy Cows with Processed Starch Rich Concentrates

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Abstract

The effect of processed cereal grain supplementation on rumen fermentation pattern of grazing, lactating Holstein-Friesian cows was examined in a 5x5 Latin square experiment. The experimental treatments were the following: control (only grazing, no supplement addition, NS), pelleted barley (PB), pelleted maize (PM), toasted and subsequently pelleted barley (TPB), and toasted and subsequently pelleted maize (TPM). The rapid and extensive ruminal fermentation of starch in all supplemented grazing cows resulted in higher concentrations of volatile fatty acids (VFA) and lower pH in ruminal fluid than in NS animals. Total VFA (TVFA) concentrations (mmol/l) were higher for cows fed processed barley (121.0 and 125.3 for PB and TPB, respectively), than for cows fed processed maize (121.7 and 115.6 for PM and TPM, respectively). Supplement feeding lowered the acetate to propionate (A:P) ratio and the non-glucogenic to glucogenic ratio (NGR) in comparison to NS animals, but the cereal grain type and processing method had no significant effect on these ratios. The acetate molar proportion in supplemented animals decreased from 66% to 61% and the propionate increased from 19% to 24% regardless the type of the supplementation or the method of processing. Concentrations of ammonia (mmol/l) and the ammonia to TVFA ratio decreased when the cows were supplemented with processed grains and toasting and subsequently pelleting resulted in numerically lower ruminal ammonia concentrations and lower ammonia to TVFA ratio as well than did pelleting only. The different processing methods resulted in similar VFA patterns, A:P ratios, and TVFA concentrations in the rumen. It seems that differences in ruminal fermentation which may have existed between maize and barley before heat processing, disappeared, moreover heat treated maize supplementation synchronised the energy with the rumen degradable protein better than did barley.

Keywords: Grazing; Heat treatment; Starch; Ruminal fermentation; Dairy

1. Introduction

High ammonia levels in the rumen of dairy cows are an indication for a shortage of energy availability or a lack of synchrony between energy and nitrogen supplies, that limits the use of available nitrogen by ruminal microorganisms (Huntington, 1990). Synchronising the ruminal fermentability of energy (starch) and nitrogen sources increases the outflow of bacterial protein from the rumen of dairy cows (Huntington, 1997). Depending on the efficiency of microbial growth, the ratio between microbial biomass and their end products may vary between 0.4 and 1.0 (Hvelplund, 1991). When degradation of carbohydrates and proteins (in g per unit of time) are synchronised and take place in a ratio of approximately 5:1, microbial protein synthesis will occur most efficiently and with little nitrogen losses from the rumen (Tamminga *et al.*, 1990). The rate of degradation largely determines the ratio in which volatile fatty acid (VFA) are formed, rapid degradation usually means a high proportion of propionic acid (sometimes lactic acid may accumulate), whereas slow degradation results in the formation of predominantly acetic acid. The ratio in which VFA are produced depends on the chemical composition and the rate of degradation of the substrate (Murphy *et al.*, 1982), further on the rumen pH. A low rumen pH or a rapidly degradable starch enhances propionate production, whereas a high rumen pH, slowly degradable substrate and fibre enhances the production of acetic and butyric acid. The rate at which VFA are produced in the rumen will to some extent determine their molar concentration in the rumen liquid, which in turn influences their rate of absorption (Dijkstra *et al.*, 1994). Rate of absorption of VFA appeared to depend on VFA concentration, rumen pH and rumen volume. The ratio in which propionic acid, acetic acid and butyric acid are provided does have a severe effect on milk fat content (Sutton, 1989). In order to synchronise rumen fermentation, degradative behaviour in the rumen should be controlled, if necessary by manipulation through feed processing. Grazing of very lush pasture will stimulate grass intake, but crude protein from this pasture is usually high, and rapidly and extensively degraded in the rumen (Lopez *et al.*, 1991). Furthermore high forage diets promote extensive absorption of ammonia from the rumen, because a greater proportion of ammonia is in the non-ionised form due to the higher pH associated with such diets (Siddons *et al.*, 1985). Under such conditions 50 % of the crude protein ingested with the grass may be

converted in the rumen into ammonia, absorbed in the blood stream, being converted into urea in the liver and excreted in the urine, resulting in poor utilisation of pasture protein. To reduce urinary N losses and to improve the efficiency of milk N synthesis in high yielding dairy cows, N intake should be reduced without decreasing the energy intake (Van Vuuren, 1993). This can be achieved by the partial replacement of grass by low protein supplements high in non-structural carbohydrate, for instance cereal grains. Cereal grains differ widely in their rate of degradation in the rumen and as such may not always match with the degradation of protein in pasture grass. A further optimisation is then possible by such a way of processing the grain, that the degradation of its starch becomes balanced to the degradation of protein in grass.

The objectives of the experiment reported were therefore to investigate the effects of various ways of processing cereal grains as a supplement to grazing, high yielding dairy cows on patterns of rumen fermentation characterised by pH, ammonia and VFA concentrations in rumen liquid.

2. Materials and methods

2.1. Animals and management

The experiment was carried out at the experimental farm 'De Ossekampen' of Wageningen University, The Netherlands. Five multiparous lactating Holstein-Friesian dairy cows previously surgically fitted with a rumen cannula (10 cm id., Bar-Diamond Inc., Parma, Idaho, USA) were used. Two cows were in their 7th and the others were in 2nd, 4th and 6th lactation, respectively. At the beginning of the experiment the cows produced 28.6 ± 4.6 kg/day milk and averaged 173 days post partum. The animals were milked twice daily at 6:30 h and 17:00 h.

2.2. Experimental design

The experiment was a 5 x 5 Latin square design with five cows, five treatments and five periods in summer time. Each experimental period consisted of 14 days. Days 1-9 were used for adaptation and days 10-14 for sample collection.

2.3. *Treatments and feed processing*

The five experimental treatments were a control treatment of grass only (no supplement addition, NS), grass with pelleted barley (PB), grass with pelleted maize (PM), grass with toasted and subsequently pelleted barley (TPB), grass with toasted and subsequently pelleted maize (TPM). Grain processing was carried out at the Wageningen Feed Processing Centre (WFPC). A laboratory scale pressurised toaster was used for pressure toasting the grains for 1.5 minutes at 135 °C. After toasting, the grains were dried in a forced air oven for 16 h at 35 °C, and followed by pelleting. Pelleting (80 °C, 10 s) was carried out with a 5 x 65 mm (bore x hole) die, using a V2-30 pelleting press (Robinson milling systems B.V., Boxtel, The Netherlands).

2.4. *Sample collection*

Next to a control treatment of grazing only, the four forms of processed grains were fed as a supplement in two equal portions of 3 kg each in the milking parlour during the morning (7:00 h) and evening milking (17:00 h). In the 10 days long adaptation period the cows were allowed to graze freely with the herd in a pasture of perennial ryegrass (*Lolium perenne*). Detailed descriptions of the experimental protocols have been published elsewhere (Tóthi *et al.*, 2002a). During the morning milking of the experimental period, the cows consumed the heat treated grains in the milking parlour, they were placed in their respective grazing plots, tethered within a circular plot of a fixed area with a radius of six meters and allowed to graze in the morning from 8:00 h to 11:00 h. After grazing each cow was moved to the barn and starved until 17:00 h, then milking and concentrated feeding was repeated. During the starvation period the animals had free access to water and mineral blocks (KNZ Liksteen).

During the grazing time, starting at 8:00 h in the morning, samples of rumen fluid were taken every 30 minutes during the next hour and subsequently at 11:00 h, 12:00 h, 14:00 h and 17:00 h (10 samples per cow per day). Rumenal fluid was obtained by suction from the ventral rumen compartment using a perforated rod and pH was immediately

determined in the sample with a portable pH meter (pH electrode type 62, Testo 252, Testo GmbH & Co., Germany).

2.5. Chemical analysis and calculations

Determinations of VFA and ammonia concentrations in the rumen fluid were by gas chromatography and spectroscopy respectively as described by Chilbroste *et al.* (1998). The ammonia concentration was determined with a spectrophotometer at a wavelength of 623 nm. The analysed VFA were: acetic acid (C₂), propionic acid (C₃), butyric acid (C₄), isobutyric acid (iC₄), isovaleric acid (iC₅), and valeric acid (C₅). The total concentration of VFA (TVFA) in the rumen fluid was calculated as the sum of C₂, C₃, C₄, iC₄, C₅ and iC₅. The Nongluconic Glucogenic Ratio (NGR) was calculated as described by Ørskov (1975).

2.6. Statistical analysis

The experimental data were analysed using the PROC GLM procedure of SAS (1995). Cow, period and treatment (supplementation, grain, heat and the interaction between heat and grain) were the class variables in the model. When significant differences due to the treatment were detected, the multiple comparison procedures (Tukey and Dunnett) were used. Results are reported as least square means and standard errors of least square means. Treatment effect within feed type was judged using PDIFF in SAS 6.12 (SAS, 1995). Differences of P<0.05 were considered to be significant.

3. Results and discussion

3.1. Ruminal pH and VFA concentrations

The ruminal pH and mean VFA concentrations and molar proportions of individual VFA in the ruminal fluid of lactating dairy cows fed the different heat processed cereal grains as supplements are presented in Table 1.

Table 1 Rumen concentrations of volatile fatty acids concentration, VFA pattern and ruminal pH (mean of 14 values measured during the day) within the ruminal fluid of dairy cows grazing grass pasture¹, and supplemented with processed cereal grains²

	NS	Barley grain		Maize grain		SEM	P ³		
		PB	TPB	PM	TPM		G	H	GxH
pH	6.35 ^S	6.10	6.09	6.11	6.13	0.06	0.8	0.9	0.7
TVFA, mM/l	114.2 ^{TPB}	121.0	125.3	121.7	115.6	3.3	0.2	0.8	0.14
VFA, mol/100 mol									
Acetate	75.7	73.8	76.8	74.5	70.1	2.7	0.3	0.8	0.2
Propionate	21.4 ^S	28.6	28.8	29.1	27.6	1.1	0.8	0.5	0.4
Isobutyrate	1.12 ^S	0.86	0.92	0.89	0.86	0.04	0.6	0.8	0.3
Butyrate	13.0 ^T	14.5	15.4	14.2	13.8	0.7	0.2	0.7	0.3
Isovalerate	1.7	1.5	1.6	1.5	1.4	0.1	0.4	0.7	0.2
Valerate	1.15 ^S	1.82	1.78	1.50	1.75	0.2	0.4	0.6	0.5
A:P ⁴	3.6 ^S	2.7	2.7	2.6	2.6	0.14	0.7	0.9	0.9
NGR ⁵	4.8 ^S	3.7	3.8	3.6	3.7	0.2	0.7	0.8	0.9

¹ NS: no supplement addition.

² PB: pelleted barley, TPB: toasted and pelleted barley,

PM: pelleted maize, TPM: toasted and pelleted maize.

³ G: effect of grain type, H: effect of type of heat treatment, GxH: effect of grain type and heat interaction.

⁴ A:P: ratio of acetate to propionate.

⁵ NGR: non-gonglucogenic to glucogenic ratio

^S figure with superscript is significantly (P<0.05) different from others.

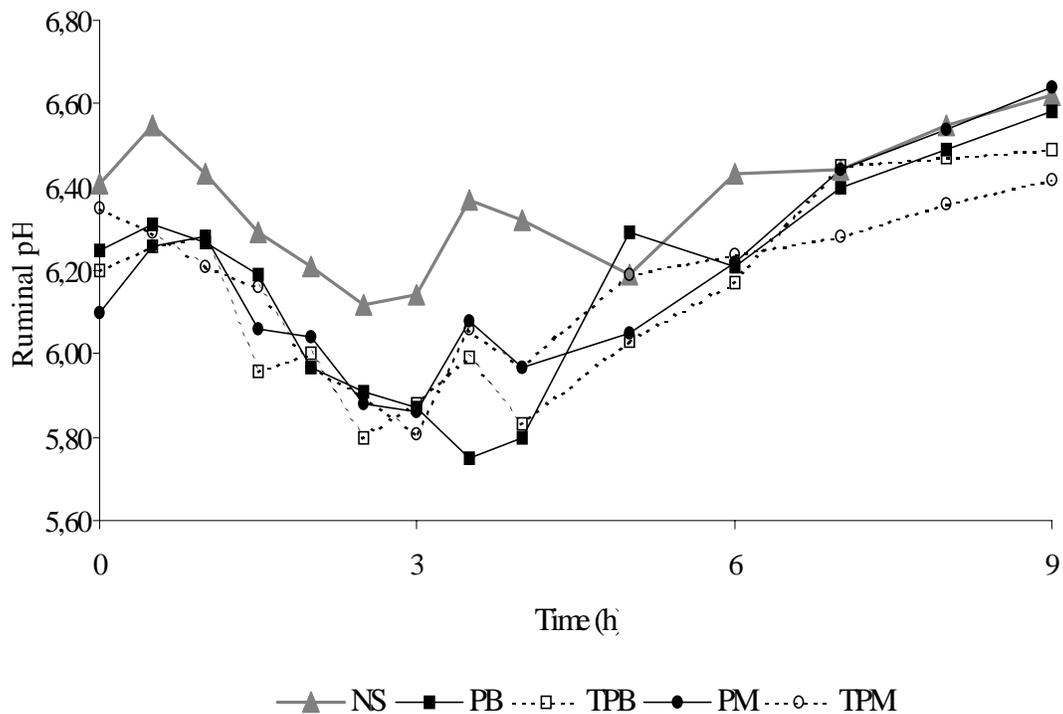
^{TPB} figure with superscript is significantly (P<0.05) different from TPB treatment.

^T figure with superscript is tend to significantly different from others (0.05<P<0.1).

The rapid and extensive ruminal fermentation of starch in all supplemented grazing cows resulted in higher concentrations of TVFA in ruminal fluid than in NS animals. However significantly (P<0.05) only NS and TPB differ. The high concentrations of TVFA in ruminal fluid depressed ruminal fluid pH (Figure 1) in comparison with NS animals to values that averaged around 6.1 during the experimental day for each supplemented animals and tended to be slightly lower for the barley (TPB and PB) than for the maize (PM and TPM) (Table 1). About 30 minutes after the start of the grazing period, ruminal pH of the supplemented animals continuously decreased in the grazing phase (Figure 1) to below 6.0 for about 2 hours which may have affected the effectively

function of enzymes necessary for fibre breakdown and the cellulolytic activity of bacteria and depressed ruminal fiber degradation.

Figure 1 Least squares means for ruminal pH determined at 30 minutes intervals in grazing time (3 h) and the starvation time (6 h) for lactating cows. Each point represents the mean of ten observations. (For abbreviations see Table 1)

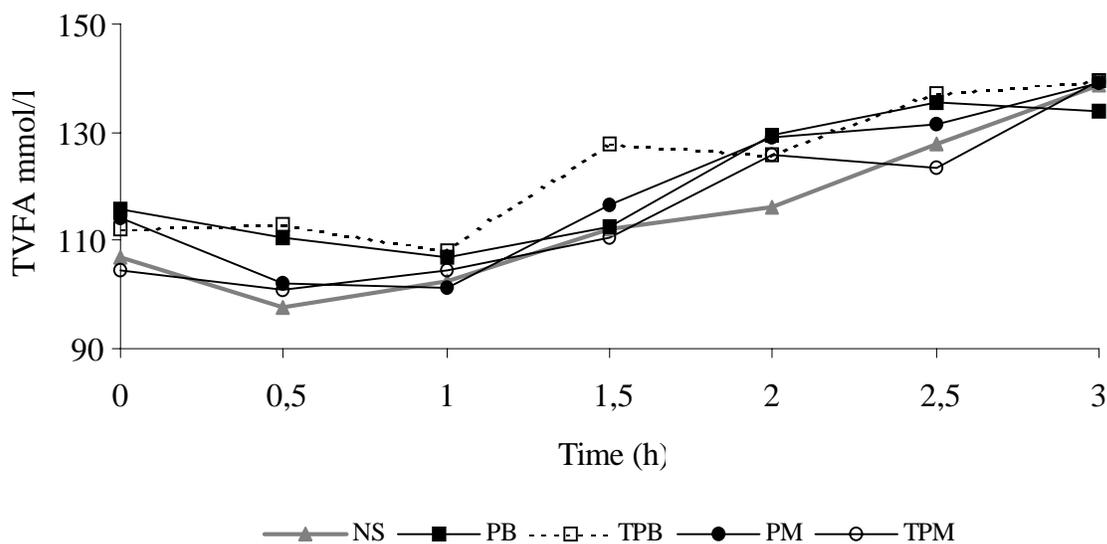


Optimal pH for cellulolytic activity of bacteria in the rumen is near 6.8 (Terry, 1969). Numerous *in vitro* studies have shown that the major cellulolytic bacteria (*Ruminococcus albus*, *Ruminococcus flavefaciens* and *Fibrobacter succinogenes*) cannot tolerate a pH below 6.0, because the bacteria are unable to maintain the pH inside their cells when ruminal pH is low (Russell and Wilson, 1996). The effects of diurnal shifts in rumen pH *in vivo* are uncertain. Yang *et al.* (1999) measured ruminal pH and fibre digestion in dairy cows fed diets that ranged in the extent to which barley grain was flattened during steam-rolling and found that ruminal and total tract fibre digestion was unaffected by grain processing. Maybe other fibrolytic organisms contribute significantly to ruminal fibre

digestion. Forster *et al.* (1999) found that over 60% of rumen bacterial species have not yet been fully characterised and some of these species may be fibrolytic.

The TVFA concentrations (mmol/l) were higher for cows fed processed barley (121.0 and 125.3 for PB and TPB, respectively), than for cows fed processed maize (121.7 and 115.6 for PM and TPM, respectively). PB and TPB had consistently higher TVFA concentrations than PM or TPM (Figure 2). These observations were in agreement with McCarthy (1989) and Casper *et al.* (1999) who reported that the TVFA concentrations were greater for cows fed maize than cows fed barley, which was correlated to the greater ruminal liquid volumes of cows fed diets based on barley.

Figure 2 Least squares means for ruminal concentration of total fatty acid (TVFA) determined at 30 minutes intervals in grazing time (3 h) for lactating cows. Each point represents the mean of ten observations. (For abbreviations see Table 1)



The processing method did not have any significant effect ($P > 0.05$) on the TVFA concentrations, especially when barley was fed. But when maize was fed, PM resulted in higher TVFA concentrations than when TPM was fed. This might have been caused by the increase in the washable fraction and degradation rate of the maize starch caused by pelleting while toasting only increases the degradation rate but not the washable fraction

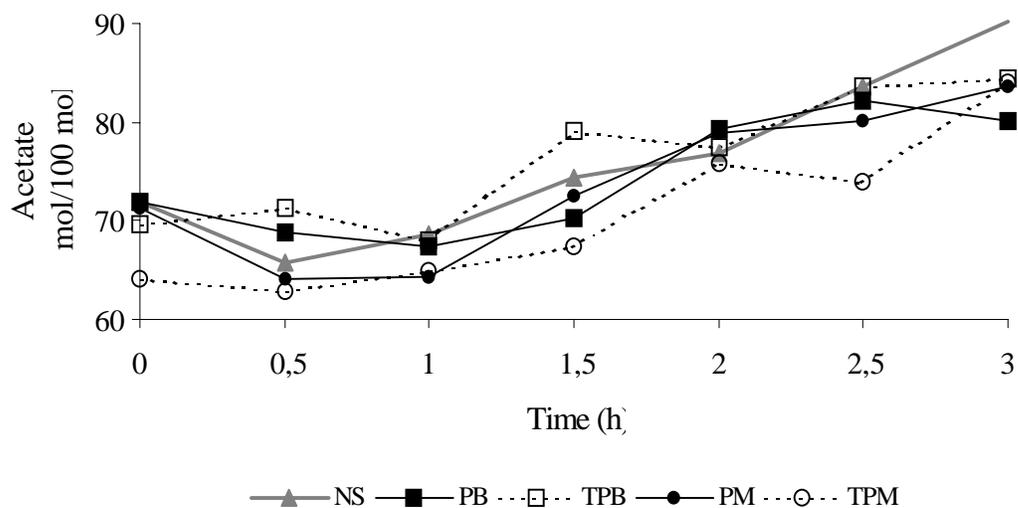
(Tóthi *et al.*, 2002b). Crocker *et al.* (1998) reported that the TVFA concentrations in ruminal fluid was unaffected by maize processing, which was consistent with a lack of the processing effect on OM digestion in the rumen. VFA concentrations in the rumen are the resultant of various processes. The concentration will be increased by VFA production, but decreased by VFA absorption, VFA passage out of the rumen with the fluid and increases in the rumen fluid content. No differences were observed in the rumen fluid volumes (Tóthi *et al.*, 2002a). Higher VFA concentrations as well as a lower pH both enhance the absorption rate of VFA (Dijkstra *et al.*, 1993). So, the increase in VFA concentrations as observed in our experiments was most likely due to increased production rates.

Supplementation of pasture grass with heat treated cereal grains significantly increased ($P<0.05$) the molar concentration of propionic acid and there is a tendency of increased ($P=0.06$) butyric acid during grazing time (Figure 4 and 5), rising its daily ratio from 19% to 24% and 11% to 12% as a percentage of the TVFA concentration (Table 1). In the literature major changes in the molar proportion of propionic acid (Visser *et al.*, 1992; Sutton *et al.*, 1987; Bargo *et al.*, 2002; Reis and Combs, 2000) and butyric acid (Bargo *et al.*, 2002; Reis and Combs, 2000) have been reported when rumen degradable starch was fed. The increase in propionic acid concentration and ratio was mainly at the expense of the acetic acid of which the contribution to the TVFA decreased from 66% to 61% and to a lesser extent to isobutyric acid of which the contribution decreased significantly from 1.1% to 0.9 %. The acetate concentration during grazing time (Figure 3) after feeding TPB seems to give a quite wavy picture, and this phenomenon was also observed for butyrate (Figure 5) and slightly for propionate (Figure 4).

Feeding supplements resulted in a significantly lower ($P<0.05$) A:P ratio and NGR. These decreases agree with those observed by Van Vuuren *et al.* (1986) for grass fed cows with 1 kg of a concentrate supplement vs. cows fed with 7 kg of a starch rich supplement. The increase in propionate concentration due to supplementing the grazing cows is caused by the rapid degradation of starch by the amylolytic bacteria, which tends to produce propionate as its end product of utilising carbohydrates, because this produces more energy per unit of time. Amylolytic activity in the rumen is stimulated by a low pH

while the acetate concentration decreases due to the lower pH, which affects the activity and the efficiency of the cell wall degrading bacteria that produce mainly acetate.

Figure 3 Least squares means for ruminal concentration of acetate determined at 30 minutes intervals in grazing time (3 h) for lactating cows. Each point represents the mean of ten observations. (For abbreviations see Table 1)

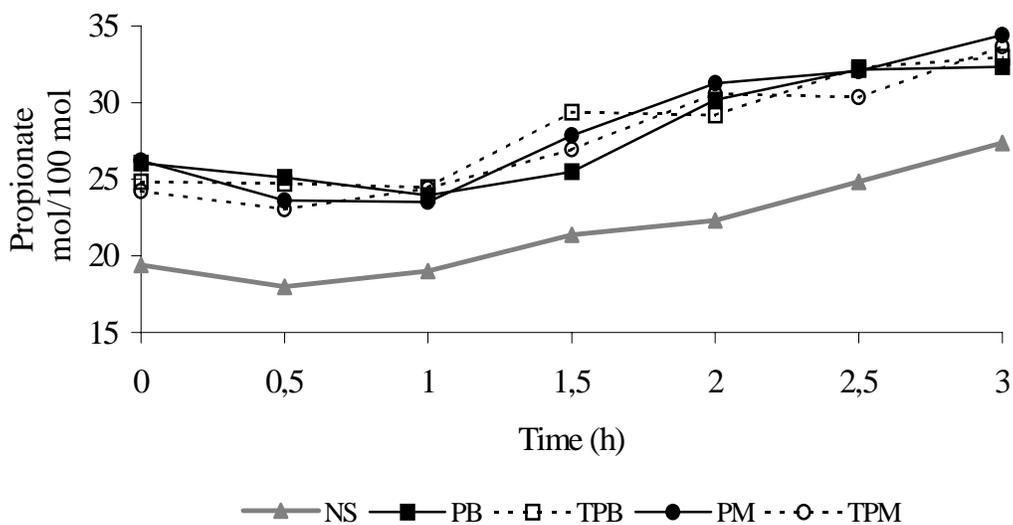


Hoover and Stokes (1991) suggested that the presence of an alternate, more readily digested carbohydrate could cause an initial inhibition of cellulose digestion. Also lower pH affects negatively the proteolytic activity of the rumen microbes and decreases protein degradation, this will cause a decrease in the branched chain VFA concentrations like isobutyric acid. Stokes *et al.* (1991), Bach *et al.* (1999) and Ariza-Nieto *et al.* (1998) also found a higher molar proportion of propionate when increasing amounts of non structural carbohydrates were supplied to ruminal microbes that were maintained in a continuous culture system. Dijkstra (1994) concluded in his review that fermentation of structural carbohydrates compared to fermentation of starch yielded high amounts of acetate and low amounts of propionate. France and Siddons (1993) showed that the fermentation pattern is determined by the basal diet, particularly the type of dietary carbohydrates. High fibre forage diets encourage the growth of acetate producing bacterial species and the acetate:propionate:butyrate ratio will be in the region of 70:20:10, whereas starch rich diets favour the development of propionate producing bacterial species, and are

associated with an increase in the proportion of propionate at the expense of acetate. Isobutyrate concentration was significantly higher ($P < 0.05$) in NS animals than in supplemented animals and isovalerate showed also numerically higher values. Isobutyrate and isovalerate are the end products of protein degradation in the rumen (Umbarger, 1978), these results indicate that rapid ruminal fermentation of starch in the rumen originating from heat treated cereal grains might have reduced protein losses.

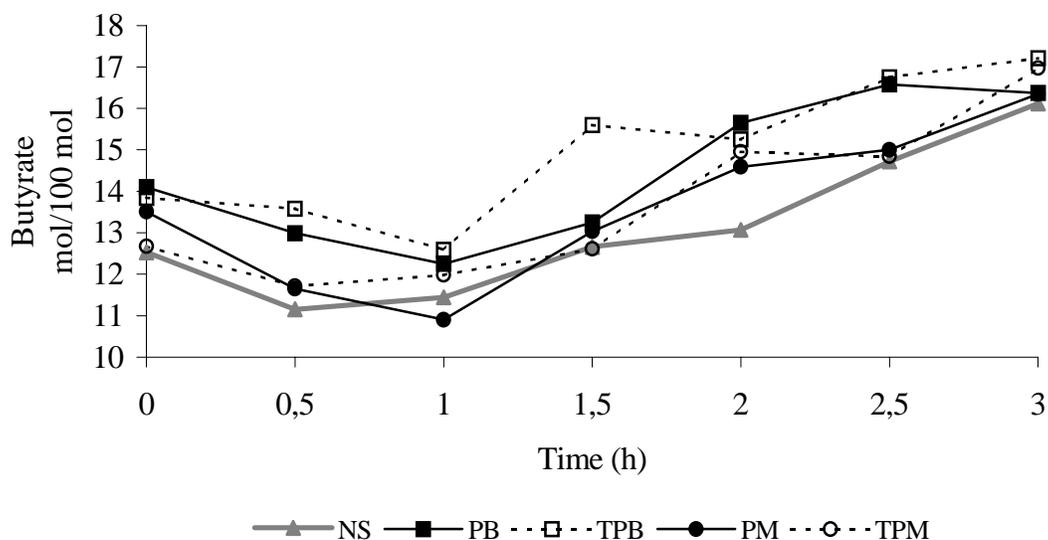
The type of starch source and heat processing method had no significant effect ($P > 0.05$) on the A:P ratio, neither on the NGR ratio. The acetate molar proportions were around 61% and the propionate around 24% regardless the type of the supplementation or the method of processing. These observations are in agreement with data reported by Casper *et al.* (1999) and DePeters and Taylor (1985) who reported similar molar proportions of VFA when cows were fed diets based on either ground maize or barley. However Casper and Schingoethe (1989), Casper *et al.* (1990) and McCarthy *et al.* (1989) reported greater propionate concentrations for early lactation dairy cows that were fed barley compared to those that were fed with maize.

Figure 4 Least squares means for ruminal concentration of propionate determined at 30 minutes intervals in grazing time (3 h) for lactating cows. Each point represents the mean of ten observations. (For abbreviations see Table 1)



These similar VFA ratios or molar proportions regardless the type of supplement, may be due to greater fractional passage rates of solids from the rumen for cows fed barley as reported by Casper *et al.* (1999), and due to the greater starch concentration in maize compared to barley. Crocker *et al.* (1998) reported a significant increase in the molar proportion of propionate and a decrease in the molar proportion of acetate, and NGR when maize was heat treated. Joy *et al.* (1997) investigated the concentration of VFA within the rumen for steam flaked and dry rolled maize, and found that the steam treatment increased the molar percentage of propionate whilst the concentrations of acetate and isovalerate declined. But as in this study the comparison is between two types of processing that both require heat treatment, no significant differences were shown according to the processing method.

Figure 5 Least squares means for ruminal concentration of butyrate determined at 30 minutes intervals in grazing time (3 h) for lactating cows. Each point represents the mean of ten observations. (For abbreviations see Table 1)



3.2. Ruminant ammonia concentration

Grazing pasture grass only resulted in significantly higher ($P < 0.001$) ruminal ammonia concentration compared to supplemented dairy cows all over the experimental day and in both phases of the experiment, grazing and starvation (Table 2). Higher values of ammonia concentration have been observed in grazing, non supplemented lactating dairy cows (Rearte and Santini, 1989; Van Vuuren *et al.*, 1986). In agreement with the observations of Chilbroste (1999) our results also show that in the morning grazing session after the starvation during milking the ammonia concentration in the rumen liquid increased with time (Figure 6).

Table 2 Ruminal ammonia concentration and ammonia to TVFA ratio of dairy cows grazing grass pasture¹, and supplemented with processed cereal grains²

	NS	Barley grain		Maize grain		SEM	P ³		
		PB	TPB	PM	TPM		G	H	GxH
NH ₃ -N mg/l (D) ⁴	180.9 ^S	87.8	79.6	72.0	66.9	9.4	0.2	0.5	0.9
NH ₃ -N mg/l (G)	178.7 ^S	81.7	78.4	67.3	66.2	8.8	0.2	0.8	0.9
NH ₃ -N mg/l (S)	185.7 ^S	104.8	81.8	87.4	72.2	15.7	0.4	0.3	0.8
NH ₃ -N mMol/l (D)	12.9 ^S	6.3	5.7	5.1	4.8	0.7	0.1	0.5	0.9
NH ₃ -N/TVFA ratio	11.7 ^S	5.4	4.6	4.5	4.5	0.5	0.1	0.5	0.8

¹ NS: no supplement addition.

² PB: pelleted barley, TPB: toasted and pelleted barley, PM: pelleted maize, TPM: toasted and pelleted maize.

³ G: effect of grain type, H: effect of type of heat treatment, GxH: effect of grain type and heat interaction.

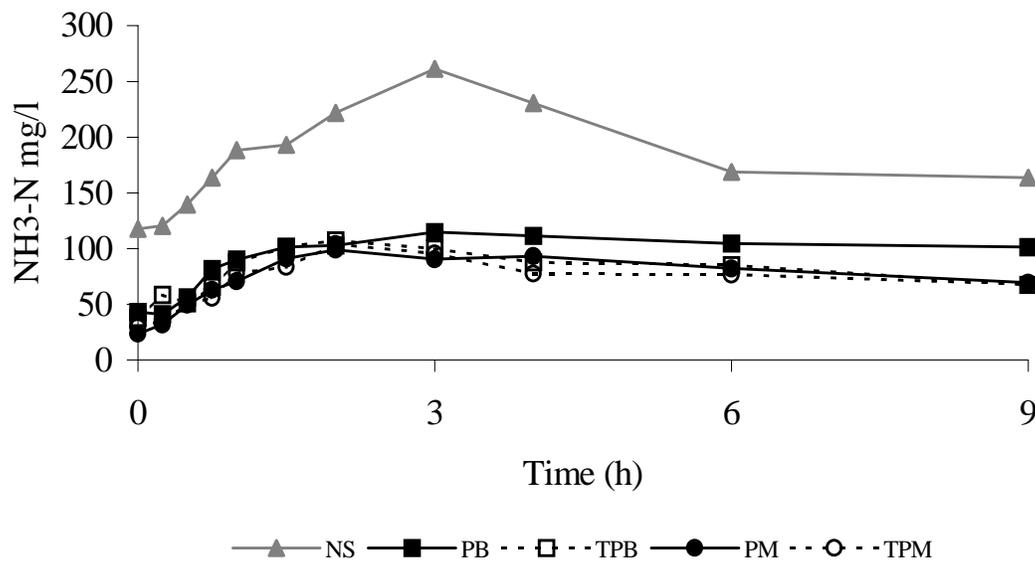
⁴ D: all experimental day (9 h), G: grazing time (3 h), S: starvation (6 h)

^S figure with superscript is significantly ($P < 0.001$) different from others.

The high ruminal ammonia concentration caused by pasture grass grazing supports the hypothesis that the protein of pasture grass is highly and extensively degraded in the rumen (Beever and Siddons, 1986; Van Vuuren *et al.*, 1986). It also supports the hypothesis that pasture grass is an unbalanced diet, which does not contain sufficient energy to make use of its degradable protein. Lopez *et al.* (1991) and Siddons *et al.* (1985) reported that protein from the pasture was rapidly and extensively degraded in the

rumen, which resulted in high ruminal ammonia concentrations, of which a large proportion was in the non-ionised form because of the higher pH associated with feeding pasture grass. Bach *et al.* (1999) also reported a high ruminal ammonia concentration associated with feeding pasture grass only, which was correlated with the rapid and extensive degradability of pasture protein.

Figure 6 Least squares means for ruminal ammonia concentration determined at 30 minutes intervals in grazing time (3 h) and hourly the starvation time (6 h) for lactating cows. Each point represents the mean of ten observations. (For abbreviations see Table 1)



Unsupplemented cows had a peak in rumen NH₃-N at the end of grazing period (Figure 6), indicating rumen proteolysis of pasture after a period of high grazing activity following the morning milking. In contrast supplemented cows had a more constant pattern of NH₃-N in the rumen, indicating the improved utilization of NH₃-N by the energy provided with concentrate or a different diurnal pattern of grazing resulting from supplementation. Feeding heat treated grains decreased pH which also affects negatively the proteolytic activity of the microbes and decreases its ability to degrade protein. These results were in agreement with Hoover and Stokes (1991). Bach *et al.* (1999) found a

decrease in ruminal ammonia concentration when they supplemented pasture with cracked maize and beet pulp. This decrease in ruminal ammonia was attributed to differences in bacterial N utilisation and to the adequate amount of energy made available to the microbes to capture most of the ammonia from ruminal fluid.

The concentrations of ruminal ammonia with the supplemented diets were more than 50 mg/l reported by Satter and Slyter (1974) as the minimum ammonia concentration required in the rumen to ensure maximum microbial growth. However Russel *et al.* (1983) found no differences in microbial growth when ammonia concentration in the rumen were below 50 mg/l or greater than 160 mg/l.

No significant differences ($P>0.05$) in ruminal ammonia concentration according to the starch source were found. But processed maize (PM and TPM) tended to have a greater effect than barley (PB and TPB), since it showed the lowest ruminal ammonia concentration all over the experimental day. However Casper *et al.* (1999) and McCarthy *et al.* (1989) found higher ruminal ammonia concentration when cows were fed maize compared with barley.

It was expected that barley would have a greater effect on the decrease of ruminal ammonia concentration than maize. Especially in the grazing phase due to the rapid degradation of its starch by microbes in comparison to maize as was reported by Herrera-Saldana *et al.* (1990) and McCarthy *et al.* (1989). But as shown in Table 2 maize had a greater effect than barley in both phases. This might have been due to the fact that barley has a higher concentration of ruminal degradable protein compared to maize (NRC, 2001) which might have been the cause of higher ruminal ammonia when barley was fed. Also the higher concentration of starch in maize compared to barley may have caused the greater effect of maize on lowering ruminal ammonia concentration during the grazing phase. Another explanation may be what was reported by Casper *et al.* (1990) that barley had the same rate of NSC degradation as maize. They concluded that differences in NSC degradability appear to exist in barley, which is caused by variety, and growing conditions. It must be kept in mind as well that the cereal grains used in this experiment were processed which is expected to have bigger effects on maize than on barley.

No significant differences ($P>0.05$) in the ruminal ammonia concentration were found between the processing methods. But in general toasting and subsequently pelleting had a

greater effect in lowering the ruminal ammonia concentration than did pelleting only, regardless of the grains. This may have been due to the higher *in situ* potentially degradable fraction (D) caused by the pressure toasting and subsequently pelleting procedure compared to pelleting only (Tóthi *et al.*, 2002a; Goelema *et al.*, 1999). Or due to the shift in protein digestion caused by pelleting, which shifts protein digestion from the intestine to the rumen making more cereal grain protein available for degradation in the rumen and higher ruminal ammonia for pelleted grains than for toasted and subsequently pelleted. Also it seems that toasted and pelleted grains synchronised the release of energy all over the grazing phase and all over the experimental day.

Regardless the type of the cereal grains it was shown clearly that ruminal ammonia concentration was higher during the starvation period than that during grazing. This might have been caused by the time the supplements were offered. The supplements were offered at the morning before grazing at 7:00 h, which made more energy available in the early morning and during the grazing phase for the microbes to make use of the ruminal ammonia. Less energy which comes through the degradation of the cell wall components of pasture grass was available during the starvation. When no supplement was offered ruminal ammonia concentration during grazing was also lower than that of the starvation. The ruminal ammonia concentration is affected by many complex integrated processes in the rumen, such as degradation of feed protein, deamination of feed amino acids, synthesis of microbial protein, absorption of ammonia through the rumen wall, recycling of ammonia and urea through saliva and rumen wall and bypass of ammonia to the omasum. So maybe energy availability and recycling of ammonia and urea were the cause of the higher ruminal ammonia concentration during starvation.

The ratio of ammonia to the TVFA was significantly higher ($P < 0.001$) when the cows were not supplemented (Table 2). This was due to the greater ruminal ammonia concentration and the lower TVFA concentrations. The high ammonia to TVFA ratio shows the unbalanced protein to energy characteristics of the feed.

Supplementation lowered the ammonia (mg/l) to TVFA (mmol/l) ratio from 12 to around 5. Because supplementing increased the TVFA production and concentration in the rumen and decreased the ruminal ammonia concentration all over the experimental

day making feed more balanced and synchronising the availability of energy with rumen degradable protein.

No significant differences ($P>0.05$) in ammonia to TVFA ratio were shown according to the heat treated grain source, neither to the processing method. Maize feeding had a slightly lower ratio than barley (4.5 and 4.5 vs. 5.4 and 4.6). The lower ammonia to TVFA ratio caused by feeding processed maize (PM and TPM) was mainly due to the decrease in ruminal ammonia concentration. While in feeding processed barley (PB and TPB), a moderate increase in the TVFA concentrations in addition to the decrease in ruminal ammonia concentration was the cause of the low ammonia to TVFA ratio. Although we did not find significant differences between the supplements in ammonia to TVFA ratio it has to be pointed out that PM and TPM had a lower ratio than PB or TPB which suggests that heat treated maize supplementation synchronised the energy with the rumen degradable protein better than did barley.

4. Conclusions

Pasture grass for lactating dairy cows is an unbalanced diet in terms of protein and energy. Its protein is rapidly and extensively degraded in the rumen. It gives rise to ammonia in the rumen resulting in a high concentration of ammonia of which a large proportion is in the non-ionised form. It has a high ammonia to TVFA ratio, which results from the high ruminal ammonia concentration and the low ruminal VFA concentration caused by its feeding. Grazing without any supplementation results in poor utilisation of its protein.

Supplementing pasture grass with differently heat processed cereal grains decreased the pH of rumen fluid and ammonia concentration, ammonia to TVFA ratio, isobutyrate proportions as a percentage of the TVFA, A:P and NGR ratio. While it increased TVFA concentrations, propionate, butyrate and valerate proportion as a percentage of the TVFA, making more energy available for the microbes to utilise and incorporate ammonia into microbial protein. And making more energy available for the host animal since the production of propionate is not associated with energy loss in the form of methane. Supplementation with processed maize had a similar VFA pattern and A:P ratio as did

supplementing with processed barley, except that barley supplementation had greater total VFA concentrations in the rumen all over the day. Ruminal ammonia concentration and ammonia to TVFA ratio were lower when pasture grass was supplemented with processed maize than with pelleted barley, which shows that processed maize with pasture grass is a more balanced and synchronised diet than only pelleted barley with pasture grass. Processing method resulted in similar VFA pattern, A:P ratio, and TVFA concentrations in the rumen.

From the results of this experiments it seems that differences in ruminal fermentation which may have existed between maize and barley before heat processing disappeared. Moreover processed maize supplementation may have synchronised the energy with the rumen degradable protein better than did barley. It should be noted that the higher solubility and degradability of starch from barley in comparison to maize that was observed with the *in situ* method (Tóthi *et al.*, 2002b), was not reflected in ruminal parameters measured *in vivo* in lactating dairy cows.

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CHAPTER 7

Effect of Feed Processing on Volatile Fatty Acid Production Rates Measured with ¹³C-acetate in Grazing Lactating Dairy Cows

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Abstract

The effects of processed cereal grain supplementation on volatile fatty acid (VFA) production rates of grazing, lactating Holstein-Friesian cows were measured in a 5 x 5 Latin square experiment. The experimental treatments were the following: control (only grazing, no supplement addition, NS), pelleted barley (PB), pelleted maize (PM), toasted and subsequently pelleted barley (TPB), and toasted and subsequently pelleted maize (TPM) as supplements. An isotope dilution technique using ^{13}C -acetate as a marker was employed for the estimation of VFA production rates. At the beginning of a 3-hour long allowed grazing time, 100 mg of 99% enriched $^{13}\text{C}_2$ Na-acetate was introduced in the rumen and this was repeated after grazing with 50 mg of 99% enriched $^{13}\text{C}_2$ Na-acetate, after which the cows were starved 6 hours until the evening milking. The marker was placed into the evacuated rumen fluid and thoroughly mixed with the evacuated rumen content mass. Rumen liquid samples were taken each half hour during grazing and every hour during the starvation period. During grazing disappearance rate (k_{dis}) and production rate (k_{prod}) of acetate, propionate and butyrate were significantly higher ($P < 0.05$) in supplemented cows than in NS cows. Moreover the effect of barley grain and pelleting treatment was higher than the effect of maize grain and toasting. During starvation significantly higher ($P < 0.05$) k_{dis} and k_{prod} of VFAs were observed in PM and TPM treatment. Total VFA production for the experimental period (grazing + starvation) were 49.5, 78.7, 69.9, 88.5, 80.8 mol/day for NS, PB, TPB, PM and TPM, respectively. The higher VFA productions measured in supplemented animals emphasise the extensive digestion that occurs in the rumen after feeding processed grains. In methodological terms, $^{13}\text{C}_2$ Na-acetate labelling, provided the results are interpreted with caution, appears to be a useful means for examining the VFA acetate production in ruminants.

Keywords: Dairy; Grazing; Supplementation; VFA production; Stable isotope

1. Introduction

Microbial activity in the rumen of dairy cows results in the degradation of hexose (arising from the digestion of water-soluble carbohydrates, starch and fibre, from amino acid deamination, and from lipid hydrolysis) and pentoses (France and Siddons, 1993). These monomers are used by the microbial population for the synthesis of microbial biomass, or to provide ATP for both microbial maintenance and growth requirements. Hexose fermentation results in the formation of volatile fatty acids (VFA), methane and CO₂. The mixture of VFA formed depends on the chemical composition of the substrate, the pH in the rumen and the microbial population that develops (Murphy *et al.*, 1982). Variation of fermentation in the rumen is much greater than in the lower gut (Drochner and Meyer, 1991). Acetate, propionate and butyrate are the predominant acids arising from ruminal fermentation of hexose. Their concentration and relative proportions is related to the level of feed intake (Sutton, 1985) and many other factors (Dijkstra, 1994). Rate of change in ruminal VFA concentrations is the difference between their rates of production from fermentation and rates of absorption and passage. The majority of the VFA produced in the rumen are absorbed from the rumen through the rumen wall by simple diffusion of the undissociated acids (Stevens, 1970; Allen, 1997). Ruminal pH and osmolality, type and concentration of VFA significantly affect absorption rates of VFA from the rumen (Thorlacius and Lodge, 1973). A certain proportion of VFA passes to the omasum and abomasum and is absorbed from these organs (Weston and Hogan, 1968, Erdrise and Smith, 1977). The absorbed VFA are important because they provide two-thirds of the energy supply of the ruminant (Sutton, 1985). Because of the importance of VFA in ruminant metabolism several methods have been developed to measure VFA production in the rumen.

Indirect methods make use of the stoichiometric relationships which exist between the production of VFA and the production of methane (Webster, 1978) or between the production of VFA and the amount and composition of the mixture of substrates fermented (Murphy *et al.*, 1982). The agreement between VFA molar proportions in rumen fluid and VFA proportions produced have been questioned, because the proportions of VFA in the rumen may not precisely reflect their relative production rates,

especially when the diet contains a substantial proportion of concentrates (Sutton, 1985). Direct methods based on various isotope-dilution techniques using ^{14}C labelled VFA have been most commonly used.

Among stable isotopes the isotopes of carbon and nitrogen are most frequently used as biological tracers. These two elements are found in the earth, the atmosphere, and all living organisms. Each has a heavy isotope (^{13}C and ^{15}N) with a natural abundance of ~1% or less and a light isotope (^{12}C and ^{14}N) that makes up all of the remainder, in the case of nitrogen, or virtually all in the case of carbon (carbon also has a radioactive isotope, ^{14}C). Stable isotopes have the advantage over other tracers that they are not radioactive. ^{14}C is hazardous and subject to many regulations, licensing requirements and rules for safe disposal of radioactive wastes (Smith, 1989). Therefore the primary advantage of using ^{13}C rather than ^{14}C as a marker is that no health and regulatory constraints are present when working with the stable isotope ^{13}C . The ^{13}C labelling technique was initially used in agronomy (Thompson, 1996; Svejcar *et al.*, 1990; Deleens *et al.*, 1994), but became gradually also applied in animal nutrition after labelling forages. Appropriate aspects to be investigated are the digesta passage, the diet digestibility and carbon metabolism in the body (Boutton, 1991a; Svejcar *et al.*, 1993; Südekum *et al.*, 1995). With ^{13}C enriched VFA the ruminal VFA production can also be measured (Chen *et al.*, 1997; Breves *et al.*, 1987; Kristensen, 2001).

Supplementation of lactating grazing dairy cows with processed cereal grains supposedly effects the ruminal fermentation and VFA production. Therefore the objective of this study was to quantify the influence of fresh grass and processed cereal grains on the production of VFA in the rumen of grazing, lactating dairy cows using $^{13}\text{C}_2$ Na-acetate as a marker.

2. Materials and methods

2.1. Animals and management

The experiment was carried at the experimental farm 'De Ossekampen' of Wageningen University, The Netherlands. Five multiparous lactating Holstein-Friesian dairy cows fitted with a large rumen cannula (10 cm id., Bar-Diamond Inc., Parma, Idaho, USA) were

used. Two cows were in their 7th and the others were in 2nd, 4th and 6th lactation, respectively. At the beginning of the experiment the cows produced 28.6 ± 4.6 kg/day milk and averaged 173 days post partum. The animals were milked twice daily at 6:30 h and 17:00 h.

2.2. Experimental design

The experiment was a 5x5 Latin Square design with five cows, five treatments and five periods. Each experimental period consisted of 14 days. Days 1 to 9 were used for adaptation and days 10 to 14 for sample collection.

2.3. Treatments and feed processing

The five treatments were the following: control (no supplement addition, NS) pelleted barley (PB), pelleted maize (PM), toasted and subsequently pelleted barley (TPB), and toasted and subsequently pelleted maize (TPM). Grain processing was carried out at Wageningen Feed Processing Centre (WFPC). A laboratory scale pressurised toaster was used for pressure toasting the grains for 1.5 minutes at 135 °C. After toasting, the grains were dried in a forced air oven for 16 h at 35 °C, and followed by pelleting. Pelleting (80 °C, 10 s) was carried out with a 5 x 65 mm (bore x hole) die, using a V2-30 pelleting press (Robinson milling systems B.V., Boxtel, The Netherlands).

2.4. Sample collection

Next to a control treatment of grazing only, the four forms of processed grains were fed as a supplement in two equal portions of 3 kg each in the milking parlour during the morning and evening milking. In the 10 days long adaptation period the cows were allowed to graze freely with the herd in a pasture of perennial ryegrass. On days 11 and 13 (also days 12 and 14), rumen evacuations were conducted after morning milking in the same sequence with a time interval of 30 minutes. After rumen evacuation, at 8:00 h, cows were allowed to graze individually, tethered within a circular plot of a fixed area

with a radius of six meters. This method was used earlier by different researchers (Forbes, 1988; Dougherty *et al.*, 1992) and the procedure was further developed by Chilibröste (1997). After the grazing period at 11:00 h each cow was removed from the experimental grazing plot and brought to the barn and rumen evacuations were repeated. Then the animals were kept inside the barn and starved until 17:00 h at which time rumen evacuations were repeated again. Manual emptying of the rumen contents (three times per cow per day) was performed according to the procedure described by Borsting and Weisbjerg (1989). After the last rumen evacuations of the experimental day the cows were allowed to graze freely in the same experimental pasture of perennial ryegrass until the next morning. During the starvation period the animals had free access to water and mineral blocks (KNZ Liksteen).

Immediately before the beginning of the grazing period, starting at 8.00 a.m., 100 mg of 99% enriched $^{13}\text{C}_2$ Na-acetate (Euriso-top, Bât, France) was introduced in the rumen for estimation of VFA production. The marker was put into the evacuated rumen fluid and this was added to the evacuated rumen content mass during thorough hand mixing of the material in the plastic container. After mixing the rumen content was backpacked to the cows. This enrichment procedure was repeated at 11:00 h after 3 hours of grazing with 50 mg of 99% enriched $^{13}\text{C}_2$ Na-acetate. Rumen liquid samples were taken each half hour starting at 8:00 h until 11:00 h then every hour until 17:00 h (12 samples per cow per day).

2.5. Chemical analyses

Rumen liquid samples were kept frozen for isolating VFA by distillation with the Kjeldahl distillation equipment. After the Kjeldahl distillation equipment had been distilled clean with water, a 100 ml volumetric flask was placed under a tygon tubing outlet of the distilling unit. An aliquot of 50 ml of rumen liquid was centrifuged at 3000 rpm for 10 minutes. After centrifugation the supernatant was poured into a weighed glass beaker. The weight of the rumen liquid in the beaker was reduced to about 40 g, by taking out the excess with a Pasteur-pipette. The residual sample in the beaker was weighed accurately and transferred to a 750 ml Kjeldahl distillation flask via a funnel. The beaker was rinsed clean with 35 ml demiwat. This water was also brought into the Kjeldahl

flask. Then about 40 g Na₂SO₄ (Merck nr. 106649) was weighed in a beaker and brought into the Kjeldahl flask using a powder funnel. Also a few boiling chips were added to the distillation mixture. Then 25 ml of 96% concentrated sulphuric acid was added to the Kjeldahl flask. The flask was rapidly attached to the distillation unit and its contents mixed. The mixture was distilled until the distillation flask was almost full with foam and white fumes were visible in the splashing device on top of the distillation flask. The distillation flask was then removed from the distillation unit and another one with boiling water was attached to it to distillate the residues of VFA into the volumetric flask. When this flask was almost filled up to the mark, it was removed from the distillation unit. To the volumetric flask about 4 drops of concentrated phosphoric acid were added and then the flask was filled up to the mark with demiwaterr and mixed well. A part of this solution was put in a small bottle and stored in the fridge for further analysis for stable isotope. After isolating VFA by distillation, samples were analysed using isotope ratio mass spectrometry (IRMS) as described by Boutton (1991b) to determine the ¹³C to ¹²C ratios.

2.6. *Mathematical treatment of the data*

2.6.1. *Calculations for ¹³C enrichment*

For the estimation of acetate production rates, the procedure based on the stable isotope ¹³C labelling technique developed by Chen *et al.* (1997) was used. This procedure is based on the following principle: in the rumen organic matter is fermented continuously and VFA is produced and enters the rumen fluid. At the same time VFA disappears from the rumen by being absorbed through the rumen wall and by passing out with the liquid phase. If a certain amount of ¹³C enriched acetate is introduced into the rumen, as a result the total acetate pool will become enriched, and ¹³C enriched acetate (like ¹²C acetate) will disappear by absorption and outflow. Acetate production is assumed to occur at a fixed fractional rate (k_{prod}) and is estimated from the decline in the ¹³C to ¹²C ratio.

The percentage enrichment with ¹³C was calculated for each collection time in each cow in each respective period. From IRMS we had the total carbon concentration in each sample of ruminal fluid and the percentage of ¹³C present into these samples. We took the

first evacuation time (8.00 a.m. as time zero) as the basal level of enrichment. All values for ^{13}C enrichment found at collection times were corrected by subtracting this basal value. Regression equation was applied to these corrected values to find the fractional rate of enrichment disappearance. All fractional rates were calculated by the NLIN procedure of the SAS package (SAS Institute INC, 1995).

The disappearance rate (k_{dis} , h^{-1}) of the acetate was calculated for the grazing and starvation period as:

$$k_{\text{dis}} = \frac{\ln QHAc_{(0)} - \ln QHAc_{(t)}}{t} + k_{\text{prod}}$$

Where $QHAc_{(t)}$ is the acetate pool in the rumen at the 2nd or 3rd evacuation time in moles, $QHAc_{(0)}$ is the acetate pool in the rumen at 1st or 2nd evacuation time in moles, k_{prod} is the acetate production rate (h^{-1}), t is time in hours (up to 3 h in the grazing period and up to 6 h in the starvation period).

During grazing associated with the effect of protein and carbohydrate availability, bacteria shift the pathways in response to changes in pH. Rumen pH influences rates of VFA absorption (Bergman, 1990; Dijkstra *et al.*, 1993), therefore we assumed that the disappearance of propionate and of butyrate was 1.5 times that of acetate. This value of 1.5 is based on results at $\text{pH} = 6.3$ and $\text{pH} = 5.4$ reported by Dijkstra *et al.* (1993). We calculated the k_{prod} for propionate and butyrate then the VFA production as follows:

$$QVFA_{\text{prod}} = \frac{k_{\text{prod}}}{(k_{\text{prod}} - k_{\text{dis}})} \times QVFA_{(0)} \times e^{(k_{\text{prod}} - k_{\text{dis}})t} - 1$$

Where $QVFA_{\text{prod}}$ is the individual VFA production (acetate, propionate or butyrate) in moles, k_{dis} is the disappearance rate (h^{-1}), k_{prod} is the production rate (h^{-1}), $QVFA_{(0)}$ is the VFA pool of the rumen at the beginning of grazing time or starvation time in moles, t is time in hours (up to 3 h in the grazing period and up to 6 h in the starvation period).

2.6.2. Calculations for hexose fermentation

To validate or refute our estimates of VFA production measured with the ^{13}C labelling technique we calculated fermentable organic matter (FOM) from *in vivo* and *in situ*

measurements and from VFA production measurements using several approaches. In the first approach our estimation was based on the assumption that the fermentation of organic matter to VFA is from protein, neutral detergent fibre (NDF) and non-structural carbohydrate (NSC), sugar and starch.

We assumed that the iso-acids are coming from protein deamination, therefore we excluded iso-butyric, methyl butyric, valeric and iso-valeric acid. Then we assumed that the entire fermented pasture grass and processed cereal grains were composed of hexose polymers, with a molecular weight of 162 per hexose unit. Based on stoichiometry we assumed that one mole of hexose produces two moles of propionate, two moles of acetate or one mole of butyrate. Finally we assumed that 20% of the substrate degraded is converted into bacterial cells (Tamminga *et al.*, 1994). Based on these assumptions total hexose (kg) fermented in the rumen of lactating dairy cows was calculated from VFA productions by using the following equation:

$$FOM_{VFA} = \frac{HAc}{2} \times 162 + \frac{HPr}{2} \times 162 + HBu \times 162 \quad (\text{Eqn. 1})$$

Where FOM_{VFA} is the amount (kg/period) of hexose fermented to principal VFA, HAc, HPr and HBu is the volatile fatty acids (mol/period) and the hexose molecule in polymer form has a molecular weight of 162.

In the second approach the available organic matter in grazing and starvation period was calculated from the *in vivo* measurements (Tóthi *et al.*, 2002a) using the following formulas:

$$\text{grazing period: } FOM_A = ((OM_{BG} + OM_{GI}) - OM_{AG})(k_d / k_d + k_p) \quad (\text{Eqn. 2})$$

$$\text{starvation period: } FOM_A = (OM_{AG} - OM_{AS})(k_d / k_d + k_p) \quad (\text{Eqn. 3})$$

Where FOM_A is the amount (kg/period) of hexose fermented to principal VFA, OM_{AG} is the organic matter pool in the rumen after grazing, OM_{BG} is the organic matter pool in the rumen before grazing, OM_{AS} is the organic matter pool in the rumen after starving, OM_{GI} is the grass intake, k_d = rate of degradation of organic matter in grazing time, k_p = rate of passage, calculated based on lignin clearance from the rumen (Tóthi *et al.*, 2002a) after correction for 86% recovery of lignin according to Van Soest (1994).

In the third approach we assumed that the water-soluble fraction of the grass was instantly and totally degraded in the rumen, therefore we modified the FOM_A calculation formula with *in sacco* data (Tóthi *et al.*, 2002b) as follows:

$$FOM_B = ((OM_{BG} + OM_{GID}) - OM_{AG})(k_d / k_d + k_p) + (OM_{GIW}) \quad (\text{Eqn. 4})$$

Where OM_{GID} is the potentially degradable part of the grass intake and OM_{GIW} is the water soluble part of the grass intake.

In the fourth approach we assumed that rumen degradation of the D fractions of each OM component (rumen content (RC), fresh grass (GR) and supplements (SU)) occur according to a first order kinetic function as measured previously in our *in sacco* experiments (Tóthi *et al.*, 2002b).

$$FOM_{RC} = OM_{BG} (Dk_d(k_d+k_p)(1-e^{-(k_d+k_p)t})) \quad (\text{Eqn. 5.1})$$

$$FOM_{GR} = OM_{GI} (W + Dk_d(k_d+k_p)(1-e^{-(k_d+k_p)t})) \quad (\text{Eqn. 5.2})$$

$$FOM_{SU} = OM_{SI} (W + Dk_d(k_d+k_p)(1-e^{-(k_d+k_p)t})) \quad (\text{Eqn. 5.3})$$

$$FOM_C = FOM_{RC} + FOM_{GR} + FOM_{SU} \quad (\text{Eqn. 5.4})$$

Where W is the water soluble fraction, D is the potentially degradable fraction, k_d is the degradation rate in the rumen of D fraction (/h), k_p is the rate of passage arbitrarily assumed to be 6%/h for supplements and 4.5%/h for grass, t is grazing time 3 h, OM_{SI} is the organic matter intake from supplements.

2.7. Statistical analysis

All data were subjected to least squares ANOVA for a 5 x 5 Latin square design using the GLM procedure (SAS Institute INC, Cary, NC). Sources of variation in the statistical analyses were cow, period and treatment (grain type, heat treatment, interaction of heat and grain). When significant differences due to the treatment were detected, the multiple comparison procedures (Tukey) were used. Results are given as least square means. Treatment effects were separated using the PDIFF statement and considered statistically different at $P < 0.05$.

3. Results and discussion

3.1. Disappearance and production rates of individual acids

Table 1 shows the disappearance rates (k_{dis}) and production rates (k_{prod}) of acetate, propionate and butyrate in the rumen of dairy cows fed only grass or grass supplemented with processed cereal grains. During the grazing period k_{dis} of VFAs were lower than the k_{prod} for each individual VFA because the VFA pool size increased during grazing time when cows were eating and new substrate entered and was fermented in the rumen. In comparison to NS animals PB, PM and TPM supplementation significantly increased ($P < 0.05$) k_{dis} of acetate, propionate and butyrate. The k_{prod} of PB, PM and TPM supplemented cows were also significantly higher ($P < 0.05$) than NS cows probably because of the availability of more fermentable substrate (starch) at grazing time. Supplementing pasture grass with pelleted cereal grains resulted in numerically higher k_{prod} and k_{dis} than those with toasted grains.

In the starvation period k_{dis} of VFAs were higher in each treatment than k_{prod} because the VFA pool size decreased during the starvation period. The differences in k_{dis} which existed in the grazing period between NS and supplemented cows with barley grain were eliminated, while k_{dis} of PM and TPM were still significantly higher ($P < 0.05$) than NS animals and than barley (PB and TPB). This phenomenon also was observed in k_{prod} of VFAs because k_{prod} of acetate, propionate and butyrate of TPM were significantly higher than those of NS and barley. A possible explanation for that might be the higher starch concentration and slower ruminal starch degradation rate of maize, which makes more fermentable substrate available for the microbes during the starvation period.

During grazing time pelleted cereal grains supplemented animals had a higher k_{dis} and k_{prod} than toasted and subsequently pelleted cereal grains supplemented cows. However this was inverted during the starvation period and toasted and subsequently pelleted cereal grains supplemented cows had higher k_{dis} and k_{prod} than pelleted cereal grains supplemented animals. This might indicate that toasting had a protective effect on the starch in maize and barley resulting in delaying its fermentation for a while.

Table 1 Disappearance (k_{dis}) and production (k_{prod}) rates (/h) of acetate (HAc), propionate (HPr) and butyrate (HBu) in the rumen of dairy cows grazing grass pasture¹, and supplemented with processed cereal grains²

	NS	Barley grain		Maize grain		SEM	P ³		
		PB	TPB	PM	TPM		G	H	GxH
GRAZING									
HAc k_{dis}	0.69 ^S	1.34	0.94	1.19	1.08	0.14	0.9	0.1	0.3
HPr k_{dis}	1.04 ^S	2.00	1.14	1.79	1.63	0.21	0.9	0.1	0.3
HBu k_{dis}	1.04 ^S	2.00	1.14	1.79	1.63	0.18	0.9	0.1	0.4
HAc k_{prod}	0.80 ^S	1.35	1.04	1.30	1.14	0.12	0.7	0.1	0.5
HPr k_{prod}	1.18 ^S	2.03	1.55	1.93	1.72	0.17	0.8	0.1	0.5
HBu k_{prod}	1.14 ^S	2.01	1.52	1.91	1.69	0.17	0.8	0.1	0.5
STARVING									
HAc k_{dis}	0.62 ^T	0.62 ^a	0.65 ^a	0.83 ^a	1.04 ^b	0.08	0.008	0.2	0.3
HPr k_{dis}	0.92 ^T	0.94 ^a	0.97 ^a	1.25 ^{ab}	1.56 ^b	0.13	0.008	0.2	0.3
HBu k_{dis}	0.92 ^T	0.94 ^a	0.97 ^a	1.25 ^{ab}	1.56 ^b	0.12	0.007	0.2	0.2
HAc k_{prod}	0.46 ^T	0.54 ^a	0.57 ^a	0.74 ^{ab}	0.97 ^b	0.09	0.01	0.2	0.3
HPr k_{prod}	0.73 ^T	0.84 ^a	0.88 ^a	1.14 ^{ab}	1.48 ^b	0.12	0.009	0.2	0.3
HBu k_{prod}	0.75 ^T	0.84 ^a	0.89 ^a	1.15 ^{ab}	1.49 ^b	0.12	0.01	0.2	0.3

¹ NS: no supplement addition.

² PB: pelleted barley, TPB: toasted and pelleted barley,
PM: pelleted maize, TPM: toasted and pelleted maize.

³ G: effect of grain type, H: effect of type of heat treatment, GxH: effect of grain type and heat interaction.

^S figure with superscript is significantly ($P < 0.05$) different from PB, PM and TPM.

^T figure with superscript is significantly ($P < 0.05$) different from PM and TPM.

^{a,b} figures with different superscript in the same row differ significantly ($P < 0.05$).

3.2. Volatile fatty acid production

In Table 2 the calculated VFA productions (mol/period) are presented. In the grazing as well as in the starvation period the total VFA (TVFA) production and the individual VFA production were higher in supplemented animals than in NS animals. Supplementation with PB resulted in significantly higher ($P < 0.05$) acetate, propionate and butyrate

productions than NS during the grazing period. PB contains the largest water soluble starch fraction (Tóthi *et al.*, 2002a) which is a fast energy source for the ruminal microbes. Based on our *in sacco* experiments (Tóthi *et al.*, 2002b) the rate of degradation of the potentially degradable fraction of OM of PB also was the highest of the grains used in this study, which plays an important role in the VFA production.

Table 2 Acetate, propionate, butyrate and total VFA production (mol/period) in the rumen of dairy cows grazing grass pasture¹, and supplemented with processed cereal grains²

	NS	Barley grain		Maize grain		SEM	P ³		
		PB	TPB	PM	TPM		G	H	GxH
Acetate									
Grazing	14.96 ^S	25.10	18.06	22.71	16.82	3.4	0.6	0.1	0.9
Starving	14.15 ^T	14.82 ^a	18.66 ^{ab}	23.58 ^{ab}	25.22 ^b	2.8	0.02	0.3	0.7
Propionate									
Grazing	6.44 ^S	16.74	10.27	13.21	10.38	2.5	0.5	0.1	0.5
Starving	6.21 ^T	9.22 ^a	10.93 ^{ab}	14.98 ^b	14.73 ^{ab}	1.7	0.02	0.7	0.6
Butyrate									
Grazing	3.86 ^S	8.01	5.84	6.60	5.23	1.1	0.4	0.1	0.7
Starving	3.86 ^T	4.81 ^a	6.10 ^{ab}	7.41 ^{ab}	8.42 ^b	0.9	0.04	0.3	0.9
TVFA ⁴									
Grazing	25.27 ^S	49.86	34.18	42.52	32.44	6.7	0.5	0.1	0.7
Starving	24.24 ^T	28.87 ^a	35.69 ^{ab}	45.98 ^b	48.38 ^b	5.4	0.02	0.4	0.7

¹ NS: no supplement addition.

² PB: pelleted barley, TPB: toasted and pelleted barley,

PM: pelleted maize, TPM: toasted and pelleted maize.

³ G: effect of grain type, H: effect of type of heat treatment, GxH: effect of grain type and heat interaction.

⁴ TVFA (total VFA) = acetate+propionate+butyrate

^S figure with superscript is significantly (P<0.05) different from PB.

^T figure with superscript is significantly (P<0.05) different from PM and TPM.

^{a,b} figures with different superscript in the same row differ significantly (P<0.05).

Compared to the grazing period, during starvation the opposite occurred. The differences between NS and PB were eliminated and due to feeding maize significantly higher ($P < 0.05$) acetate, propionate, butyrate and TVFA productions were observed. Supplementing pelleted cereal grains resulted in higher VFA productions during the grazing period than did toasted grains. In the starvation period TPM resulted in numerically higher values than PM for acetate, butyrate and TVFA production, while PM resulted in slightly higher propionate productions than did TPM. This might mean that toasting has a protective effect on cereal grains starch. In the starvation period the different processing of barley supplementation did not result in differences in acetate and TVFA productions, while TPB resulted in higher propionate, and butyrate productions than PB. Barley supplementation seemed to result in higher TVFA and individual VFA production during grazing than maize. This was reversed in the starvation period as maize grain resulted in higher TVFA and individual VFA production. This is consistent with the fact that even processed maize starch is more slowly degraded in the rumen than barley starch. TVFA productions for the whole experimental period (grazing period + starvation period) were 49.5, 69.9, 78.7, 80.8 and 88.5 mol/experimental day of 9 hours for NS, PB, TPB, PM and TPM, respectively.

The molar proportions of acetate : propionate : butyrate in the grazing period (and in the starvation period) were 60:25:15 (58:26:16) for NS and 50:34:16 (51:32:17), 53:30:17 (52:31:17), 53:31:16 (51:33:16) and 52:32:16 (52:30:17) for PB, TPB, PM and TPM, respectively. These results indicate that supplementation of pasture grass with processed cereal grains favours the development of propionate producing bacterial species and are associated with an increase in the proportion of propionate at the expense of acetate, although acetate is always the most abundant of the acids (France and Siddons, 1993). The higher VFA productions measured in supplemented animals emphasise the extensive digestion that occurs in the rumen after feeding processed cereal grains.

3.3. Hexose fermentation

Table 3 shows the results of different ways of estimating the amount of substrate required for VFA productions. This was done to validate or refute the VFA production

rates we obtained using the ^{13}C labelling technique. The results of FOM_{VFA} which was calculated based on our VFA production data are higher than $\text{FOM}_{\text{A, B, C}}$ values in each experimental period. Calculated FOM_{B} in the grazing period for NS was closer (the differences is 0.47) to FOM_{VFA} than FOM_{A} or FOM_{C} , while for supplemented animals FOM_{C} values are closest to FOM_{VFA} (the differences is 1.55, 0.12, 1.52, 0.45 kg for PB, TPB, PM and TPM, respectively). A number of possibilities might have caused this result.

Table 3 Estimated amount of fermented carbohydrate (kg/period) in the rumen of dairy cows grazing grass pasture¹, and supplemented with processed cereal grains²

	NS	Barley grain		Maize grain	
		PB	TPB	PM	TPM
Grazing					
$\text{FOM}_{\text{VFA}}^3$	2.36	4.69	3.24	3.98	3.05
FOM_{A}^4	1.59	2.35	1.91	1.32	1.96
FOM_{B}^5	2.22	2.87	2.50	1.69	2.43
FOM_{C}^6	1.89	3.14	3.12	2.46	2.60
Starvation					
$\text{FOM}_{\text{VFA}}^3$	2.27	2.73	3.38	4.32	4.60
FOM_{A}^4	1.59	2.57	2.99	2.39	2.16

¹ NS: no supplement addition.

² PB: pelleted barley, TPB: toasted and pelleted barley, PM: pelleted maize, TPM: toasted and pelleted maize.

³ FOM_{VFA} : amount (kg/period) of hexose fermented to principal VFA, calculation based on the results VFA production (see Eqn.1)

⁴ FOM_{A} : amount (kg/period) of hexose fermented to principal VFA, calculation based on rumen OM pools (see Eqn.2)

⁵ FOM_{B} : amount (kg/period) of hexose fermented to principal VFA, calculation based on rumen OM pools, (see Eqn.4)

⁶ FOM_{C} : amount (kg/period) of hexose fermented to principal VFA, calculation based on rumen OM pools, (see Eqn. 5.4)

It seems that we have overestimated the VFA production when using ^{13}C labelled acetate as a marker. But we have to look carefully at the $\text{FOM}_{\text{A,B,C}}$ values as well. The estimation of FOM degradation characteristics requires an assumption for the passage rate. Different components of the OM appear to have different passage rates, and the cell wall fraction

probably does not follow first order kinetics (Tamminga *et al.*, 1989), which makes an assumption on passage rate of total OM more difficult. In this study the assumed values in calculating FOM_A and FOM_B were based on the lignin clearance values measured during the starvation period in our former study (Tóthi *et al.*, 2002a), while in the calculation of FOM_C we used the commonly used k_p values (Tamminga *et al.*, 1994). The k_p values for grass were the same in the calculations of FOM_{A, B, C} ($k_p=0.045$); in supplemented cows these values were different which resulted in lower FOM_B than in FOM_C. There is a possible difference in passage rate between grazing period and starvation. Therefore FOM_A calculations based on *in vivo* values resulted in slightly lower values than FOM_{VFA}. The relatively high differences between the FOM estimated from VFA production and FOM estimated from rumen evacuations in maize during the starvation periods could be explained with the lower amount of the marker we introduced in the rumen at the onset of the starvation period, which resulted in difficulties in calculating the enrichment curves .

Production rates of VFA as measured by isotope dilution techniques showed a wide variability and errors as has been discussed previously by Sutton (1985) and Dijkstra (1994). In some other studies (Leng and Brett, 1966; Gray *et al.*, 1952) carbon exchange between acetate and butyrate was considered a source of error because acetate label was detected in butyrate. Label from acetate has also previously been detected in propionate, valerate and other VFA (Kristensen, 2001). It makes it more difficult to estimate the real production of the different individual VFA actually synthesised in the rumen especially when we used only $^{13}\text{C}_2$ Na-acetate. Van Soest (1994) concluded that a substantial amount of endogenous acetate is produced from the metabolism of other substances, particularly from long chain fatty acids and amino acids and this endogenous amount might also increase the acetate pool in the rumen.

There could also be another internal carbohydrate source for microbes resulting in VFA. It is known that the dairy cow secretes up to 190 litres of saliva per day (Pond, 1995) and about 70% of the water entering the rumen comes from salivary secretion (Church, 1988). Apart from water, bicarbonate salts, immunoglobulins, secreted proteins, enzymes, saliva contains mucin. Salivary mucin can be defined structurally as large viscous glycoproteins composed of approximately 75% carbohydrate and 25% amino acids linked via O-glycosidic bonds between N-acetylglactosamine and serine or threonine residues (Bansil

et al., 1995). Salivary oligosaccharides range in complexity from simple mono- and disaccharide to highly branched structures (Tabak, 1995). Therefore mucin might be an extra carbohydrate source for the ruminal microbes, which results in VFA production. No information could be found in literature to quantify its contribution.

Also the ^{13}C to ^{12}C ratio and the k_{prod} calculations might be effected by the naturally occurring differences in the ratio of the stable carbon isotopes ^{12}C and ^{13}C between plant species belonging either to the C_3 group (cool-season, barley) or to the C_4 group (warm season, maize). The ^{13}C content of C_4 plants is higher than that of C_3 plants (O'Leary, 1981), which is probably an explanation for the high values calculated for maize. More research is needed to elucidate the uncertainties and possible variation in the assumptions needed to make this validation.

4. Conclusions

Supplementation of pasture grass with different processed cereal grains significantly elevated VFA production in dairy cows compared to the non supplemented animals. In the grazing period the pelleted cereal grains resulted in higher production rates and total productions of acetate, propionate and butyrate while in the starvation we observed the opposite and maize grain resulted in apparently higher values than did barley grain. Toasting and subsequently pelleting of cereal grains might have a protective effect on barley and maize starch, which might delay its ruminal fermentation or shift a certain amount of starch to the small intestine.

From a methodological point of view $^{13}\text{C}_2$ Na-acetate labelling appears to be an easy and useful way for examining the VFA production of ruminants but estimates of VFA production should be treated carefully. Notably the uncertainty of the assumptions needed to be made for its validation needs further attention. Higher enrichments than those used here (100 mg +50 mg) and the use of other labelled VFA than $^{13}\text{C}_2$ Na-acetate (^{13}C Na-propionate and ^{13}C Na-butyrate) may improve the reliability of this labelling technique.

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CHAPTER 8

General Discussion

1. Introduction

Pasture harvested by dairy cows in intensively managed grazing systems as commonly used in Western Europe has a high feeding value. However, the fresh grasses are generally low in readily fermentable carbohydrates and high in rumen degradable protein (RDP). This imbalance may lead to an inefficiency in nitrogen (N) and energy utilisation (Valk, 1994; McCormick *et al.*, 2001) because despite the efficient synthesis of microbial protein, preduodenal losses of N can account for up to 30% of ingested N (Beever *et al.*, 1996). Furthermore, Peyraud *et al.* (1995) and Delagarde *et al.* (1997) reported that lowering N fertilizer applications reduced the supply of amino acids in animals fed fresh herbage. Better nutrient utilization, especially of N, may be possible by matching the supply of RDP and carbohydrates. *In vivo* studies suggest that 25 g of N/kg of OM fermented (Van Vuuren *et al.*, 1990) optimizes the efficiency of ruminal ammonia utilization.

In most situations dietary energy input is the most limiting nutritional component for profitable milk production and normal reproductive performance when using pasture as the main source of forage. Therefore, in the past 15 to 20 years feeding concentrates, as a supplement to lactating cows, has become common practice in dairy systems based on grazing of temperate pastures. Supplements can serve as a source of carbohydrates and provide the energy to utilise RDP in pasture. The types of energy rich supplements for forages fall apart into three categories: starch (e.g., cereal grains), sugars (e. g., molasses) and fiber (e.g., sugar beet pulp). In practice the types of concentrates used as a supplement to pasture grass (mainly perennial ryegrass) are mainly grain-based (mixtures of grains about 2 to 7 kg/day) and in addition other supplements including hay, silage and by-products are used. As nowadays more emphasis is put on milk protein yield by the genetically improved dairy cows (e.g., higher body weight of the cows, higher milk production since the seventies), the rationale for using processed cereal grains in dairy nutrition increased, especially in the last decade. Milk protein yield may to some extent be controlled by synchronisation of ruminal nitrogen and energy availability and therefore processing of cereals may increase the production capacity. An additional advantage is that processing affects the physical, nutritional and hygienic quality of the

produced feed (Chapter 2). The research described in this thesis is focused on the processing of cereal grains and was initiated because of two main reasons.

Firstly current knowledge of hydrothermal processing (pelleting, expanding and toasting) on cereal grains raised the question whether the ruminal behaviour of starch of these cereal grains will differ after the heat processing.

Secondly it was hypothesized that processed cereal grains could help to synchronise rumen fermentation and reduce ammonia levels in the rumen of grazing, lactating dairy cows.

Accordingly, the aims of the investigations were to find a hydrothermal grain processing method for maize and for barley, which can result in an optimal addition of sources of readily available energy to the ruminal microbes, and result in increases in the production response of a grazing lactating dairy cow.

In the following sections of this General Discussion the results of the experiments are evaluated, considering the initial objectives. In the first section the effect of hydrothermal processing on ruminal behaviour of cereal grains will be discussed in detail and the next section summarises the effect of supplementation of processed cereal grains for grazing dairy cows on rumen fermentation synchrony.

2. Effect of hydrothermal processing on ruminal degradation of cereal grains

In this thesis two different cereal grains and four different processing methods were studied in the experiments discussed in the different Chapters (Table 1).

Barley and maize were chosen because of their different patterns of starch and protein fermentation in the rumen. In general, starch and protein are more degradable in barley than in maize. Differences in degradability is primarily attributed to the different properties of the protein matrix that limit, to a variable extent, the access of ruminal bacterial enzymes to starch granules (McAllister *et al.*, 1993). Based on the differences in starch (570 ± 22 g/kg DM and 715 ± 25 g/kg DM, for barley and for maize, respectively) and protein (127 ± 14 g/kg DM and 98 ± 6 g/kg DM, for barley and for maize, respectively) content and starch structure and composition (Chapter 2), barley and maize were expected to respond in different ways to the heat treatments, which was confirmed by the results (Chapter 3, 4, 5, 6, 7).

Table 1 Cereal grains and processing methods and conditions applied in this study

Grain/ processing	Grinding	Pelleting	Expanding	Toasting and pelleting
Barley	untreated	80 °C, 10 sec	105 °C, 2 min	135 °C, 1.5 min
Maize	untreated	80 °C, 10 sec	95 °C, 2 min	135 °C, 1.5 min
<i>Abbreviation</i>	<i>UB and UM</i>	<i>PB and PM</i>	<i>EB and EC</i>	<i>TPB and TPM</i>
<i>Chapter No.</i>	3	4,5,6, 7	3	4,5,6, 7

In the conventional pelleting process first of all cereal grains are ground which disrupts the pericarp, reduces the size of feed particles and increases the surface area for rumen microbes. During grinding a proportion of the starch is damaged by fracturing the hydrogen bonds between, and covalent bonds within starch molecules. This damaged starch subsequently absorbs water more and faster than undamaged starch. After grinding the mash is pelleted in a roller-and-die pellet press, both vertical and horizontal. Before entering the pellet press, the mash is usually subjected to some form of granulation for example by the use of an expander to increase temperature and moisture level during the treatment. The moist heat serves to swell the starch granule and to gelatinise the starch.

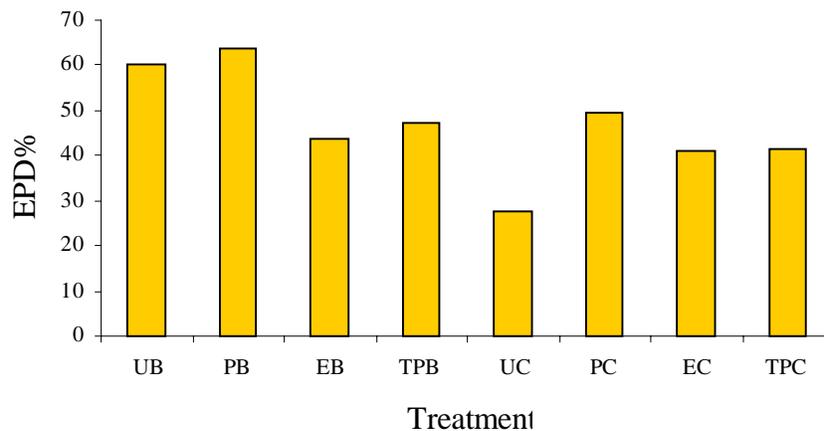
Starch gelatinization is characterised by several changes in its granular structure, such as swelling of granules, exudation of some complex molecules, dissolution of some granules, loss of the degree of crystallinity, etc. The processing temperature must allow sufficient disruption of the granules to generate these structural changes. Gelatinization of the starches improves their digestibility by increasing their rate of enzymatic hydrolysis into lower molecular weight sugar constituents, which are more water-soluble. It is well established that the gelatinization and swelling properties of starches are starch specific. Maize starch has a higher range of gelatinization temperature (62 to 72 °C) than barley (51 to 60 °C) starch.

Shear forces during expander treatment further disrupt the granular structure of starch, affect particle size and modify the chemical structure of the grain. In contrast with expander treatment, when cereal grains are toasted, there is usually no grinding process before the treatment and the whole seeds are more slowly hydrated and heated than

ground mashes. The presence of seed hulls and the lack of shearing action causes the seeds to “blow up” in the pressure toaster. The outlet material of the toaster therefore has a different physical structure than the expanded mashes.

All *in situ* experiments reported in this thesis showed that the heat processing of barley grains in comparison to untreated barley had not changed (expander treatment) or even decreased (pelleting, toasting) the effective nylon bag degradation of starch and above 100 °C the degradation of protein even decreased (Figure 1). Arieli *et al.* (1995), Weisbjerg *et al.* (1996), Lund (1999) and Prestløkken (1999a; 1999b) also found that the expander treatment of barley considerably reduced ruminal degradation of protein measured *in situ*. Cone *et al.* (1991) found the same effect with steam rolling of barley. Prestløkken (1999) observed that the ruminal degradation of barley protein reached a point at which any additional increase in treatment intensity did not result in a further decrease of ruminal degradation of protein. It seems that for barley this point is reached at the applied temperature of expander processing (105 °C).

Figure 1 Effective protein degradability (ESD %) of heat treated cereal grains. For abbreviations see Table 1.



The effects of all heat processing on maize were more robust than on barley. All heat treatments used elevated the washable fractions of organic matter, protein and starch in maize while the size of the potentially degradable fraction (D) decreased. The rate of

degradation of the D fraction was elevated and this resulted in higher effective degradabilities of OM, CP (Figure 1) and starch. These results suggest that the heat treatment of maize resulted in the gelatinization of maize starch, making it more accessible to enzymatic breakdown. This in turn results in more ruminal available starch (as energy source for microbes) than ground maize and in this case it seems that the expander treatment is more effective than the other processing methods (pelleting or toasting). The elevated effective degradation of protein is rather less important with regard to microbial aspects, because maize contains the smallest amount of CP among the cereal grains (9 to 10%).

We observed that the losses of fine particles through the pores of the nylon bag associated with washing of the nylon bags in the washing machine are considerable (Chapter 3). The reason of these losses could be the grinding procedure prior to the incubations (Weisbjerg *et al.*, 1990; Prestløkken, 1999). These fine particles lost from nylon bags are possibly and likely degraded in the rumen at the same rate as the particles left in the nylon bags. Therefore losses of particles in the washing procedure will lead to an overestimation of the *in situ* ruminal degradation of starch (Michalet-Doreau and Ould-Bah, 1992; Weisbjerg *et al.*, 1990). Goelema (1999) showed that only a part of the washable fraction (W) of starch is immediately available and incorporated in microbial mass. When the washable fraction is large (30 to 40%) like in barley (see Table 2) a substantial part of the W fraction of starch escapes fermentation via rumen outflow.

Therefore the *in situ* degradability of starch seems to slightly overestimate the digestibility of the rapidly degraded barley and underestimates the slowly degraded maize compared to the *in vivo* results (Chapter 3). It was suggested in Chapter 3 and in the result of the calculation tabulated in Table 7 in Chapter 3 and Table 2 in this General Discussion that a correction is needed for *in situ* values to predict the true rumen starch digestibility. Decreasing the fractional rate of degradation of the D fraction to 0.03 h^{-1} or assuming that 10% of the W fraction passes out of the rumen undigested results in degradability figures in close agreement with *in vivo* rumen digestibility (Table 2).

Toasting and subsequently pelleting decreased the rumen protein and starch degradability of barley, by decreasing the water-soluble fractions, as well as the rate of degradation (k_d) of the potentially degradable fraction (Table 2). For maize the effect of

toasting was the opposite, rumen starch availability was elevated after toasting. It is possible that at a temperature of 135 °C the level of gelatinization of maize starch is more complete than in barley grain.

Table 2 *In situ* characteristics of processed cereal starch¹

Cereal grain	Starch (g/kg DM)	W (%)	D (%)	k_d (h ⁻¹)	ESD 1 (%)	ESD 2 (%)	ESD 3 (%)
Barley							
Untreated	567	55	45	63	96	93	92
Pelleted	572	57	41	21	89	88	87
Expanded and pelleted	542	75	24	36	96	92	90
Toasted and pelleted	597	49	49	19	87	88	87
Maize							
Untreated	743	23	77	5	58	68	68
Pelleted	683	40	58	3	65	72	71
Expanded and pelleted	725	40	60	7	72	77	76
Toasted and pelleted	710	35	59	6	66	73	72

¹ W is the rapidly soluble fraction, D the potentially digestible fraction, k_d the rate of digestion of D fraction. Effective starch degradability (ESD 1) was calculated as $W+D k_d / (k_d + k_p)$, where k_p is the rumen outflow rate (0.06/h) or (ESD 2) assuming that the W fraction is available for passage. Fractional rate of degradation for W fraction: 1.00 h⁻¹, fractional rate of passage 0.08 h⁻¹ and fractional rate of passage of D fraction: 0.03 h⁻¹ or (ESD 3) assuming 10% of the W fraction pass out of the rumen undigested and fractional rate of passage of D fraction: 0.03 h⁻¹.

Goelema (1999) found in legume seeds that above a certain level of gelatinization an increased degree of starch gelatinization results in an increased k_d of starch, whereas an increased degree of retrogradation is responsible for the decreased water soluble fraction of starch. During feed manufacturing, in order to decrease moisture and latent heat, pellets need to be cooled. The free water content of the feed is decreased in the cooling process, making it possible to store the feed for a sufficiently long period of time. During cooling and storage gelatinised starch exhibits shrinkage, water syneresis and as a result the texture can become tough. This phenomenon is known as recrystallisation or retrogradation. Retrogradation has been used to describe changes in physical behaviour

following gelatinization and due to the crystallisation of amylose, and some of the amylopectin. Variation in the molecular structure of starch can result in altered retrogradation behaviour. Structural modification, by means of physical modification of starch, has been employed to alter the process of retrogradation. Because starch retrogradation is a kinetically controlled process (Slade *et al.*, 1997) the alteration of time, temperature, and water content during processing can produce a variety of end products. After processing, the properties of the metastable starch–water system can also be influenced by moisture content, the botanical source of starch, storage time, and storage temperature (Gudmundsson and Eliasson, 1990). The difference in retrogradation behaviour is most likely related to the different amylopectin structures. Maize starch contains approximately 73% amylopectin and 27% amylose (Brown *et al.*, 1984). Barley starch contains generally 75 to 85% amylopectin (Ullrich *et al.*, 1986) and the amylose content of barley varies from zero in certain waxy barley varieties (Morrison *et al.*, 1986) to about 45% in high amylose Glacier (Bhatty and Rossnagel, 1997). Normally in barley starch the amylose content is about 25%. However, maize amyloses are composed of a smaller number of chains per molecule than barley amyloses, and their amylopectins are different in the distribution of short side-chains. Moreover, the protein distribution in the genetically variable maize endosperm may affect starch availability for enzymatic hydrolysis in the rumen (Philippeau *et al.*, 2000).

In conclusion:

Because of the particle loss of starch, and the supposed differences in fractional rate of degradation for barley and maize starch, it is necessary to correct *in situ* values if we want to accurately predict the actual *in vivo* digestibility. After correction of *in situ* values it seems that in comparison to simple physical treatment (grinding) the proportion of starch available as substrate for rumen microbes is significantly elevated after heat treatment of maize regardless the type of the processing, but decreases the ruminal starch degradability of barley. Because of the differences between maize and barley in starch structure and protein-starch matrix it is hypothesised that heat processing between 80 °C and 135 °C is more effective in maize than in barley.

3. Balance between ruminal available organic matter and nitrogen

3.1. Calculation of the synchrony index

Bacterial growth in the rumen of dairy cows largely depends on the amount of degradable N and fermentable OM in the rumen. Some studies have confirmed that synchronising the ruminal fermentability of starch and nitrogen sources, increases the outflow of bacterial protein from the rumen (Hererra-Saldana and Huber, 1989; Aldrich *et al.*, 1993). Synchronisation of available N and available OM for rumen microbes seems advantageous for an optimum N economy of a grazing cow (Van Vuuren *et al.*, 1993) because of the conditions of extensive absorption of ammonia (Chamberlain and Choung, 1995). In grazing situations the release of ammonia from the (often high) content of crude protein in fresh grass is very rapid, and this requires a similarly rapid release of energy in the rumen to ensure the most efficient microbial capture of ammonia. To be able to estimate the possible rumen synchrony after feeding processed cereal grains as a supplement to grazing dairy cows there is a possibility to use the so-called synchrony index.

Sinclair *et al.* (1993) developed a synchrony index (I_S) based on the fermentation characteristics of feeds that is calculated from the hourly releases of nutrients to the rumen microbes. This I_S seems to be a useful tool to examine the impact of supplement feeding on synchronising the release of OM and pasture nitrogen in the rumen of dairy cows. With the calculated grass intake, grain intake (Chapter 4) and *in situ* degradation results of grass, processed grains (reported in Chapter 3, 5 and Lund *et al.*, 1999) and rumen content (Tóthi *et al.*, unpublished data) we estimated on an hourly basis, the degradation of OM and N for over 24 hours. The patterns of the first grazing event is based on our own observations (Chapter 4, 5, 6 and 7) and more information for the later hours was adapted from other work about grazing behaviour of dairy cows (Rook *et al.*, 1994; Gibb *et al.*, 1997; Gibb *et al.*, 1998; Gibb *et al.*, 2002; Orr *et al.*, 2001).

Therefore the following assumptions we made:

Throughout the 24 h of grazing two fixed time points seem to exist. Removal of the grazing cows from the pasture twice-daily for milking and feeding them with processed concentrates in the milking parlour during milking time and their subsequent return

(between 6:45 h and 7:45 h and between 16:30 h and 17:30 h). It is further assumed that in the paddock-grazing systems the dairy cows was given their daily pasture allocation in the mornings. In our observations at 8:00 h following the morning milking, cows had an initial meal of approximately 2 h duration (first grazing event). Then we assumed a relatively small grazing period (1 h duration) in the late afternoon (from 14:00 h to 15:00 h) before the evening milking because during hot periods (summer) cows reduce the afternoon grazing and increase evening-time grazing. Evening meal after milking until sunset was assumed to last for 4.5 h (second large grazing event) with minor or no resting periods. After sunset the cows start to ruminate and rest until sunrise. Cattle have demonstrated little directional grazing after darkness (Walker and Heitschmidt, 1988). Evidence is mounting that cattle rely on vision to move about in their environment so as darkness sets in, they lose many of their visual cues so they do not venture far from night time bedding areas.

In summer a significant grazing bout was observed at sunrise (Chilibroste, personal communication) early in the morning from 5:00 h, which lasted until the removal of the cows from the pasture to the milking parlour (approximately after 1.5 h grazing). We also assumed that the total grazing time in 24 hours is affected by the supplementation. McGilloway and Mayne (1996) reported reductions in grazing time of 15 to 22 min/kg concentrates. Sayers (1999) reported a reduction in grazing time of 16 to 20 min/kg of concentrate when the amount of supplement was increased from 5 to 10 kg/d. Pulido and Leaver (2001) and Bargo *et al.* (2002) reported a lower reduction in grazing time per kilogram of concentrate (4 to 11 min). According to literature information we assumed not more than 560 min/day average grazing time for NS animals with 13 kg of OM intake if we consider the summer time and pasture condition (Chapter 4). Because the reduction in grazing time is a common effect of supplementation, we assumed 500 min/day grazing time for supplemented animals. Observed reduction in duration of the first meal following the morning milking with supplement may result from perturbation of the rumen environment due to the rapid digestion of the cereal grains (Sutton, 1981), rather than physical limitation of capacity. According to the non-significant differences in DMI (Chapter 4) in the first meal between supplemented and non-supplemented animals we assumed the same tendency in the other meals after feeding concentrates.

The effective ruminal degradabilities (ERD) of OM and N were calculated for every 15 minutes and cumulated in every hour through the day using the fractional outflow rate (k_p) of solids from the rumen (assumed passage rates were 4.5 and 6.0% for grass and supplements, respectively).

ERD was calculated as follows:

$$ERD = W + ((D * k_d) / (k_d + k_p))(1 - e^{-(k_d + k_p)t})$$

Where W is the washable fraction; D is the potentially degradable fraction; k_d is the fractional degradation rate of D; k_p fractional outflow rate of the solids; t is time of degradation in hours.

The synchrony index (I_s) which describes the synchrony of nitrogen and organic matter degradation in the rumen was calculated according to an equation similar to the one proposed by Sinclair *et al.* (1993).

$$I_s = \frac{25 - \sum_{1-12} \frac{\sqrt{(25 - \text{hourly N/OM})^2}}{24}}{25}$$

Where 25 = 25 g N/kg OM truly digested in the rumen which was assumed to be the optimal ratio (Czerkawski, 1996).

An I_s of 1.0 represents perfect synchrony between nitrogen and energy supply throughout the day whilst values less than 1.0 indicate the degree of asynchrony (Sinclair *et al.*, 1993). The supply of N and OM was calculated from the sum of the hourly degradation and the ratio of N to OM calculated.

3.2. Effect of fresh grass as sole feed on the synchronisation level

The predicted hourly ratio of N to OM degraded in the rumen throughout the day in the paddock-grazing system where the dairy cows were given their daily pasture allocation after the morning milking, is illustrated in Figure 2, Figure 3 and Figure 4.

Figure 2 Predicted ratio of nitrogen to organic matter degraded in the rumen of non supplemented (NS) and supplemented dairy cows. Supplement (3 kg per day) consumed two equal meals per day, times of supplement feeding are indicated by the arrows. For abbreviations see Table 1.

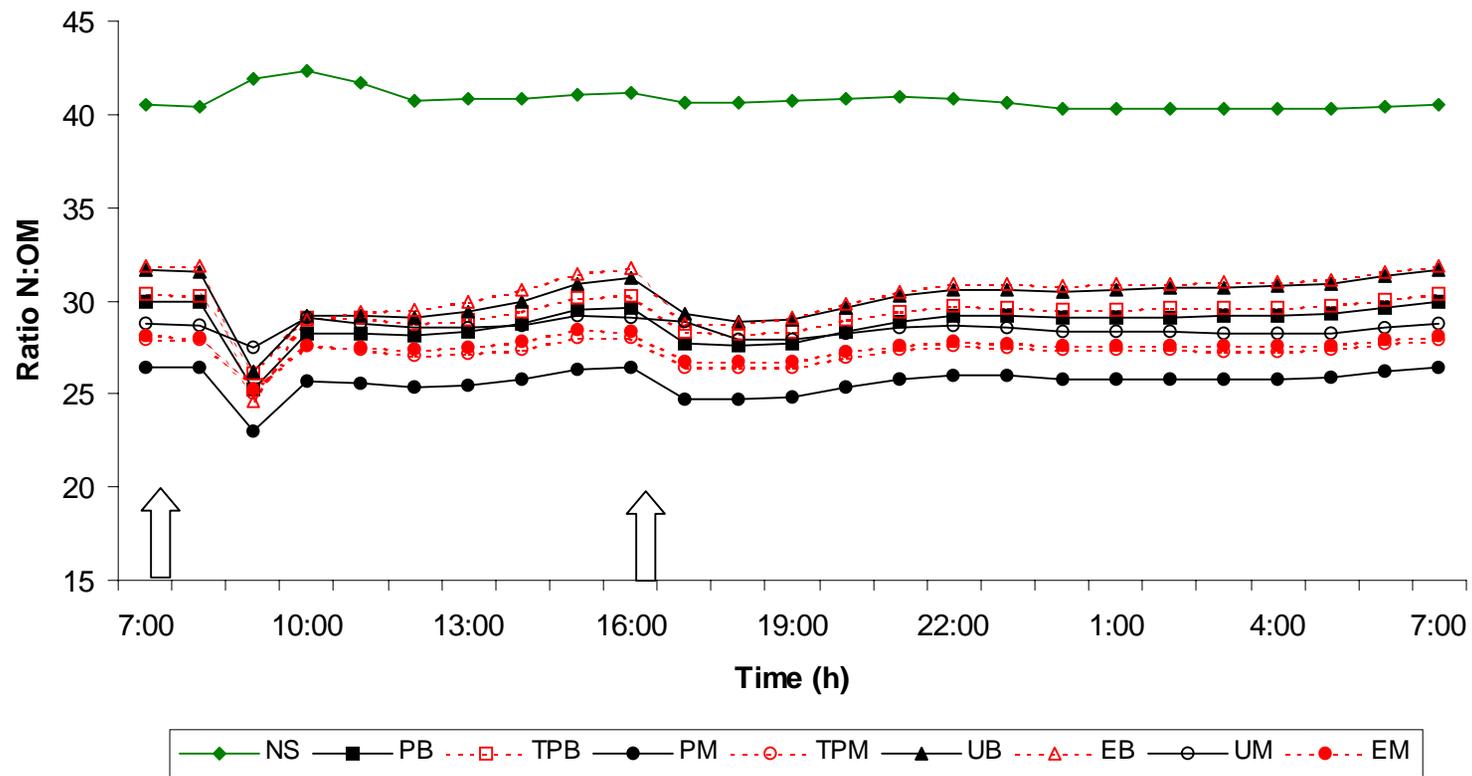


Figure 3 Predicted ratio of nitrogen to organic matter degraded in the rumen of non supplemented (NS) and supplemented dairy cows. Supplement (6 kg per day) consumed two equal meals per day, times of supplement feeding are indicated by the arrows. For abbreviations see Table 1.

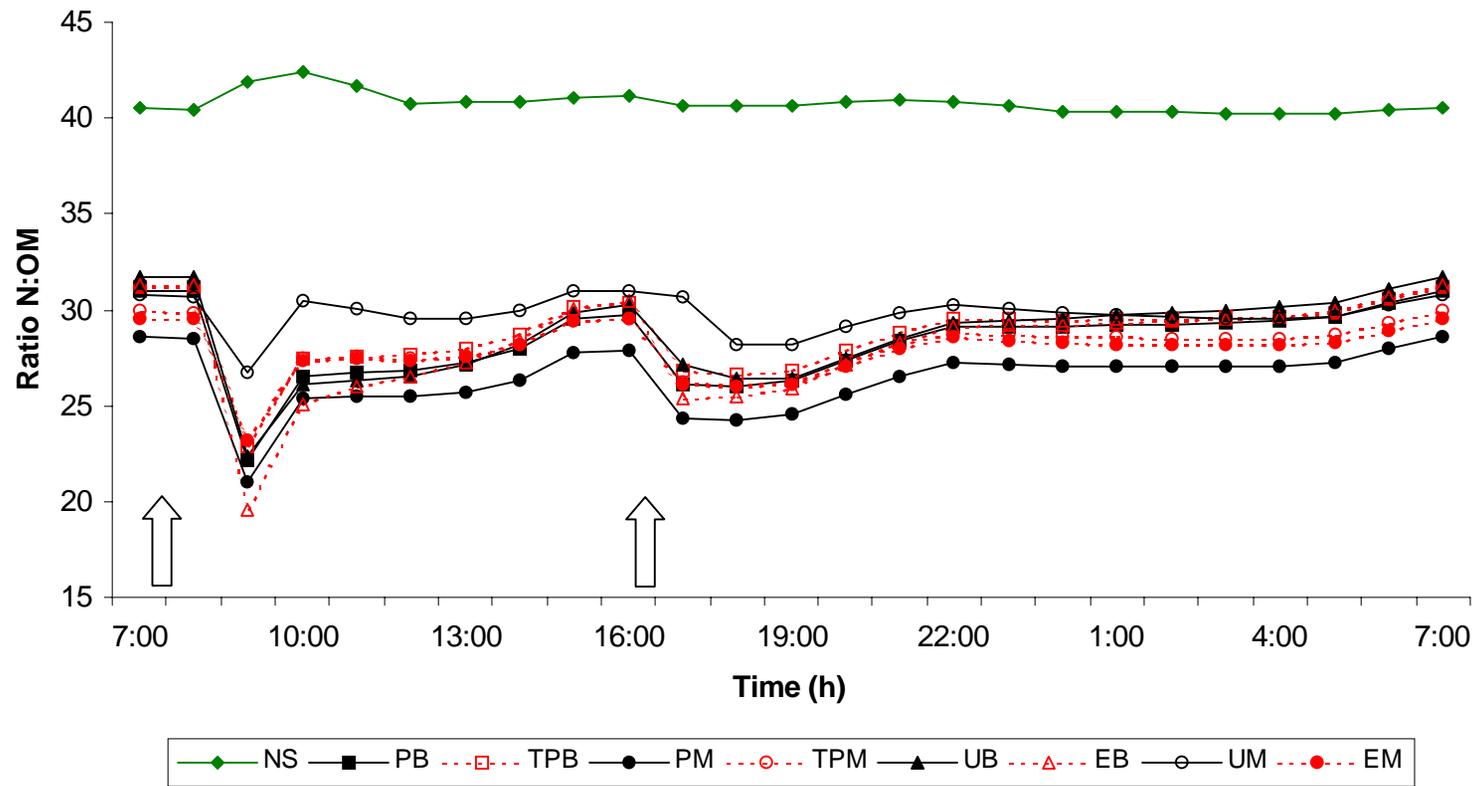
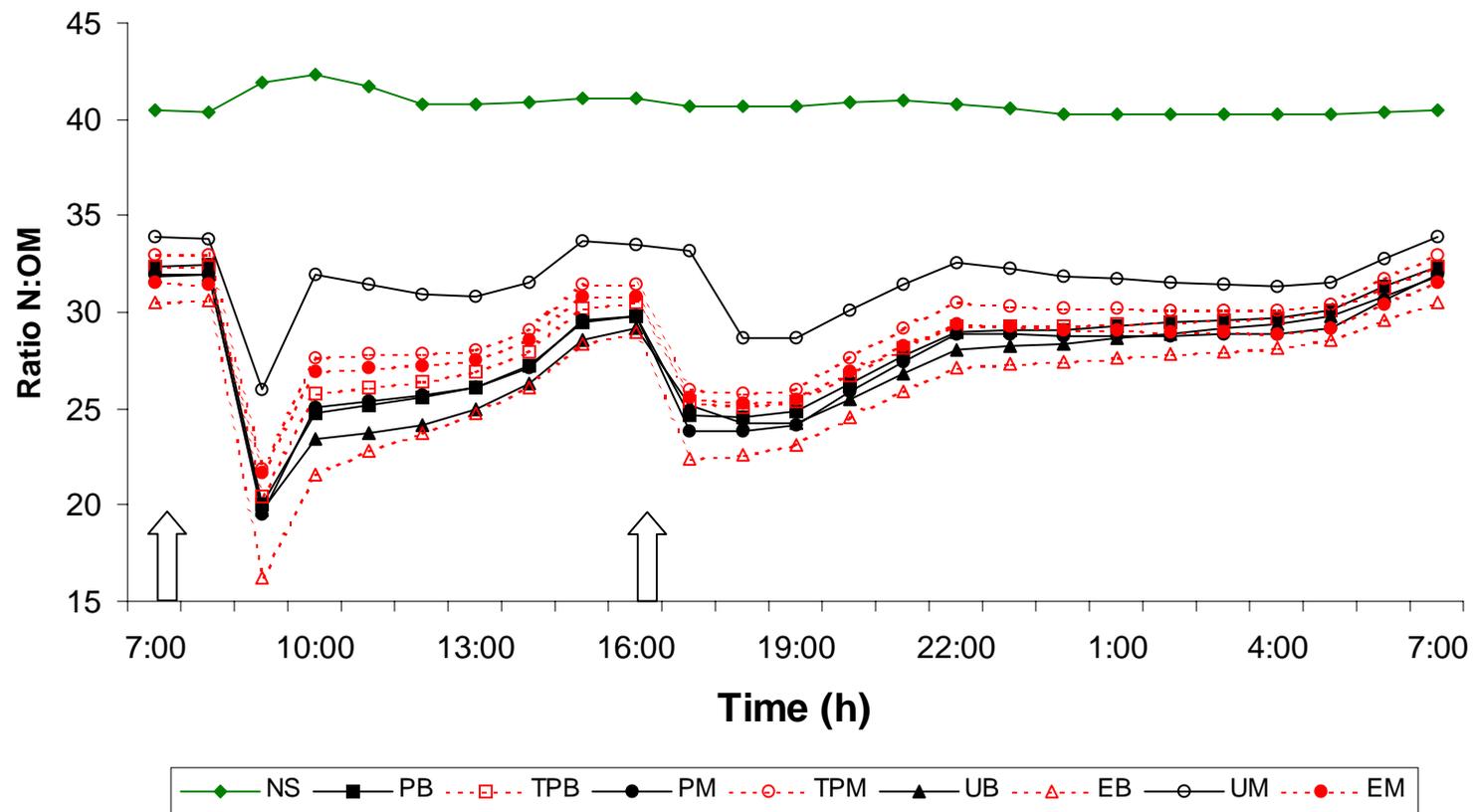


Figure 4 Predicted ratio of nitrogen to organic matter degraded in the rumen of non supplemented (NS) and supplemented dairy cows. Supplement (9 kg per day) consumed two equal meals per day, times of supplement feeding are indicated by the arrows. For abbreviations see Table 1.



As could be expected the non supplemented animals (NS) have a higher N to OM ratio throughout the day than the supplemented animals. The most likely explanation for this phenomenon is that the concentration of readily fermentable carbohydrates (sugars) in highly fertilised ryegrass is relatively low in comparison to structural carbohydrates (Visser *et al.*, 1997). Structural carbohydrates, though highly digestible are not immediately available for degradation. The cell machinery of rumen bacteria has been developed around the premise that energy will be first limiting (Russell, 1998). Ruminant bacteria have only a limited capacity to store intracellular polysaccharides that in turn limits their ability to buffer the effects of mismatching of energy and nitrogen release.

The daily change of the sugar content of perennial ryegrass is one of the possible explanations for the daily changes in the N to OM ratio in NS animals. Due to photosynthesis the concentration of total sugars increases during daylight and decreases during the night (Smith, 1973). During the first hours grazing in the morning, when the sugar concentrations is lower than later in the day (Van Vuuren *et al.*, 1986), and because of the effect of starvation during milking, resulting in intensive grazing after milking, the N intake from grass is high, and the N to OM ratio is high as well. The concentration of total sugars in fresh grass increases and becomes highest in the late afternoon (after 16:00 h to 17:00 h) which may result in a more balanced nutrient supply to the microbes in the afternoon grazing event during the dusk than in the first grazing event. During night time grazing activities are scarce and concentrations of fermentation products decrease. During the early morning grazing event before milking, the ammonia concentrations in the rumen raise and this raise continues during the grazing after the milking (Chapter 6). The high concentrations of ammonia, the high predicted N to OM ratio and the calculated synchrony index ($I_s = 0.37$, for 24 h) indicate a high degree of asynchrony and a substantial degradation of protein in the rumen.

3.3. Effect of different supplementation level on synchronization

Compared with the non supplemented diet, supplementation with 6.0 kg of processed cereal grains fed in two equal amounts per day in the milking parlour during milking results in a lower ratio of N to OM release in the rumen during the day (Figure 3).

The ratio N to OM can decrease by changing the release of either N or OM and each manipulation has a somewhat different impact on the utilisation of N. In this feeding situation the total OMI of a grazing dairy cow increases with supplemental feeding of processed cereal grain. The quantity of N shows a tendency to decrease (Chapter 5) compared to non-supplemented animals and simultaneously the release of OM increases. Microbial protein will likely increase and ruminal ammonia N decrease (Chapter 6) as result of an increased incorporation of ammonia N in microbial protein synthesis. In the morning, after feeding 3 kg of concentrates, the easily available water soluble fraction of cereal starch increases the OM release in the rumen, therefore the N to OM ratio drops.

The first two active grazing hours after milking result in a fast rate of release of herbage nitrogen compared with the release of OM. With all diets rumen ammonia concentrations increase during the first grazing event, but the ammonia concentrations in the rumen of supplemented animals remain significantly lower than in unsupplemented cows (Chapter 6). Microbial ammonia-N utilization in the rumen is primarily carbohydrate-limited (Russell *et al.*, 1992). Thus, the increase of carbohydrate availability in the rumen has the potential of improving the efficiency of microbial utilization of ammonia. It should be taken into account that rumen ammonia concentration can vary not only with variation in N release and rate of carbohydrate release but also with variation in rumen outflow rate, rumen volume, rate of absorption, and nitrogen recycling (Nolan, 1975; Russell *et al.*, 1983). The ruminal degradation of processed cereals is quite intensive a few hours after feeding and this may result in high amounts of OM available for the microbes and higher propionate and butyrate concentrations (Chapter 6) and VFA productions (Chapter 7) than with NS. Rumen VFA production rates are a reflection of the rate of substrate supply, but the molar ratios are more a reflection of the dominant microbial species present (Chesson, 1990). Supplementation of fresh grass may cause a more stable population of microorganisms in the rumen resulting in less variation in VFA molar ratios. However, the effects of processed cereal grain supplementation on rumen microbes requires further research.

In the milking parlour at the evening milking time the dairy cows receive the second part (3 kg) of their concentrate meal. In the rumen starch from the morning feeding is still present, because until this time point around 80% of the starch from barley had

disappeared and 30 to 35% of the starch from maize (Chapter 3 and 4). However, the drop in N to OM ratio is smaller than the observed fall in the morning. The reason may be the degradation of protein from the cereal grains, which also elevate the N pool in the rumen. In the first hours of the second large grazing event in the afternoon, the available N and OM for the microbes seems more synchronised than in the morning grazing event. This could be the result of the remaining starch, the new starch intake and probably the increased sugar content of the grass.

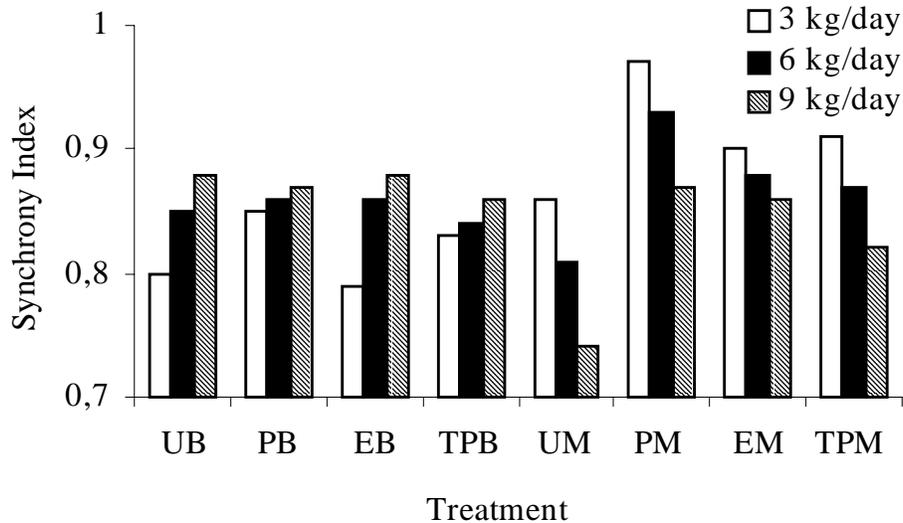
If the cows were given their daily allocation of ryegrass pasture after the afternoon milking, this would result in a more synchronised availability of N and OM for the ruminal microbes than in the situation when the cows are given their daily pasture allocation after the morning milking. This is because the sugar content of the pasture is 5 to 7% higher than in the morning and the dry matter content is also higher. This elevated amount of sugar is a readily available energy source for microorganisms.

The synchrony index (6 kg of concentrate per day) is highest in dairy cows supplemented with pelleted ($I_s = 0.93$) or expanded maize ($I_s = 0.88$) and lowest in untreated (ground) maize ($I_s = 0.81$), with the barley supplements in between ($I_s = 0.84$ to 0.86) (Figure 5).

Differences in I_s between untreated and processed barley are smaller than between untreated and processed maize. The differences between untreated and processed maize grain can be explained by the higher water-soluble fraction and the faster rate of degradation of heat-treated maize.

The effects of processed grains on the synchrony in the rumen may be more pronounced when higher (Figure 4) or lower (Figure 2) levels of concentrates are used in the same grazing circumstances. An elevated amount of cereal grains (9 kg per day) will reduce grass intake (possible substitution effect) resulting in a lower N to OM ratio during the day (Figure 4). More water soluble starch results in more fast energy for the microbes, but higher intakes of grain may reduce the digestion of grass (Mould *et al.*, 1983) and may also increase the amount of starch escaping digestion (Stockdale *et al.*, 1987).

Figure 5 Calculated synchrony index (I_S) of supplemented grazing dairy cows over the day. For abbreviations see Table 1.



Therefore the ruminal N and OM available for the microbes seems less synchronised with 9 kg per day supplementation of maize. Slowly degradable untreated maize shows a less balanced N to OM ratio during the day than the other supplements, and the calculated synchrony index for unprocessed maize is lower ($I_S = 0.74$) than for the other treatments. I_S of processed maize decreases also when the supplementation is raised to 9 kg per day. Barley behaves opposite to maize, while I_S is still increasing with a higher level of supplementation, although the differences between the processing methods are eliminated.

A reduced level of supplementation (3 kg per day) shows a higher I_S for maize and a lower for barley than the 6 or 9 kg. At the low level of supplementation the grass intake from pasture is probably also higher and the OM release from maize will likely result in a smaller decrease of the ruminal pH and a smaller decrease of the NDF digestibility.

The starch disappearance of barley is higher than that of maize, which synchronises the rumen shortly after the feeding but the starch release from the lower amount of barley during the day does probably not provide a sufficient balance of protein and energy for the microbes.

In conclusion:

Based on *in situ* degradation characteristics of the ingredients the fresh grass only diet is less synchronous for the rumen microbes than supplemented diets. With the grass only diet there is more rumen degradable protein than the microbial population needs, therefore much of the ammonia will be absorbed into the bloodstream and be converted to urea in the liver, with a greater portion being excreted in the urine.

From this models it appears that supplementing grass with processed cereal grains can alter microbial growth and efficiency of utilization of nutrients and results in a more synchronous substrate for the microbes.

Supplementation with processed maize results in a more synchronised substrate for the microbes than with untreated maize, independent of the level of supplementation. Supplementing 3 kg per day of maize grain results in a more synchronised diet than higher levels of supplementation. Higher levels of supplementation decrease the I_s of maize, and these decreases are larger with unprocessed than with processed maize. It seems that maize pelleting or expanding has more advantage than the simple mechanical treatment and toasting is also more effective.

For barley grain an elevated level of supplementation from 3 kg per day to 6 kg per day and then to 9 kg per day results in a more synchronised substrate for the microbes. The differences between untreated and processed barley are more pronounced with 3 kg/day supplementation than with higher levels of supplementation that result in a quite similar synchrony index. It is suggested that processed barley is more appropriate as a supplement for lactating, grazing dairy cows at higher level of feeding.

4. General conclusions

The main conclusions to be drawn from of this dissertation are:

- *In situ* studies showed that all hydrothermal treatments (pelleting, expanding, toasting) increased the ruminal starch availability of maize but decreased that of barley.

- Expanding and toasting decreased, but pelleting increased ruminal protein availability of barley and all hydrothermal treatments (pelleting, expanding, toasting) increased the ruminal protein availability of maize.
- Compared to untreated grains, expander treatment significantly ($P < 0.05$) increased the apparent rumen and total tract digestibility of maize starch but did not significantly ($P > 0.05$) affect the digestibility of barley.
- Supplementation of grazing dairy cows with pelleted and pressure toasted cereal grains did not influence significantly ($P > 0.05$) the DMI of grass in the first grazing event (in the morning after milking).
- Feeding of heat treated cereal grains decreased the $\text{NH}_3\text{-N}$ level in the rumen.
- Supplementing pasture grass with pelleted and pressure toasted cereal grains decreased the pH, the ammonia concentration of ruminal fluid, ammonia to total VFA (TVFA) ratio, isobutyrate proportion, acetate to propionate and non-glucogenic to glucogenic ratio. Simultaneously it increased TVFA concentrations, propionate, butyrate and valerate proportions as a percentage of the TVFA.
- It seems that the effect of heat processing is more effective on maize than on barley.
- Differences in ruminal fermentation, which existed between maize and barley before pelleting or toasting disappeared.
- Pelleting as well as toasting followed by pelleting did affect production responses in dairy cows, by elevating milk protein and decreasing milk fat production, and milk urea nitrogen but no significant differences ($P > 0.05$) between these two heat treatments were found.
- Lactation studies to determine the value of expanded or toasted barley or maize grain have not been reported. Therefore lactation performance studies with dairy cows need to be conducted to clarify the value of this cereal processing for dairy cows.
- The need of synchrony is specially important with diets based on fresh grass, in which markedly asynchronous rates of release of energy and nitrogen occur in the rumen ($I_s = 0.39$).
- Using processed cereal grains to generate a better ruminal N and OM synchrony to improve microbial N yield and N utilization seems feasible, but direct measurement of microbial yield is needed.

- Factors effecting estimates of OM and N synchronisation that require further investigations are the pattern of pasture intake, rumen turnover rate and frequency of supplement feeding .
- Responses obtained from cereal grain supplementation are very dependent on the quality and degradation characteristics of the pasture consumed, which change through the grazing session and which makes the synchronisation of ruminal degradation of supplemental carbohydrate with pasture nitrogen difficult.

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SUMMARY

Under north-western European conditions where the dairy husbandry is characterised by a high production level per cow, approximately 30 to 50% of the forage consumed by dairy cows is fresh grass, primarily perennial ryegrass (*Lolium perenne L.*). Much of the pasture protein is highly degraded in the rumen mainly due to its large fraction of highly soluble protein and non-protein nitrogen. Therefore the grazed fresh, perennial ryegrass may possibly cause asynchrony between N and energy availability for microbial synthesis. This generates a rumen environment with a high concentration of ammonia nitrogen, leading to inefficient use of the dietary protein and resulting in a net loss of amino acids potentially available to the animal. Efficient use of rumen degradable protein by rumen microbes is dependant on the presence of readily available carbohydrate sources in the rumen. There is evidence in the literature that for lactating dairy cows ruminal conditions could be improved by supplying more energy to the rumen. This could be achieved by supplying highly degradable starch to the rumen. Processing cereal grain presents a possibility to manipulate the ratio of rumen available protein to fermentable carbohydrate, and increase the efficiency of nitrogen utilisation.

In Chapter 1 the aim and the outline of this thesis is described. Chapter 2 is a literature review on the importance of cereal grains in dairy diets. It describes and discusses the effects of different processing methods of cereal grains. Attention is given to the effect of processing on salmonella reduction, on ruminal and intestinal starch digestion, on rumen fermentation and on dry matter intake and production. Wet (hydro-) thermal processing causes gelatinization of starch, denaturization of protein, bonding of lipids, inactivation of anti-nutritional factors, and pasteurisation of processed material. Based on the literature data it is concluded that wet (hydro-) thermal processing, effectively controls Salmonella in feed, increases the percentage of bypass protein, increases ruminal starch degradability, affects the site of digestion of starch and protein, improves the intestinal rate of degradation, increases the total digestibility of grain starch, increases of milk yield and improves the pellet quality if the feed is to be pelleted.

Chapter 3 discusses the effects of expander treatment on ruminal and intestinal digestibility of starch in barley and maize grains. Four lactating Danish Holstein Friesian cows, fitted with ruminal, duodenal and ileal cannulae, were offered grass-clover silage and grass-clover hay based diets, supplemented with soybean meal and either untreated

barley, expanded barley (105 °C), untreated maize or expanded maize (95 °C). Ruminal degradation characteristics of starch for untreated and expanded grains were determined *in situ* using nylon bags with two pore sizes (15 or 36 µm). Rate of starch degradation in the rumen was also determined *in vivo* based on series of total rumen evacuations. *In vivo* digestibility was estimated by sampling duodenal and ileal contents and faeces. Ruminal degradation of starch determined *in situ* was higher for barley than for maize, regardless heat treatment. *In situ* studies (36 µm) showed that expanding increased effective maize starch degradability in the rumen from 0.60 to 0.72, mainly due to an increased soluble fraction. Effective degradability of barley starch, however, was unchanged at 0.96 as an increase in the soluble fraction was counterbalanced by a decrease in the rate of degradation from 0.63 to 0.36 h⁻¹ with 36 µm nylon bags. *In vivo* degradation characteristics based on rumen evacuations and the starch pools corrected for rumen outflow, showed that heat treatment increased the fractional rate of degradation of starch in both barley and maize, resulting in a slight increase in the effective degradability of barley starch from 0.81 to 0.85 and an increase in rumen effective degradability of maize starch from 0.71 to 0.78. *In situ* studies seemed to overestimate ruminal degradation of rapidly fermentable starch in barley, and to underestimate degradation of slowly fermentable starch in maize, although *in situ* results were highly dependant on assumptions made on digestion and passage of the soluble fraction. Total tract digestibility of barley starch was not affected by heat treatment (0.99), whereas total digestibility of starch in maize increased from 0.84 to 0.96. Apparent rumen digestibility of starch was higher for barley than for maize. Duodenal flow of starch in barley was highest 4 h post feeding, whereas a much larger peak was found for maize at 10–12 h post feeding, which indicates a passage time lag for undegraded maize starch in the rumen, or possibly the abomasum. Fractional rate of passage of starch from the rumen was not constant, indicating that passage of starch does not follow first order kinetics.

The research described in Chapter 4 to Chapter 7 focused on grazing conditions. In this experiments lactating Holstein-Friesian dairy cows fitted with a large rumen cannula were allowed to graze perennial ryegrass (*Lolium perenne*) swards. Next to a control treatment of grazing only, pelleted barley, pelleted maize, toasted and subsequently pelleted barley, and toasted and subsequently pelleted maize were fed as a supplement in

two equal portions of 3 kg each in the milking parlour during the morning and evening milking. Before and after 3 hours of grazing the rumen content was evacuated, weighed, sampled and returned to the animals. Then the cows were kept inside the barn and starved for 6 hours, after which rumen evacuations were repeated.

In Chapter 4 the grass intake, rumen pool sizes, ruminal kinetics and the performance of the grazed and supplemented dairy cows were studied. Feeding of heat treated grains numerically, but not significantly reduced dry matter intake (DMI) of grass in the first grazing bout. Compared to unsupplemented animals the apparent ruminal clearance of nitrogen was significantly ($P < 0.001$) reduced. The estimated clearance rates of starch significantly differ between grain types, but the effect of toasting more pronounced on barley. Supplementation with processed grains significantly ($P < 0.001$) decreased milk fat percentage and milk urea content. Milk production and protein percentage increased for supplemented animals compared to the performance of the animals that had not received supplementation. It is concluded that supplementing grass based diets with high-energy low protein feeds, such as grains substantially improves the N utilisation and reduces the urea output in milk.

In Chapter 5 results are reported of experiments using the nylon bag technique. It discusses the effects of different heat treatments of cereal grains on the *in situ* degradability of protein and starch and the *in situ* degradability of fresh perennial ryegrass (*Lolium perenne*). Pelleting and pressure toasting increased the undegradable fraction of dry matter and organic matter and decreased the *in situ* protein degradability of the cereal grains. Undegraded intake protein increased after toasting from 36.4 to 52.4% for barley and from 50.3 to 58.2% for maize, respectively. Undegraded intake starch increased from 14.9 to 16.7% after toasting barley and decreased from 36.1 to 31.3% after toasting maize, respectively. After pressure toasting compared to pelleting alone, the washable fractions of each cereal grain decreased for both constituents, the fractional rate of degradation of protein decreased, while the fractional rate of degradation of starch increased for maize, but decreased for barley. The chemical composition and *in situ* degradation characteristics of ryegrass samples (in summer months, from June 2 to July 31) did not result in significant differences between sampling times but with maturation the rate of degradation of potentially degradable, but insoluble

organic matter and crude protein tended to decrease. It is concluded that the crude protein of fresh grass is highly degradable in the rumen, therefore supplementation with processed cereals gives a more balanced ruminal protein and energy availability, but the differences are more effective between grain types than between the heat treatments applied in this study.

Chapter 6 reports on the effect of processed cereal grain supplementation on rumen fermentation. The rapid and extensive ruminal fermentation of starch in all supplemented grazing cows resulted in higher concentrations of volatile fatty acids and lower pH in ruminal fluid than in non supplemented animals. Total volatile fatty acid concentrations (mmol/l) were higher for cows fed processed barley (121.0 and 125.3 for pelleted barley and toasted and pelleted barley), than for cows fed processed maize (121.7 and 115.6 for pelleted maize and toasted and pelleted maize, respectively). Supplement feeding lowered the acetate to propionate ratio and the non-gongluconic to gluconic ratio in comparison to non supplemented animals, but the cereal grain type and processing method had no significant effect on these ratios. The molar proportion of acetate in supplemented animals decreased from 66% to 61% and the proportion of propionate increased from 19% to 24% regardless the type of the supplementation or the method of processing. Concentrations of ammonia (mmol/l) and the ammonia to total volatile fatty acid ratio decreased when the cows were supplemented with processed grains. Toasting and subsequently pelleting resulted in numerically lower ruminal ammonia concentrations and a lower ammonia to total volatile fatty acid ratio than did pelleting only. The different processing methods resulted in similar volatile fatty acid patterns, acetate to propionate ratios, and total volatile fatty acid concentrations in the rumen.

In Chapter 7 the effects of processed cereal grain supplementation on volatile fatty acid production rates were studied. An isotope dilution technique using ^{13}C -acetate as a marker was employed for the estimation of volatile fatty acid production rates. At the beginning of a 3-hour long allowed grazing time, 100 mg of 99% enriched $^{13}\text{C}_2$ Na-acetate was introduced in the rumen and this was repeated after grazing with 50 mg of 99% enriched $^{13}\text{C}_2$ Na-acetate, after which the cows were starved for 6 hours until the evening milking. During grazing disappearance rate and production rate of acetate, propionate and butyrate were significantly higher in supplemented cows than in non

supplemented cows. Moreover the effect of barley grain and pelleting treatment was higher than the effect of maize grain and toasting. During starvation significantly higher disappearance and production rates of volatile fatty acids were observed in maize. The higher VFA productions measured in supplemented animals emphasise the extensive digestion that occurs in the rumen after feeding processed grains.

In the General Discussion (Chapter 8) it was concluded that based on *in situ* degradation characteristics of the ingredients the fresh grass only diet is less synchronous for the rumen microbes than supplemented diets. With the grass only diet there is more rumen degradable protein than the microbial population needs, therefore much of the ammonia will be absorbed into the bloodstream and be converted to urea in the liver, with a greater portion being excreted in the urine. Supplementing grass with processed cereal grains can alter microbial growth and efficiency of utilization of nutrients and results in a more synchronous substrate for the microbes.

Supplementation with processed maize results in a more synchronised substrate for the microbes than with untreated maize, independent of the level of supplementation. Supplementing 3 kg per day of maize grain results in a more synchronised diet than higher levels of supplementation. Higher levels of supplementation decrease the synchrony index of maize, and these decreases are larger with unprocessed than with processed maize. It seems that maize pelleting or expanding has more advantage than the simple mechanical treatment of grinding and toasting is also more effective.

For barley grain an elevated level of supplementation from 3 kg per day to 6 kg per day and then to 9 kg per day resulted in a more synchronised substrate for the microbes. The differences between untreated and processed barley were more pronounced with 3 kg per day supplementation than with higher levels of supplementation that result in a quite similar synchrony index. It is suggested that processed barley is more appropriate as a supplement for lactating, grazing dairy cows at higher level of feeding.

Main conclusions

In situ studies showed that all hydrothermal treatments (pelleting, expanding, toasting) increased the ruminal starch availability of maize but decreased that of barley. Expanding and toasting decreased, but pelleting increased ruminal protein availability of barley and

all hydrothermal treatments (pelleting, expanding, toasting) increased the ruminal protein availability of maize. Compared to untreated grains, expander treatment significantly ($P < 0.05$) increased the apparent rumen and total tract digestibility of maize starch but did not significantly ($P > 0.05$) affect the digestibility of barley. Supplementation of grazing dairy cows with pelleted and pressure toasted cereal grains did not influence significantly ($P > 0.05$) the DMI of grass in the first grazing event (in the morning after milking). Feeding of heat treated cereal grains decreased the $\text{NH}_3\text{-N}$ level in the rumen. Supplementing pasture grass with pelleted and pressure toasted cereal grains decreased the pH, the ammonia concentration of ruminal fluid, ammonia to total VFA (TVFA) ratio, isobutyrate proportion, acetate to propionate and NGR ratio. Simultaneously it increased TVFA concentrations, propionate, butyrate and valerate proportions as a percentage of the TVFA. It seems that the effect of heat processing is more effective on maize than on barley. Differences in ruminal fermentation, which existed between maize and barley before pelleting or toasting disappeared. Pelleting as well as toasting followed by pelleting did affect production responses in dairy cows, by elevating milk protein and decreasing milk fat production, and milk urea nitrogen but no significant differences ($P > 0.05$) between these two heat treatments were found. Lactation studies to determine the value of expanded or toasted barley or maize grain have not been reported. Therefore lactation performance studies with dairy cows need to be conducted to clarify the value of this cereal processing for dairy cows. The need of synchrony is specially important with diets based on fresh grass, in which markedly asynchronous rates of release of energy and nitrogen occur in the rumen ($I_s = 0.39$).

Using processed cereal grains to generate a better ruminal N and OM synchrony to improve microbial N yield and N utilization seems feasible, but direct measurement of microbial yield is needed. Factors effecting estimates of OM and N synchronisation that require further investigations are the pattern of pasture intake, rumen turnover rate and frequency and time of supplement feeding. Responses obtained from cereal grain supplementation are very dependent on the quality and degradation characteristics of the pasture consumed, which changes through the grazing session and which makes the synchronisation of ruminal degradation of supplemental carbohydrate with pasture nitrogen difficult.

SAMENVATTING

Onder de omstandigheden in Noordwest Europa, waar de melkveehouderij wordt gekenmerkt door een hoge melkproductie per koe, bestaat het opgenomen ruwvoer voor ongeveer 30 tot 50% uit vers gras, voornamelijk Engels raaigras (*Lolium perenne L.*). Een groot deel van het in weidegras aanwezige eiwit wordt in de pens gemakkelijk en snel afgebroken, vooral als gevolg van een grote fractie oplosbaar eiwit en niet-eiwit N. Dit leidt er toe dat bij koeien die op Engels raaigras grazen, de N en energie die nodig zijn voor microbiële groei niet synchroon beschikbaar komen. Dit veroorzaakt in de pens een hoog gehalte aan ammonia N, wat leidt tot een weinig efficiënt gebruik van het opgenomen voereiwit en tot gevolg heeft dat er een netto verlies optreedt van aminozuren die potentiëel voor de koe beschikbaar zijn. Een efficiënte benutting van pensafbreekbaar eiwit door de pensbacteriën is afhankelijk van de aanwezigheid van snel beschikbare koolhydraten in de pens. De literatuur geeft aan dat bij melkgevende koeien de omstandigheden in de pens verbeterd kunnen worden als er meer energie wordt verstrekt. Het technologisch behandelen van granen maakt het mogelijk om de verhouding waarin in de pens eiwitten en koolhydraten beschikbaar komen, te beïnvloeden en op die manier de efficiëntie van de microbiële benutting te verbeteren.

Hoofdstuk 1 van dit proefschrift beschrijft de doelstellingen van het onderzoek en de indeling van het proefschrift. Hoofdstuk 2 is een literatuuroverzicht over het belang van granen in de voeding van melkkoeien. Het beschrijft en bespreekt de invloed van diverse methoden van technologisch behandelen van granen. Hierbij wordt aandacht besteed aan de invloed van technologisch behandelen op een vermindering van een besmetting met *Salmonella* bacteriën, op de vertering van zetmeel in pens en darm, op pensfermentatie en op drogestof opname en productie. Verhitten onder vochtige omstandigheden (hydrothermisch) veroorzaakt verstijfseling van zetmeel, denaturatie van eiwit, het binden van lipiden, het inactiveren van anti nutritionele factoren (ANF) en het pasteuriseren van het behandelde materiaal. Uit de gegevens in de literatuur wordt geconcludeerd dat het hydrothermisch behandelen van voer effectief een besmetting met *Salmonella* onder controle houdt. Daarnaast wordt het percentage bestendig eiwit verhoogd, verhoogt het de afbraak van zetmeel in de pens, beïnvloedt het de plaats van vertering van zowel eiwit als zetmeel, en verbetert het de snelheid van vertering in de darm. Tenslotte wordt de totale

verteerbaarheid van zetmeel verbeterd, verhoogt het de melkproductie en bij gepelleteerd voer verbetert het de pellet kwaliteit.

Hoofdstuk 3 bespreekt de effecten van expanderen op de verteerbaarheid van zetmeel uit gerst en mais in pens en darm. Aan vier melkgevende Deense Holstein Friesian koeien, all voorzien van canules in pens, duodenum en ileum, werd een basisrantsoen van gras/klaver silage of gras/klaver hooi verstrekt dat werd, aangevuld met sojaschroot en onbehandelde gerst, geëxpandeerde gerst (105 °C), onbehandelde mais of geëxpandeerde mais (95 °C). De karakteristieken van zetmeelafbraak in de pens werden bepaald in niet behandelde en geëxpandeerde granen met behulp van *in situ* incubaties van met het voer gevulde nylon zakjes met twee poriëngroottes (15 of 36 micron). De snelheid van zetmeelafbraak werd ook *in vivo* bepaald met seriëel uitgevoerde evacuaties van de pensinhoud. De verteerbaarheid *in vivo* werd bepaald door het bemonsteren van de inhoud van duodenum, ileum en faecale uitscheiding. Ongeacht de behandelingsmethode was de *in situ* bepaalde afbraak van zetmeel in de pens hoger voor gerst dan voor mais. *In situ* studies (36 micron) lieten zien dat expanderen de effectieve afbraak van maiszetmeel in de pens verhoogde van 0,60 naar 0,72, voornamelijk als gevolg van een toename van de uitwasbare fractie. De effectieve afbraak van gerstzetmeel bleef echter onveranderd op 0,96, omdat hier een afname in de afbraaksnelheid van 0,63 tot 0,36 h⁻¹, gemeten met nylon zakjes met poriën van 36 micron, opwoog tegen de toename van de uitwasbare fractie. De *in vivo* afbraak karakteristieken, gebaseerd op pensevacuaties en een correctie voor passage van zetmeel uit de penspool, liet zien dat hittebehandeling de fractionele afbraaksnelheid van zowel gerst als mais verhoogde. Dit resulteerde in verhogingen van de effectieve pansafbraak van gerstzetmeel van 0,81 tot 0,85 en van maiszetmeel van 0,71 naar 0,78. De *in situ* methode leek de pansafbraak van snel afbreekbaar zetmeel in gerst te overschatten en de afbraak van langzaam afbreekbaar zetmeel in mais te onderschatten. Hierbij moet bedacht worden dat de resultaten van de *in situ* methode sterk afhankelijk zijn van de aannames die gedaan worden over de vertering en passage van de uitwasbare fractie. Verteerbaarheid in het totale maagdarmkanaal van gerstzetmeel werd niet beïnvloed door de hittebehandeling (0,99); die van zetmeel in mais werd verhoogd van 0,84 tot 0,96. De *in vivo* bepaalde schijnbare pensvertering van zetmeel was hoger voor gerst dan voor mais. De passage van zetmeel uit gerst door het duodenum bereikte 4

uren na het voeren een maximum, terwijl voor zetmeel uit mais een veel grotere piek in de passage werd gevonden tussen 10 en 12 uren na het voeren. Dit verschil wijst op een tijdsvertraging voor niet afgebroken maiszetmeel in de pens of mogelijk in de lebmaag en is tevens een aanwijzing dat zetmeelpassage niet verloopt volgens een eerste orde kinetiek.

Het onderzoek beschreven in de hoofdstukken 4 tot en met 7 heeft haar focus op de omstandigheden onder begrazing. In deze proeven werd aan melkgevende Holstein Friesian koeien, voorzien van een grote penscanule, de mogelijkheid geboden een veld met Engels *raaigras* (*Lolium perenne*) te begrazen. Naast een controle behandeling van alleen grazen, werd aan de dieren een supplement verstrekt van per melkbeurt 3 kg krachtvoer, bestaande uit gepelleteerde gerst, gepelleteerde mais, getoaste en daarna gepelleteerde gerst en getoaste en daarna gepelleteerde mais. Voorafgaand aan en na afloop van een graasperiode van 3 uren in de ochtend werd de pensinhoud geëvacueerd, gewogen en na bemonstering in het dier teruggeplaatst. Na de tweede pensevacuatie werden de dieren gedurende 6 uren binnen gehouden en gevast, waarna de evacuaties van de pensinhoud werden herhaald.

In hoofdstuk 4 werden het verloop bestudeerd van de grasopname, de poolgroottes in de pens, de penskinetiek en de productie van de controledieren en de met krachtvoer gesupplementeerde dieren. Het voeren van granen die een hittebehandeling (toasten) hadden ondergaan hadden numeriek maar niet significant een lagere drogestof opname uit gras in de eerste graasperiode na de ochtendmelking. In vergelijking met de controledieren was de schijnbare verdwijning van N uit de pens verlaagd. Tussen de gesupplementeerde krachtvoerders werden geen significante verschillen in verdwijning van N uit de pens waargenomen. Het verstrekken van technologisch behandelde granen resulteerde in een significante verlaging van de gehalten aan vet en ureum in de melk. Melkproductie en percentage eiwit in de melk in de gesupplementeerde dieren waren hoger dan in de controledieren. Er werd geconcludeerd dat het verstrekken van uit granen bestaand energierijk, eiwitarm krachtvoer aan met gras gevoerde melkkoeien leidde tot een behoorlijke verbetering van de N benutting en een verlaging van de ureum uitscheiding in de melk.

In hoofdstuk 5 worden de resultaten weergegeven van de *in situ* uitgevoerde nylon bag incubaties. Resultaten worden gepresenteerd van de effecten van verschillende hittebehandelingen op de *in situ* afbraak van eiwit en zetmeel van granen en die van vers Engels raaigras (*Lolium perenne*). Pelleteren en druktoasten verhoogde de onverteerbare fractie van droge en organische stof (OS) en verlaagde de *in situ* eiwitafbraak van de granen. Door toasten werd het percentage bestendig eiwit in gerst verhoogd van 36,4 naar 52,4 en voor mais 50,3 naar 58,2. Het percentage bestendig zetmeel werd door toatsten in gerst verhoogd van 14,9 naar 16,7 en in mais verlaagd van 36,1 naar 31,3. In vergelijking met alleen pelleteren werd na pelleteren en toasten de omvang van de uitwasbare fractie van beide granen voor zowel eiwit als zetmeel verlaagd. De fractionele afbraaksnelheid van eiwit werd verlaagd, terwijl voor mais de fractionele afbraaksnelheid van zetmeel werd verhoogd en die in gerst werd verlaagd. De chemische samenstelling en *in situ* afbraak karakteristieken van de raaigras monsters (geoogst in de zomermaanden in de periode tussen 2 juni en 31 juli) leidde niet tot significante verschillen tussen oogstdata, maar met het ouder worden werd er wel een tendens waargenomen van een afname van de afbraaksnelheid van de potentieel afbreekbare niet uitwasbare fracties van organische stof en eiwit. Er werd geconcludeerd dat eiwit in vers gras in de pens voor een groot deel afbreekbaar is en dat daardoor het extra verstrekken van technologisch behandelde granen een beter gebalanceerde beschikbaarheid van eiwit en energie geeft, maar dat de verschillen tussen typen graan groter zijn dan de verschillen tussen technologische behandelingen.

Hoofdstuk 6 rapporteert over het effect van graan supplementen op de pensfermentatie. In vergelijking met de controledieren resulteerde het verstrekken van supplementen in een snelle en uitgebreide fermentatie van zetmeel, met als gevolg hogere concentraties aan vluchtige vetzuren (VFA) en een lagere pH in de pensvloeistof. De concentraties (mmol/L) aan totaal VFA waren hoger in koeien gevoerd met technologisch behandelde gerst (respectievelijk 121,0 en 125,3 voor gepelleteerde gerst en getoaste en gepelleteerde gerst) dan bij koeien gevoerd met technologisch behandelde mais (respectievelijk 121,7 en 115,6 voor gepelleteerde mais en getoaste en gepelleteerde mais). Het voeren van supplementen verlaagde de azijnzuur/propionzuur verhouding en de Nonglucogenic Glucogenic Ratio (NGR) ten opzichte van niet gesupplementeerde dieren, maar

graansoort of type technologische behandeling hadden geen effect. Het aandeel azijnzuur in de totale VFA bij de gesupplementeerde dieren daalde van 66 naar 61% en het gehalte aan propionzuur steeg van 19 naar 24% ongeacht graansoort of type technologische behandeling. Het verstrekken van een supplement verlaagde de concentratie aan ammonia (mmol/l) en de verhouding tussen ammonia en totaal VFA. In vergelijking met alleen pelleteren gaf toasting gevolgd door pelleteren numeriek lagere ammonia concentraties in de pens en gaf het een lagere verhouding tussen ammonia en totaal VFA. De VFA patronen, de zijnzuur propionzuur verhouding en het gehalte aan totaal VFA werd niet beïnvloed door het type technologische behandeling.

In hoofdstuk 7 zijn de effecten van technologische behandeling op de producties van VFA bestudeerd. Hiervoor werd gebruik gemaakt van een isotoop verdunnings methode waarbij met ^{13}C verrijkt azijnzuur werd gebruikt als merker. Vlak voor de start van een graasperiode van 3 uren werd 100 mg 99% verrijkt ^{13}C Na-acetaat in de pens gebracht en dit werd na afloop van de graasperiode herhaald met nog eens 50 mg 99% verrijkt ^{13}C Na-acetaat, waarna de koeien 6 uren werden gevast tot het middagmelken. Tijdens de graasperiode waren de productiesnelheden van azijnzuur, propionzuur en boterzuur significant hoger in gesupplementeerde dan in niet gesupplementeerde dieren. Ook was het effect van gerst groter dan van mais en was het effect van pelleteren groter dan van toasten plus pelleteren. Gedurende de periode van vasten waren alleen bij mais de snelheden van verdwijning en productie van VFA hoger. De hogere VFA producties bij de gesupplementeerde dieren was een afspiegeling van de uitgebreide fermentatie in de pens die optreedt na het voeren van technologisch behandelde granen.

In de algemene discussie in hoofdstuk 8 werd op basis van de met de *in situ* techniek verkregen resultaten geconcludeerd dat het rantsoen op basis van alleen gras een minder synchroon afbraakpatroon had voor de pensbacteriën dan de gesupplementeerde rantsoenen. Een supplement van 3 kg mais per dag gaf een betere synchronisatie dan hogere niveaus van supplementatie. Hogere niveaus van supplementatie verlaagden de synchronisatie index bij mais en deze verlaging was groter bij niet technologisch behandeld dan bij technologische behandelde mais. Dit suggereert dat het pelleteren of expanderen van mais meer voordeel biedt dan eenvoudige mechanische behandelingen

zoals breken of malen en dat toasten na pelleteren betere resultaten geeft dan pelleteren alleen.

Voor gerst gaf het verhogen van het supplementatieniveau van 3 kg per dag naar 6 kg per dag en vervolgens naar 9 kg per dag telkens een toename van de mate van synchronisatie. Voor gerst waren, in vergelijking met de hogere niveaus van supplementatie de verschillen tussen onbehandeld en technologisch behandelde meer uitgesproken bij een niveau van supplementatie van 3 kg per dag. De hogere niveaus van supplementatie gaven een ongeveer gelijke synchronisatie index (I_s). Dit doet vermoeden dat gerst geschikter is als supplement naast gras bij hogere voeropnameniveaus.

Belangrijkste conclusies

In situ studies toonden aan dat alle hydrothermische behandelingen (pelleteren, expanderen, toasten) de beschikbaarheid voor microbiële afbraak in de pens van zetmeel uit mais verhoogden, maar die van gerst verlaagden. Expanderen en toasten verlaagden, pelleteren daarentegen verhoogde de beschikbaarheid voor microbiële afbraak in de pens van eiwit, daarentegen verhoogden alle hydrothermische behandelingen de eiwitbeschikbaarheid van mais. In vergelijking met onbehandeld graan werd door expanderen de schijnbare vertering in de pens en de darmvertering van maiszetmeel verhoogd, maar was er geen significante invloed bij gerst. Het supplementeren met gepelleteerd en onder druk getoaste granen had geen significante invloed op de drogestof opname van vers gras in de eerste graasperiode ('s ochtends na het melken). Het voeren van granen die een hittebehandeling hadden ondergaan verlaagde het ammonia N niveaus in de pens. Het supplementeren van weidegras met gepelleteerd en onder druk getoaste granen verlaagde de pH, de ammonia concentratie in de pens, de verhouding ammonia totaal VFA, het aandeel isoboterzuur in de totaal VFA, de azijnzuur propionzuur verhouding en de NGR. Tegelijkertijd verhoogde het de concentraties aan totaal VFA, en de aandelen propionzuur, boterzuur en valeriaanzuur in de totaal VFA. Het lijkt erop dat het effect van hittebehandeling effectiever is bij mais dan bij gerst. Door pelleteren of toasten verdwenen de tussen onbehandelde mais en gerst bestaande verschillen in pensfermentatie. Bij het voeren van granen als supplement naast gras had zowel pelleteren als toasten plus pelleteren een invloed op de productie respons in

lacterende koeien. Het melkeiwitgehalte werd verhoogd en de melkvetproductie en het ureumgehalte in melk werden verlaagd, maar tussen de beide hittebehandelingen werden geen verschillen gevonden. In de literatuur zijn geen proeven bekend waarin de waarde van geëxpandeerd of getoaste gerst of mais is onderzocht. Er moeten dus productieproeven worden uitgevoerd om de waarde van op deze wijze behandelde granen te bepalen. De noodzaak van pensynchronisatie is speciaal van belang voor rantsoenen gebaseerd op gras, waarin de afbraaksnelheden van energie en eiwit sterk asynchroon verlopen ($I_S=0.39$). Technologische behandelingen van granen leidt tot een meer synchronoos verlopen van de afbraak van energie en eiwit waardoor een hogere opbrengst aan microbiëel eiwit en een betere N benutting mogelijk wordt. Echter om dit te evalueren zijn directe metingen nodig van de microbiële eiwit produktie. Invloedsfactor op de schattingen van de synchronisatie van het in de pens beschikbaar komen van OM en N die meer onderzoek vergen zijn de patronen van ruwvoeropname, de turnoversnelheid van de pensinhoud en de frequentie en tijd van supplement verstrekking. Onder begrazing zijn de responsen als gevolg van graan supplementatie erg afhankelijk van de kwaliteit en afbraakkenmerken van het opgenomen gras. Deze veranderen met het vorderen van het seizoen, waardoor het synchronoos laten verlopen van de pensafbraak van koolhydraten uit het supplement en eiwit uit het gras extra wordt bemoeilijkt.

ÖSSZEFOGLALÁS

Európa észak-nyugati területein a nagy tejtermelésű tehén állományok szálatakarmányainak 30 – 50 %-át legelőfü teszi ki, mely elsősorban angolperje (*Lolium perenne L.*). A legelőfü sok könnyen oldódó fehérjét és nem fehérje nitrogént tartalmaz, ami a bendőben nagy mértékben és gyorsan lebomlik. Ezért a tehenek által lelegelt angolperje a bendőmikrobák nitrogén és energiaellátásának aszinkron állapotát idézheti elő. Ennek következtében a bendőben megnő az ammónia koncentráció és a takarmány eredetű fehérje mikrobiális felhasználásának hatékonysága csökken, így a mikrobás fehérje szintézisre potenciálisan rendelkezésre álló aminosavak veszteségével is számolhatunk. A bendőben lebontható fehérje hatékony mikrobiális hasznosíthatósága elsősorban a bendőben rendelkezésre álló szénhidrát forrástól függ. A szakirodalmi adatok azt bizonyítják, hogy a tejtermelő tehenek bendő fermentációja javítható ha a bendőt több energiával látjuk el, s ez úgy érhető el például, ha jó bendőbeli lebonthatóságú keményítőt juttatunk a bendőbe. A keményítőforrásként felhasználható gabonamagvak hőkezelésével lehetőséget teremthetünk a bendőbeli fehérje és szénhidrát arány manipulálására úgy, hogy a mikrobák nitrogén hasznosításban növekedés következzen be.

Az értekezés első fejezetében a dolgozat célkitűzései és a disszertáció felépítése került leírásra. A második fejezet szakirodalmi áttekintés a különböző gabonamagvaknak a tejelő tehenek takarányozásában betöltött fontos szerepéről. Bemutatásra és részletezésre kerülnek a különböző hidrotermikus gabonamag előkészítési eljárások. Kiemelt figyelem fordul a hőkezelésnek a szalmonellák csökkentésében játszott szerepére, a hőkezelt keményítő bendőbeli és vékonybélbeli emészthetőségére, valamint a hőkezelt gabonamagvaknak a tehenek szárazanyagfelvételére és tejtermelésére gyakorolt hatására. A hidrotermikus előkészítési eljárások ugyanis a keményítő zselatinizációját okozzák, denaturalizálják a fehérjét, inaktiválják az antinutritív anyagokat, csíramentesítik a hőkezelt takarmányt. A szakirodalmi adatok áttekintése alapján arra a következtetésre juthatunk, hogy a gabonamagvak hidrotermikus előkészítése hatékonyan csökkenti a takarmány szalmonella fertőzöttségét, megnöveli a bendőben lebontható keményítő mennyiségét és az un. bypass fehérje mennyiségét, valamint megváltoztatja keményítő és a fehérje emésztésének a helyét. További hatásként növeli a keményítő vékonybélbeni lebontásának mértékét, a gabonamagvak teljes emésztőtraktuson mért emészthetőségét, a

megtermelt tej mennyiségét és nem utolsó sorban a pelletált takarmány minőségét is javítja, ami abban az esetben fontos, ha a hőkezelt gabonamag pelletált formában kerül felhasználásra.

A harmadik fejezet a hőkezelési eljárások közül az expandálás árpa és kukorica bendő- és vékonybél emészthetőségére gyakorolt hatását tárgyalja. A kísérletbe négy dán Holstein Fríz tejtermelő tehenet állítottunk be, amelyeket bendő-, duodenum- valamint ileum fisztulával láttunk el. Az angolperje-fehérhere szilázsából és szénából álló alaptakarmányt szójadarával, valamint hőkezeletlen árpával, expandált árpával (105 °C), hőkezeletlen kukoricával, illetve expandált kukoricával (95 °C) egészítettük ki. A hőkezeletlen, illetve hőkezelt gabonamagvak keményítőtartalmának bendőbeli lebonthatóságát *in situ* módszerrel határoztuk meg. A vizsgálat során kétféle pórusméretű (15 és 36 μm) műanyagszövetből készült zsákocskákat használtunk. A keményítő bendőbeli lebomlásának mértéke *in vivo* módszerrel, valamint a bendő teljes manuális kiürítésén alapuló technikával is meghatározásra került. További *in vivo* emészthetőségi vizsgálatok céljából a duodenális chimuszból, az ileális chimuszból valamint a bélsárból is mintát vettünk. Az *in situ* vizsgálatok eredményei szerint az árpa keményítő bendőbeli lebontása nagyobb volt, mint kukoricáé függetlenül attól hogy hőkezelve volt-e a gabonamag vagy sem. Az *in situ* vizsgálatok arra is fényt derítettek, hogy az expandálás megnövelte a kukorica keményítő bendőbeli lebomlását, a hőkezeletlen kukoricához képest 60%-ról 72%-ra, ami elsősorban azzal magyarázható, hogy a keményítő vízoldható frakciója hőhatásra megnövekedett. Az árpa keményítő bendőben lebomló hányada a hőkezeletlen árpához képest nem változott és expandálást követően is 96% maradt. Ez azzal magyarázható, hogy bár megnövekedett a vízben oldható keményítő frakció, a lassan lebomló keményítőhányad óránkénti lebomlási sebessége lecsökkent 63%-ról 36%-ra (36 μm -os nejlonzsákocskák használata esetében). A bendő manuális kiürítésén alapuló és a bendőn való áthaladás mértékével korrigált *in vivo* lebomlási vizsgálatok eredményei szerint az expandálás megnövelte a lassan lebomló keményítőhányad óránkénti lebomlási sebességét mindkét vizsgált gabonamagnál, így a keményítő effektíve lebontható hányada is növekedett az árpa (81%-ról 85%-ra) és a kukorica (71%-ról 78%-ra) esetében is. Az eredmények alapján megállapítható, hogy az *in situ* vizsgálatok túlbecslik a bendőben gyorsan lebomló árpa keményítő bendőbeli

lebomlásának mértékét és alábecslik a lassan fermentálható kukoricáét. Azonban meg kell állapítani, hogy az *in situ* eredmények nagymértékben függenek a vízoldható keményítő frakció bendőn való áthaladásának és emészthetőségének a kiszámítása során alkalmazott becslések értékétől. A vizsgálatok további eredményei szerint az árpa keményítő látszólagos emészthetősége nagyobb volt, mint a kukoricáé. Azonban az árpa keményítő teljes emésztőtraktuson mért emészthetőségét nem befolyásolta az expandálás (99%), míg a kukorica esetében megnövelte azt 84%-ról 96%-ra. A keményítő duodénumon való áthaladása árpa esetében az etetést követően 4 óra, míg a kukorica esetében 10-12 óra elteltével volt a legnagyobb mértékű. Ez arra utal, hogy az hőkezeletlen kukorica keményítőnek a bendőn, illetve az oltón való áthaladási ideje az árpához képest késleltetett. A bendőn való frakcionális áthaladás mértéke úgy tűnik nem állandó, ami arra utal, hogy a keményítőnek az előgyomrokon illetve az oltón történő áthaladása valószínűleg nem követi az un. elsődleges kinetikai szabályt.

A negyedik a hetedik fejezetben leírt vizsgálatokba bendő kanüllel ellátott holland Holstein-Fríz tejelő teheneket állítottunk be és az állatokat angolperjés (*Lolium perenne*) legelőterületen legeltettük. A kizárólag legelőfüvet fogyasztó kontroll kezelés mellett, hőkezelt gabonamagvakkal (pelletát árpa, tószolt majd pelletát árpa, pelletált kukorica, tószolt majd pelletált kukorica) egészítettük ki a tehenek napi takarmányadagját. Az abrakot napi két egyenlő 3 kg-os részre osztva etettük a fejőházban a fejések során. A reggeli fejest követő három óra időtartamú legeltetés előtt és után a bendőtartalmat kézzel eltávolítottuk, lemértük, mintáztuk. A 3 óra időtartamú legeltetést követően az állatokat az istállóban hat órán át éhezettük, majd ezt követően harmadszor is elvégeztük a bendő manuális kiürítését.

A negyedik fejezetben a legelőfü hőkezelt gabonamagvakkal történő kiegészítésnek a fűfelvételre, a bendőtartalom nagyságára, a bendő kinetikára és a tehenek tejtermelésére gyakorolt hatását vizsgáltuk. A hőkezelt gabonamagvak etetése csökkentette a tehenek legelőfü felvételét a reggeli fejest követő, első legelési időszakban. Eredményeink szerint az abrakot nem fogyasztó állatokkal szemben a hőkezelt gabonamagvakat fogyasztó tehenek bendőjéből a nitrogén kiürülésének mértéke csökkent, amit az abraktakarmány hőkezelési módja nem befolyásolt szignifikánsan ($P > 0,05$). A hőkezelt gabonamagvakkal történő kiegészítés szignifikánsan ($P < 0,05$) csökkentette a tejjel termelt zsír mennyiségét

és a tej karbamid tartalmát, valamint szignifikánsan növelte ($P < 0,05$) a termelt tej mennyiségét és növelte tejjel termelt fehérje mennyiségét is. Megállapítható tehát, hogy a legelőfü hőkezelt gabonamagvakkal történő kiegészítése során javulhat a nitrogén bendőbeli hasznosíthatósága, ami így csökkentheti a tej karbamid tartalmát.

Az ötödik fejezet tartalmazza a műanyagszövetből készült zsákocskákkal elvégzett, a legelőt alkotó angolperje (*Lolium perenne*) *in situ* lebonthatósági vizsgálatára vonatkozó eredményeket, valamint tárgyalja a különböző hőkezelések hatását a gabonamagvak *in situ* fehérje- és keményítő lebonthatóságára. Az eredmények szerint a pelletálás és a tósztolás megnövelte a gabonamagvak szárazanyag és szerves anyag tartalmának bendőben lebonthatatlan hányadát és csökkentette az *in situ* fehérje lebonthatóságot. Tósztolást követően a pelletáláshoz képest a lebontatlan fehérje hányad megnövekedett az árpa esetében 36,4%-ról 52,5%-ra, kukorica esetében pedig 50,3%-ról 58,2%-ra, míg a lebontatlan keményítő hányad árpa esetében emelkedett 14,9%-ról 16,7%-ra, kukorica esetében pedig csökkent 36,1%-ról 31,3%-ra. A pelletáláshoz képest a tósztolás mindkét vizsgált gabonamag esetében csökkentette a fehérje és a keményítő a bendőben gyorsan lebomló vízoldható frakcióját és a lassan lebomló fehérjehányad óránkénti lebomlási sebességét. Míg a keményítő lebomlás óránkénti sebessége a kukorica esetében nőtt, az árpa esetében csökkent. Az angolperje kémiai összetétele és az *in situ* lebomlási jellemzői a vizsgált időszakban (július 2 és július 31 között) történő mintavétel eredményei szerint szignifikánsan nem különböztek ($P > 0,05$), de a vegetációs idő előre haladtával a potenciálisan lebontható, vízben nem oldható szerves anyag és nyers fehérje lebontásának mértéke csökkenő tendenciát mutat. Megállapítható, hogy a legelőfü fehérjetartalma a bendőben nagymértékben lebomlik, ezért a hőkezelt gabonamagvakkal történő kiegészítés a bendőmikrobák számára sokkal kiegyensúlyozottabb fehérje és energia ellátást tesz lehetővé. A bendőbeli lebomlást jellemző tulajdonságok közötti különbségek azonban sokkal kifejezettebbek a gabonamagvak típusa között, mint a vizsgálatok során alkalmazott hőkezelési módok között.

A hatodik fejezet a hőkezelt gabonamagvak etetésének a bendőfermentációra kifejtett hatását vizsgálja. A gabonamagvak keményítőjének gyors és extenzív bendőbeli fermentációja minden abrakot fogyasztó tehén esetében az abrakot nem fogyasztó állatokhoz képest a bendőfolyadék nagyobb illó zsírsav koncentrációját és annak

alacsonyabb pH-ját eredményezte. A bendőfolyadék összes illózsírsav koncentrációja magasabb volt az árpával etetett (121,0 és 125,3 mmol/liter a pelletált árpa illetve a tószolt árpa esetében), mint a kukoricával etetett állatok bendőjében (121,7 és 115,6 mmol/liter a pelletált kukorica illetve a tószolt kukorica esetében). Az abraketetés csökkentette a bendőbeli ecetsav:propionsav arányt és a nem glükogén:glükogén illózsírsavak arányát is, azonban a gabonamag típusa vagy az előkészítés módja nem hozott szignifikáns különbségeket ($P > 0,05$). A bendőemésztés során a gabonamag típusától vagy az előkészítés módjától függetlenül fokozódott a propionsavas erjedés (a propionsav moláris aránya 19%-ról 24%-ra nőtt), ami az ecetsav csökkent mértékű keletkezésével járt együtt (az ecetsav moláris aránya 66%-ról 61%-ra csökkent). A különböző előkészítési eljárások azonban a bendőben azonos illózsírsavak képződését, azonos ecetsav és propionsav arányt és azonos összes illózsírsav koncentrációt eredményeztek. Az ammónia koncentráció (mmol/liter) valamint az ammónia és az összes illózsírsav aránya az abrakot fogyasztó állatok bendőjében csökkent. Ez a csökkenés a tószolt gabonamagvakat fogyasztó teheneknél nagyobb mértékű volt, mint a pelletált abrakkal etetett tehenek esetében.

A hetedik fejezetben a hőkezelt gabonamagvak etetésének az illó zsírsavak termelődésére gyakorolt hatása került bemutatásra. Izotóp technika segítségével vizsgáltuk az illózsírsavak keletkezésének mértékét. A kísérlet során ^{13}C -acetátot használtunk jelölő anyagként. A három óra időtartamú legeltetési periódus előtt 100 mg, majd a legelést követően újabb 50 mg 99% -os $^{13}\text{C}_2$ Na-acetátot kevertünk a teljes, evakuált bendőtartalomhoz. Az eredményeink szerint a legeltetés három órája alatt az ecetsav, a propionsav és a vajsav keletkezett mennyisége és a bendőből való eltávozásának (felszívódás és passage) mértéke szignifikánsan nagyobb ($P < 0,05$) volt az abrakot fogyasztó állatoknál, mint az abrak kiegészítésben nem részesülő teheneknél. Az árpa, mint gabonamag és a pelletálás, mint hőkezelési mód hatása nagyobb mértékű volt a keletkezett illózsírsavak mennyiségére és a bendőből való eltávozásának mértékére, mint a kukorica etetése, valamint a tósztolás hatása. A legeltetést követő éheztetés alatt az illózsírsavak keletkezése és a bendőből való eltávozásának mértéke a vizsgált gabonamagvak közül a kukoricánál volt szignifikánsan ($P < 0,05$) nagyobb. Megállapítható, hogy a hőkezelt gabonamagvakat fogyasztó tejelő tehenek bendőjének

magasabb illózsírsav koncentrációja feltehetően a gabonamagvak etetésének következtében bekövetkező extenzívebb bendőemésztési folyamatokra utal.

A nyolcadik fejezetben, arra a következtetésre jutottunk, hogy az *in situ* lebomlási jellemzők alapján a fű alapú takarmányadag kiegészítése hőkezelt gabonamagvakkal lehetőség a tejelő tehének táplálóanyagellátásának változtatására. A zöld legelőfű több, bendőben könnyen lebomló fehérjét tartalmaz, mint amennyire a mikrobiális populációnak az adott időszakban szüksége van, így jelentős mennyiségű ammónia abszorbeálódhat a véráramba, alakulhat át karbamiddá a májban és így jelentős mennyiségű nitrogén távozhat a vizelettel karbamid formában. Az elvégzett vizsgálatok alapján úgy tűnik, hogy a legelőfű mellett a hőkezelt gabonamagvak etetése befolyásolhatja a mikrobiális növekedést és a táplálóanyagok hasznosításának mértékét is. A legelőfű mellett etetett valamilyen formában hőkezelt kukorica sokkal szinkronizáltabb szubsztrát a mikrobák számára, mint a legelőfű mellett etetett hőkezeletlen kukorica, függetlenül attól, hogy napi 3, 6 vagy 9 kg-os adagban etetjük. A legelőfű mellett etetett napi 3 kg mennyiségű kukorica kiegészítés viszont szinkronizáltabb takarmány a mikrobák számára, mint a nagyobb szintű kiegészítés (6 vagy 9 kg). A nagyobb mennyiségben etetett kukorica csökkenti az un. szinkronizációs indexet (I_s). Ez a csökkenés a hőkezeletlen kukorica esetében jelentősebb, mint a hőkezelt esetén. Megállapítható, hogy a kukorica pelletálása vagy expandálása illetve tósztolása több előnnyel járhat, mint a kukorica egyszerű mechanikai előkészítése.

Az árpának a napi adagban 3, 6, illetve 9 kg-ra történő emelése az elfogyasztott legelőfűvel egyre szinkronizáltabb takarmányt eredményez a bendőmikrobák számára. A szinkronizációs indexszel kifejezhető különbségek a hőkezeletlen és a hőkezelt árpa közt sokkal kifejezettebbek a napi 3 kg mennyiségű kiegészítés során, mint nagyobb mennyiségben etetve (6 illetve 9 kg), ahol a szinkronizációs index már közel azonos. A hőkezelt árpa nagyobb adagban etetve sokkal előnyösebb abraktakarmány a legelő tejelő tehének számára, mint a csak mechanikailag előkészített vagy az előkészített, de napi kisebb adagban etetett árpa.

A dolgozat alapján a következő főbb következtetéseket lehet levonni:

- Az *in situ* vizsgálatok alapján a hőkezeletlen (csak darált) gabonamagvakhoz képest minden hidrotermikus kezelés (pelletálás, expandálás, tósztolás) megnövelte a kukoricakeményítő illetve csökkentette az árpakeményítő bendőbeli lebomlását. Az expandálás és a tósztolás csökkentette, de a pelletálás megnövelte az árpafehérje bendőbeli lebomlását, amíg valamennyi hidrotermikus kezelés (pelletálás, expandálás, tósztolás) megnövelte a kukorica fehérje bendőbeli lebomlását.
- A hőkezeletlen gabonamagvakkal összehasonlítva az expandálás szignifikánsan ($P < 0,05$) növelte a látszólagos bendő- és a teljes traktuson mért emészthetőséget, de nem volt szignifikáns hatással ($P > 0,05$) az árpa keményítő emészthetőségére.
- A legelőfü alapú takarmányadag kiegészítése pelletált és tósztoló gabonamagvakkal (árpa illetve kukorica) nem befolyásolta szignifikánsan ($P > 0,05$) a legelőfűből történő szárazanyag felvételt a legelés első, a reggeli fejest követő időszakában. A hőkezelt gabonamagvak etetése csökkentette a bendő ammónia koncentrációját, a bendő pH-t, az ammónia és összes illószírsav mennyiségének arányát, a bendőfolyadék izovajsav tartalmát, az ecetsav:propionsav arányt és a keletkezett nem glükogén:glükogén illószírsavak arányát. Mindezzel párhuzamosan megnövelte az összes bendőben keletkezett illószírsav mennyiségét, a propionsav, a vajsav és a valeriansav mennyiségét. A fermentációs vizsgálatok szerint úgy tűnik, hogy a kukorica és az árpa bendőfermentációra gyakorolt hatásának különbségei a hőkezelést követően csökkennek, valamint a kukorica hőkezelése hatásosabb az árpáénál.
- A gabonamagvak pelletálása, csakúgy, mint a tósztolása és az ezt követő pelletálása szignifikáns hatással van a tejelő tehének teljesítményére. A hőkezelt gabonamagvak etetése növeli a tejfehérje és csökkenti a tejsír mennyiségét és a tej karbamid tartalmát. A két különböző hőkezelési mód között azonban szignifikáns különbséget ($P > 0,05$) nem találtunk. Olyan vizsgálatok, amelyek az expandált vagy tósztoló árpa illetve kukorica a tejtermelési eredményekre gyakorolt pozitív hatását igazolták volna üzemi körülmények között, nem születtek. Ilyen irányú kísérletekre a hőkezelt gabonamagvak pontos takarmányozási értékének megállapításához a jövőben mindenképpen szükség van.

- Legelőfűre alapozott takarmányozás esetében a bendőmikrobák energia- és fehérje ellátásának szinkronizációja különösen fontos, ugyanis vizsgálataink szerint a legelőfű kifejezetten aszinkron táplálóanyagellátást jelent a bendőmikrobák számára ($I_s=0,39$). A fű alapú takarmányadag kiegészítése hőkezelt gabonamagvakkal a mikrobák jobb bendőbeli nitrogén és szerves anyag szinkronizációját eredményezheti, de ennek bizonyítására a bendőben szintetizálódó mikrobafehérje további vizsgálata szükséges.
- Azok a tényezők, amelyek hatással vannak a bendőmikrobák táplálóanyagellátására, a rendelkezésre álló szerves anyag és a nitrogén szinkronizációjára - mint a legelőfű felvétel mértéke vagy az abraketetés mértéke és gyakorisága -, további vizsgálatot igényelnek. A fű alapú takarmány hőkezelt gabonamagvakkal történő kiegészítésének hatékonysága nagymértékben függ a lelegelt fű minőségétől és annak bendőbeli lebontásától, amelyek a legeltetési szezon alatt változhatnak és így a mikrobiális táplálóanyagellátás szinkronizációját a fű eredetű nitrogén és a gabonamag eredetű szénhidrát között bonyolulttá tehetik.

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