PROCESSING OF LEGUME SEEDS: EFFECTS ON DIGESTIVE BEHAVIOUR IN DAIRY COWS

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J.O. Goelema

Proefschrift

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Processing of legume seeds: effects on digestive behaviour in dairy cows.
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Abstract

In this study, effects of toasting, expander treatment and pelleting on in situ rumen
degradability and intestinal digestibility of legume seeds are described. Toasting
decreases protein degradability of peas, lupins, faba beans and soybeans and starch
degradability of peas and faba beans, especially when broken instead of whole seeds are
processed. Toasting of a mixture of peas, lupins and faba beans resulted in a higher starch
degradability than expected on the separately treated feedstuffs. Toasting the mixture
decreased protein degradability, whereas pelleting and expander treatment increased protein
degradability. The observed effects for the latter two treatments were related to particle size
reduction during processing. Combinations of toasting, expander treatment and pelleting
resulted sometimes in interactions, but the order of the treatments hardly affected the effects
on protein and starch degradability. Peas and faba beans showed great resemblance in
chemical composition. This was reflected in their sensititivy to the processing conditions.
Likewise, lupins and soybeans showed similar behaviour after toasting.
The rumen degradability of amino acids (AAN) in lupins decreased, and showed a curvi-linear
response to processing time and temperature. Effects of toasting were similar for all amino
acids. Correction for microbial contamination based on diaminopimelic acid resulted in a higher
degradability of AAN compared to uncorrected N. Moreover, corrections affected the ranking of
treatments with respect to degradability of individual amino acids.
Laboratory measurements showed that protein dispersibility index (PDI) and protein
denaturation enthalpy, as measured by differential scanning calorimetry (DSC) are useful
indicators for protein denaturation, and can be used for evaluation of effects of toasting on
intestinal digestible rumen undegraded protein.

Stellingen

1. Ondanks alle tekortkomingen is de nylon zakjes incubatie methode de beste beschikbare methode voor het bepalen van de bestendigheid van afzonderlijke voercomponenten.
    Dit proefschrift

2. Hittebehandelingen leiden niet per definitie tot een verlaging van de zetmeelbestendigheid.
    Dit proefschrift

3. De hittebehandeling die tijdens pelleteren van mengvoeders onder praktijkomstandigheden plaatsvindt leidt niet tot een verhoging van de eiwitbestendigheid.
    Dit proefschrift

4. De huidige onnauwkeurige berekeningswijze voor het gehalte aan darmverteerbare aminozuren in voedermiddelen is voldoende betrouwbaar voor de evaluatie van praktische rantsoenen voor melkvee.

5. Het te sterk benadrukken van de aannames die ten grondslag liggen aan wetenschappelijk (model-)onderzoek verslechtert de kans op implementatie van de resultaten in de praktijk.

6. De mogelijkheid om internetpagina's als literatuur referentie te gebruiken vergroot de kans op fraude in wetenschappelijke artikelen.

7. De zorgelijke ontwikkeling dat politieke beslissingen steeds vaker op emotionele in plaats van op rationele gronden worden genomen wordt in hoge mate veroorzaakt doordat wetenschappers in het verleden een te afwachtende houding hebben aangenomen.

8. Promotieonderzoek vertoont grote gelijkenis met het vullen van een rugzak: slechts een beperkt deel van de bagage blijkt nodig, maar juist het doorgemaakte proces van verzameling en selectie is van onschatbare waarde.

9. De recente wijziging van het promotiereglement inzake de inhoud van stellingen belemmert promovendi in het geven van een persoonlijk niet-wetenschappelijk accent aan hun proefschrift.

Jacob Goelema
Processing of legume seeds: effects on digestive behaviour in dairy cows
Wageningen, 29 maart 1999.

Aan mijn ouders,
Carolien

Voorwoord

Voor u ligt een proefschrift over de effecten van technologische behandelingen van vlinderbloemigen. Tijdens de geweldig leerzame periode van vijf jaar promotie onderzoek werd ik gecoached door Huug Boer, Germ Hof, Thomas van der Poel, onder supervisie van mijn deskundige promotor Seerp Tamminga. Dankzij hen is het proefschrift geworden wat het nu is en ik ben hen daar zeer veel dank voor verschuldigd. Het onderzoeksproject werd financieel ondersteund door het Productschap Diervoeder, ACM U.A. te Meppel en Cargill B.V. te Amsterdam. Ik wil de begeleiders van het project, in het bijzonder de leden van de Werkgroep Voedingsonderzoek Herkauwers en de landbouwkundigen van de ACM U.A. en Cargill B.V. hartelijk danken voor hun bijdragen. Ik heb de met hen gevoerde discussies over opzet van de proeven en de interpretatie van de resultaten voor de praktische veevoeding altijd als zeer verhelderend ervaren en hoop daar in mijn verdere loopbaan mijn voordeel mee te kunnen doen.

De uitvoering van de dierexperimenten en de daaruit resulterende analyses was een geweldige klus, die nu nog niet geklaard zou zijn zonder de grote groep studenten en collega's die mij in de afgelopen periode hebben bijgestaan. Allereerst wil ik de studenten bedanken die in het kader van hun afstudeervak hebben meegewerkt aan dit project. In chronologische volgorde waren dit: Bas Philipse, Cécile van Zadelhoff, Gerard Brandsma, Wilbert Hilkens, Guido Habets, Carina Steendam, Peiqiang Yu, Bertho Boswerger, Mirjam Spreeuwenberg, Alfred Smits, Leon Vaessen, Aart Wemmers, Claudia Ferreira, Ine van Deurzen en Mieke Roth. Ik denk met veel plezier terug aan de tijd die we samen hebben doorgebracht in het Wageningen Feed Processing Centre (WFPC), op 'de Koesteeg' of op 'de Ossekampen' om de behandelingen en incubaties uit te voeren, en dank jullie hartelijk voor de geweldige inzet en jullie inbreng in de discussies.

Naast de studenten zijn er tal van andere mensen betrokken geweest bij de uitvoering van de experimenten. Piet Roeleveld en Carel Siebers van TNO, afdeling ILOB, wil ik bedanken voor het mengen, malen en opzakken van de grondstoffen. Jan Hagens, Ries Verkerk en Andre Jansen wil ik bedanken voor hun assistentie bij het 'bulk'transport van de grondstoffen van en naar het WPFC en de opslag ervan op 'De Haar'. Jan Lenaers heeft mij ingewijd in geheimen van de nylon zakjes incubaties en daarnaast ettelijke keren geholpen bij het maken, vullen, incuberen en legen van de zakjes. De dagelijkse verzorging van de fistelkoeien kon ik altijd met een gerust hart

overlaten aan de medewerkers van 'de Ossekampen', onder de supervisie van Leen Bijl en Peter Mekking. Voor de darmincubaties werd vaak gebruik gemaakt van de dieren van ID-DLO te Lelystad. Ik wil de medewerkers van de stofwisselingseenheid en het laboratorium, in het bijzonder Roel Terluin, Peter Rasing en Wim Vlaar hartelijk bedanken voor de prettige samenwerking.

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De meest intensieve discussies werden zonder twijfel gevoerd met mijn kamergenoten in het Basement Intelligence Centre en paranimfen, Menno Thomas en Jos Houdijk. Dat wij met de verhuizing van de Haagsteeg naar Zodiac van Jos gescheiden werden, was echter geen enkel probleem. Door het stemvolume wat op te schroeven, werd Jos op de discussie geattendeerd, wat soms een dichtgeslagen deur opleverde, bijvoorbeeld als de discussie een wat al te technologisch tintje kreeg.

My Norwegian friends from the Agricultural University in Ås deserve a special 'thank you'. Odd Magne Harstad, I want to thank you very much for giving me the opportunity for participating in your research program. During the experiments, I closely worked together with Trine Gotvassli, who passed away much too soon. Trine, together with Odd Magne, Egil, Kari, Hege, Mette, Liv-Torunn and Elise, you made my stay in Ås an experience I will never forget.

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Jacob

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General introduction

Feed processing as a means to improve feed utilization by ruminants

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Introduction

Ruminants derive their special place as farm animals from their capacity to digest and utilize feeds that are not or less suitable for monogastric animals and men. This is possible because of a symbiotic relationship between ruminants and the microbial population inhabiting their forestomachs. Throughout this thesis, the microbial breakdown in the rumen is referred to as degradation, whereas digestion refers to (enzymatic) breakdown. Advantages in comparison with digestion in monogastric animals are the degradation of structural carbohydrates resulting in the formation of easily absorbable and highly utilizable volatile fatty acids (VFA), the synthesis of microbial protein, the elimination of antinutritional factors, and the synthesis of B-vitamins. Disadvantages may be the degradation of feed protein and non-structural carbohydrates and the loss of potential energy in methane (CH_4) and fermentation heat.

In the feeding of ruminants, particularly that of dairy cattle, the interest in digestive behaviour of feed in forestomach and intestines is increasing, because of at least two reasons:

1)   The degradation of feed protein and microbial protein synthesis are rarely balanced. This imbalance results in unnecessary N losses from the rumen, because ammonia, resulting from deamination of amino acids and degradation of non-protein N, accumulates shortly after the feed is ingested, and may have left the rumen before it can be incorporated into microbial protein.

2)   The ratio in which the different VFA (acetic, propionic, and butyric acid) and lactic acid are formed in the rumen is largely depending on the rate at which the substrate (structural carbohydrates, non-structural carbohydrates, and protein) is degraded.

The imbalance between microbial degradation and synthesis as well as the ratio in which

VFA are formed are important determinants of the type of nutrients which come available for the animal, which in turn are important factors determining the level and composition of ruminant production. It is easy to understand that it would be advantageous to maximize the degradation of structural carbohydrates in the rumen, but to minimize or at least optimize the extent to which proteins and non-structural carbohydrates are degraded in the rumen. Ruminal degradation of the latter two feed components should be restricted to what is necessary for maintaining an efficient microbial activity and growth, provided that the rumen undegraded fraction is digested in the small intestine. Digestion of feed protein and non-structural carbohydrates in the small intestine has also the advantage that less nitrogen and energy is lost.

Among feed ingredients, a large variation exists in degradation characteristics of cell walls, protein and starch in roughages as well as concentrate ingredients. The fraction of the cell walls which is completely undegradable is often quite large and the rate of degradation is relatively low. In the majority of concentrate ingredients the size of the soluble fraction of protein or starch is (too) large and the rate of degradation (too) high. These characteristics can be influenced by processing, in positive as well as in negative sense. In the Netherlands, a large part of the feedstuffs which are included in concentrates for dairy cows are byproducts from food production processes. These byproducts are imported from all over the world. After removal of products such as for instance of juices or edible oils for human consumption, products such as citrus pulp and meal and expeller of soybean, coconut, palmkernels and sunflower seeds remain, products that are valuable feedstuffs for ruminants. Their relatively low prices make them attractive for inclusion in concentrates. The use of legume seeds and rapeseed in cattle feeding, however, is limited, despite their attractive protein content. An important reason why legume seeds are not commonly used as protein sources for ruminants, is the very rapid availability of nitrogen in the rumen. One the other hand, the VFA production is also low. This results in an imbalance between the availability of protein and energy for microbial protein synthesis, and results in unnecessary N-losses from the rumen. Therefore, processing may improve the protein value of these feedstuffs.
Treatments with moisture and heat (steam), chemicals (aldehydes, reducing sugars), temperature (heating), pressure (pelleting) or combinations of these (extrusion, expander treatment) have great potential to reduce the rumen degradability. Many extensive reviews on the effect of physical and chemical processing of ruminant feeds have been published

(Hale, 1973, Ferguson, 1975; Ørskov, 1976; Theurer, 1986; Van Belle et al., 1991; Fahey et al., 1993).

In this introductory chapter, some general aspects are given which seem relevant for legume seeds and rapeseed (meal). First, some structural characteristics of protein and starch are discussed, which are thought to be related to the differences between feedstuffs in degradation behaviour of protein and starch. These differences may also explain differences in the susceptibility of feedstuffs to processing, which is described in another paragraph. Furthermore, the changes after processing with respect to ruminant nutrition are described, as well as the characteristics which are used to quantify these changes. Emphasis has been put on the effects of physical treatments on digestion and utilization characteristics of protein and starch. For a limited number of processing methods a brief description is given, together with some literature results. Finally, the aim and outline of the thesis are described.

Compositional and structural aspects of protein and starch important for their digestive behaviour.

Protein

Proteins are macromolecular polypeptides, consisting of covalently bound a-amino acid residues. The sequence of these peptide-bound amino acids form the primary structure of the protein. The secondary structure of the polypeptide chain comprises helical coil, hold together by non-covalent bonds, such as hydrogen bonds. This structure has several forms, the a-helix, a ß-pleated sheet and the triple helix. Side chains in the a-helix are projected outward. Even in water-soluble protein, 40% of these chains are hydrophobic. To minimize the contacts between their hydrophobic groups in aqueous solution, the a-helices position themselves as best as possible to the inside, away from water, leaving the hydrophilic chains exposed to water. The tertiary structure is the folded and twisted positioning of the secondary structure, which is also stabilized by hydrogen bonds. When two polypeptide -SH groups containing chains are close together, covalent disulfide bonds can occur, which cannot be easily broken down. The way two or more polypeptides are merged together, often involving for instance nonpolypeptides groups, is the quaternary structure (Holum, 1982).

Depending on its function in the plant, a varying proportion of the protein is present in the soluble fraction (Robinson and Tamminga, 1984). This protein fraction is assumed to be

easily and rapidly degraded in the rumen. Part of the protein in growing plants is associated with the cell wall, an important part of which is extensin, a protein firmly cross-linked to cellulose. Protein in seeds can be divided between protein from the hull, seed coat and embryo, respectively. The protein content of the hull is usually low. High levels of cutin, silica or tannins in some hulls will impede proteolysis and as a result rumen degradation of hulls is variable. Seed coat protein is usually only a small portion of total seed protein and its resistance to degradation in the rumen is relatively high. Protein in the embryo is quantitatively an important fraction of total protein. Because part of the embryo is in effect a small plant, it might be expected that the characteristics of these proteins would be similar to that of growing plants. Storage proteins are often contained in protein bodies and are to be utilized by the embryo in its initial growth phase. Depending on their structure, susceptibility to proteolysis varies. Probably the primary characteristic of the structure of native protein that decreases susceptibility of proteins to proteolysis, is the number of disulfide bonds. They are known to be of particular importance in animal protein.

Starch

Starch is an important storage carbohydrate in many plants, and can make up for more than 70% in cereals. It is present as granules in leaves, stems, tubers and roots (potato, tapioca), fruits and seeds (cereals, legumes), and varies in shape and size. In most plants, a single starch granule is formed inside the amyloplast, where in some plants (*e.g.* oats) several small granules (4-10 μm) are formed, which aggregate to a much large complex (20-150 μm).

Starch contains two macro molecules, amylose and amylopectin, which are organized in a semi-crystalline structure. The linear amylose molecule is build up of 900-3000 D-glucopyranose resides, linked together by α-1,4 bonds. Amylopectin is a high-molecular weight polymer (10^4-10^5 D-glucopyranose residues), consisting of a large number of chains of 16-28 glucose units, linked together by α-1,4 bonds. These chains are interconnected by -1,6 linked branching points (Kotarski et al., 1992).

Starch granules are surrounded by a layer which consists of proteinaceous material, as well as some fat. The surface sometimes has indentations, grooves, fissures and pores, which may serve as starting points for microbial attack.

A wide variation in the digestive behaviour of starch (granules), depending on the starch source and processing conditions are reported in vitro (Wolters and Cone, 1992), as well

as in situ or in vivo (Nocek and Tamminga, 1991; Sauvant et al., 1994). Alternatively, digestive behaviour may differ between cultivars and within cultivars also due to growing conditions. In general, cereal starch is more easily digested than root/tuber starches, although tapioca starch is more susceptible than other tuber starches. Moreover, intact, native starches are less digestible than starches which have lost their crystalline structure (the so-called gelatinized starches) (Dreher et al., 1984). Most of the starch in seeds is located in the endosperm, which is surrounded by an aleuron layer which consists of autolytic enzymes (amylase inhibitors and protease inhibitors), vitamins, minerals, lipids and protein. Three types of endosperm are distinguished: peripheral, corneous and floury endosperm. Peripheral and corneous starch granules are surrounded by protein storage bodies, and embedded in an inaccessible matrix which consists mainly of protein and non-starch carbohydrates, whereas corneous starch has less cellular structure and a higher starch content.

The digestion of starch may be obstructed by fibre components, such as cellulose, hemicellulose and lignin and in turn, starch digestion may affect the utilization of other nutrients, such as protein, fat, vitamins and minerals. Differences in digestive behaviour between starch sources can be caused by several factors such as their amylose and amylopectin content, crystallinity, granular size and shape and the presence of enzyme inhibitors (Cone and Wolters, 1990). The amylose/amylopectin ratio varies from 1 to 4 (Cone and Wolters, 1990). This ratio may also influence the susceptibility of starches to enzymatic degradation. Starches containing a high amylose content generally are less susceptible than the feedstuffs high in amylopectin content. Smaller granules have a larger surface to volume ratio, and therefore are more susceptible to enzymatic digestion than large granules.

Aspects of digestion and nutrient utilization in ruminants

Proteolysis in the rumen results from the action of proteolytic enzymes from bacteria, protozoa and fungi. Bacterial proteases in the rumen are mainly cysteine proteases, but metallo- and serine proteases are also present. Protozoa also exhibit proteolytic activity, mainly cysteine and aspartate proteases, whereas rumen fungi have an extracellular metalloprotease (Wallace, 1991). As a result of proteolytic digestion, polypeptides are released which are then broken down into dipeptides and amino acids. Some of the amino acids are incorporated into microbial protein, another part of the amino acid N is converted

to ammonia by deamination. Part of the ammonia is reincorporated into microbial protein, but much is lost by diffusion across the rumen wall (Wallace, 1991). Soluble proteins are more susceptible to degradation than insoluble proteins, but exceptions to this rule do exist (Mahadevan et al., 1985). Cross-linking, particularly through disulfide bonds, has a major influence on protein degradability.

Proteolytic activity in the rumen varies, amongst others due to differences in the level of feed intake and due to dietary composition. An increased proteolytic activity with an increased feed intake can easily be explained by an increased biomass in the rumen. End products of fermentation are microbial mass, volatile fatty acids, B-vitamins, CO_2, CH_4 and fermentation heat. The ratio in which VFA are produced depends on the chemical composition of the substrate (Murphy et al., 1982), on the rate of degradation, and on the rumen fluid pH. A low pH, a rapidly degradable substrate and starch usually enhances propionate production, whereas a high pH, slowly degradable substrate or fibre enhances the production of acetic and butyric acid.

Despite the importance of starch in ruminant diets in Western-Europe and North-America, the mechanism of starch degradation by rumen micro-organisms has been only marginally studied. Isolated enzymes from various strains of rumen bacteria proved to be similar to amylases from mammalian or other bacterial sources. Yet, no accurate prediction of starch degradation could be obtained by using purified enzymes instead of freeze-dried, cell free preparations of rumen fluid (Cone, 1991). Part of the amylolytic activity is cell bound, but this aspect has not been studied intensively. Although not an essential element in starch utilization by ruminants, protozoa may play a role as stabilizing agent. Engulfment of starch granules by protozoa limits the amount of starch available for the usually rapid bacterial fermentation, and so helps to prevent lowering of rumen pH which, in severe cases may become detrimental to cell wall degrading bacteria (Chesson and Forsberg, 1988). According to Tamminga et al. (1989), amylolytic activity in the rumen is stimulated by a low pH as well as by the presence of starch.

Important degradation characteristics in the rumen are:
1.   The soluble or washable fraction (S or W) which is assumed to be degraded rapidly and completely.
2.   The fraction which will not be degraded (U) irrespective of the time it is incubated in the rumen.

3.   The fraction which is not soluble, but potentially degradable (D).

4.   The fractional rate of degradation (k_d) of fraction D.

From these characteristics the fraction of rumen undegraded intake protein (UIP) can be calculated for a chosen rumen passage rate (k_p) (equation 1). Taking into account the digestibility of UIP, the amount of intestinal digestible protein (DUP) can then be calculated.

$$UIP = U + D \times k_p / (k_d + k_p) \qquad (1)$$

For starch, it is assumed that 10% of the W escapes fermentation and that starch is completely degradable in the rumen (U = 0) (equation 2).

$$UIS = 0.1 \times W + D \times k_p / (k_d + k_p) \qquad (2)$$

The degradation characteristics mentioned above have been described for carbohydrates (structural as well as non-structural carbohydrates) and proteins (Tamminga et al., 1990; Sauvant et al., 1994) in several feeds. Recently, the digestibility of UIP in the intestines of some feedstuffs has been reported as well (Erasmus et al., 1994).

In order to optimize rumen fermentation, the degradative behaviour in the rumen should be controlled, if necessary by manipulation through processing. A careful and appropriate selection of concentrate ingredients, in combination with forages, will help to optimize milk production and -composition. In addition, feed processing, either by physical, chemical or biological means, can be used to manipulate site of digestion as well as degradation characteristics in the rumen and is therefore a helpful tool in optimizing ruminant production.

Feed processing

Before concentrate feedstuffs are fed to the animal, they usually have undergone several treatments which are applied to make the feedstuffs suitable for storage (drying), easy to handle (particle size reduction) and less bulky or dusty (pelleting). Moreover, their nutritional value can be altered by changing the shape and size to a form which facilitates intake, or prevents selective intake of concentrate ingredients (pelleting), by inactivation of components which hamper digestibility or absorption (heat treatment), or by shifting the

digestion of nutrients from the rumen to the small intestine (heat treatment)

Drying/cooling

Usually a lot of feedstuffs are cooled and/or dried, mainly to prevent microbial activity during storage. The conditions during cooling or drying vary greatly (Voragen et al., 1995), from cooling pelleted feed with ambient air, to mildly drying grains after harvesting by ventilation with heated air at approximately 35°C, to more intensive treatments (80-95°C) of corngluten, soybeans, rapeseed and palmkernels before milling, extraction or expeller treatment. Drying temperatures for citrus pulp may even exceed 100°C.

Particle size reduction

Particle size reduction includes breaking, cracking and grinding. The different ways of grinding affect the mean particle size, but also particle size distribution. Hammermilled meals of feedstuffs usually show a skewed particle size distribution, while roller milling generally results in a normal distribution, with a relatively smaller portion of fine particles. Feedstuffs show a different breaking behaviour during grinding, resulting in differences in mean particle size and particle size distribution. Grinding requires fossil energy, but may make the feed less bulky, saving in the cost of transport.

Steam treatment, extrusion, expander treatment, roasting, pelleting

Steam treatments are carried out in various degrees and for different reasons. A common treatment is the application of steam during conditioning of feed mashes in barrel type conditioners and in expanders (Thomas et al., 1997). After steam addition, part of the steam condenses on the colder feed mash, which results in a higher temperature and an increased moisture level. Conditioning may also involve the addition of water. It is carried out to improve the hygienic quality, binding properties and physical quality after pelleting. Depending on throughput, rotation speed of the paddle bar and the degree of fill, residence time in barrel type conditioner may vary from 20 to 255 s. Steam is also applied during toasting and extrusion.

Toasting can be carried out at atmospheric pressure (resulting in product temperatures close to 100°C), or in pressurized barrels such as an autoclave. In the latter case, there is a positive relation between steam pressure and the temperature in the autoclave. Processing times can be varied, although during autoclaving very short treatment times

are difficult to achieve due to the fact that the pressure has to be built up after closing the autoclave. The same difficulty is met for during the completion of the treatment. Consequently, it is difficult to evaluate the exact processing temperature and time during autoclaving. To be able to control processing time and temperature more precisely, special equipment was developed (Van der Poel et al., 1990), which enables perfect control of processing temperatures and times. Pressurized toasting is carried out with horizontal or vertical cylindrical vessels, with paddles or conveyor belts (Melcion and Van der Poel, 1993). Van der Poel et al. (1990) were able to vary processing temperature from 100 to 140°C, and carry out treatments batch wise as well as continuously.

Extruders consist of barrels with one or two screws, which transport the feed mash (Melcion and Van der Poel, 1993). The screw configuration can be varied by addition of reverse screw elements, pressure rings or air locks, in order to alter the amount of shearing action during transport. Water can be used to adjust the moisture content to the required level before processing. Although friction may be sufficient to increase temperature during extrusion, the barrel wall can be additionally heated by steam or electrically. The combination of temperature, pressure, moisture and shear, followed by expansion when the material leaves the barrel, changes the properties of the material, including its digestive behaviour in the rumen. Processing time in extruders varies from 30 to 150 s, while temperatures range from 80 to 200°C. Extrusion can be considered as a high shear treatment.

Expanders are similar to single screw extruders, but have an annular discharge valve, instead of a die. Expander treatment should be considered as an extra conditioning phase, which enables the feed manufacturer to increase the length of conditioning, as well as its temperature. An electrically or hydraulically adjustable cone is used to increase the pressure during operation up to 3800-4000 kPa (Pipa and Frank, 1989). Steam can be used for heating the barrel wall, as well as for injection in the feed mash to increase processing temperature. Mixing is carried out by mixing bolts, which results in shear action on the feed mash. Residence time in the expander varies from 5 to 15 s, while temperatures are ranging from 80 to 140°C. Shear action during expander treatment is much less than during extrusion.

Roasting is a dry heat treatment, in which heat is transferred by conduction, convection

and radiation. Heat can originate from gas burners or from electrical heaters. Moisture levels should be adjusted before the treatment, for instance by soaking. Processing temperature can be up to 200°C, while residence time is unlimited and may exceed several hours. Often, heated feedstuffs are removed from the roaster and kept in an insulated holding barrel to increase the processing time, before being cooled to ambient temperature.

Recently, Melcion and Van der Poel (1993) estimated production costs for twin-screw extrusion, single-screw extrusion, expander treatment, infra-red irradiation and pressure toasting at respectively Dfl. 51, 26, 17, 13 and 16 per ton. This indicates that extrusion is relatively costly compared to the other processes. However, it should be kept in mind that irradiation is usually carried out at a much lower production capacity than extrusion, expander treatment and pressurized toasting.

Pelleting involves the compression of a conditioned feed mash through a die. Residence time in the die does usually not exceed 15 s. Conditioning can be carried out by the addition of water and/or steam, which influences the amount of friction between feed particles, the barrel wall and in the die. Apart from the processing conditions, feed components influence the physical quality of pelleted animal feeds (Thomas et al., 1998[a]). In the Netherlands, pelleted feeds make up approximately 95% of the concentrates for dairy cows. The pelleted mashes are usually pre-conditioned at temperatures ranging from 65 and 90°C. Pelleting makes the feed less bulky, which facilitates transport. In addition, pelleting reduces selective intake and ingredient segregation, it destroys pathogenic organisms and the feed becomes less dusty and more palatable. As a result it can facilitate feed intake (Behnke, 1996).

Effects of heat treatment on protein and starch
Heat treatment of proteins results in structure stabilization and cross-linkages to carbohydrates, which protects them from ruminal hydrolysis or at least slows down their rate of degradation (Satter, 1986; Cleale et al., 1987). Such linkages may increase nutrient escape from degradation in the rumen, but, unless they are reversible in the intestine, the overalll digestibility may be reduced. The mechanism of altering the degradative behaviour of proteins with heat treatment involves principally denaturation. In structural terms, denaturation is a disorganization of the overall molecular shape of a protein. It can occur as an unfolding or uncoiling of a coiled or pleated structure, or as the

separation of the protein into its subunits, which may then unfold or uncoil (Holum, 1982). Any temperature change in the environment of the protein which can influence the non-covalent interactions involved in the structure may lead to an alteration of the quaternary, tertiary and secondary structure. More extreme forms of processing may lead to the destruction of the primary structure, often called 'degradation' of the protein (Finley, 1989). Depending on the processing conditions several processes may occur (Table 1), although it is obvious that not only temperature during treatment plays a role, but also factors such as residence time and moisture level. Very common are reactions of the early Maillard type. Lysine reacts with carbonyl compounds, usually originating from reducing sugars such as glucose, xylose and fructose. Other reactions also occur, including the formation of isopeptide cross-links between lysine and asparagine or glutamine. Additionally methionine, cystine and tryptophane may be involved in the isopeptide cross-linking (Broderick et al., 1991).

Table 1 Effects of heat treatment on denaturation and degradation of protein[1]

Temperature (°C)	Effect of heating
≤ 50	increase hydration, some loss of crystalline structure
70-80	disulfide splitting, loss of tertiary structure
80-90	loss of secondary structure disulfides
90-100	intermolecular disulfides formed
100-150	lysine and serine loss, isopeptide formation
150-200	peptidization and more isopeptide formation
200-250°C	pyrolysis of all amino acid residues

[1] Finley, 1989.

According to Satter (1986), heat treatment increases both the undegradable and undigestible protein fraction and the maximum intestinal supply of absorbable protein

depends on the time and temperature of the treatment.

With regard to starch, several processes may play a role upon heat treatment, such as swelling, gelatinization, and retrogradation. The magnitude of these processes depends on the particle size, but also largely on the temperature, the treatment time and the moisture level. Swelling results from the exposure of starch to water, combined with gradual heating. At low temperature (below 60 to 80°C) swelling is reversible after cooling and drying. At a certain higher temperature, depending on the moisture level, gelatinization may take place (Lund, 1984). At this temperature, the granular structure is altered from semi-crystalline to amorphous, which results in a different X-ray pattern and a loss of its birefringence. Gelatinization of individual starch granules occurs in a range of 1 to 2°C, but due to variation between granule fractions, it results in a 10 to 15°C range for the total starch. At low moisture content (< 35% moisture) the gelatinization temperature (T_m) dramatically increases (Colonna et al., 1992) from 50-60°C in excess moisture to more than 100°C at limited moisture levels.

Retrogradation of starch is the reassociation of starch molecules after gelatinization, in which hydrogen bonding between amylose and amylopectin is re-established. Retrograded starch does not completely regain the native starch character, and may even result in the formation of a starch fraction which is less digestible than native starch. On the other hand, retrograded starch may gelatinize again after subsequent (re-)heating.

Effects of processing on digestive behaviour of protein and starch

Particle size reduction increases the surface and therefore facilitates microbial attack. This applies to cell walls, starches as well as proteins. Particle size reduction therefore results in a substantially increased rate of solubilization and rate of fermentation. In several studies, this resulted in an increased rumen degradability of protein (Dixon and Hosking, 1988; Michalet-Doreau and Cerneau, 1991; Tice et al., 1993) and starch (Thomas et al., 1988; Cerneau and Michalet-Doreau, 1991). Grinding often results in differences in mean particle size between feedstuffs. After correction for particle size, N degradability was similar among feedstuffs (Michalet-Doreau and Cerneau, 1991).

Smaller particles are degraded faster, but probably also have a higher passage rate from the rumen. Therefore, for smaller particles total tract fibre digestibility generally reduces, but CP and starch digestibility increases. Grinding also reduces the role of rumination. The advantage of that is that it does require less energy for chewing, but it also reduces the amount of saliva produced. Therefore, buffering of the rumen contents is reduced, which

reduces pH, and may result in a shift from acetate towards propionate production. The reason for the latter is thought to be related to the depressing effect of the low pH on fibre degrading bacteria, and the direct effect of pH on the VFA production of cellulolytic bacteria. Toasting is generally applied to inactivate proteinaceous antinutritional factors (ANF) which are abundant in legume seeds. Although important for monogastrics, ANF do usually not cause problems for ruminants (Dixon and Hosking, 1988) and may even have beneficial effects, for example tannins can reduce rumen protein degradability.

For inactivation of ANF, toasting is generally carried out for 15-30 min at atmospheric pressure. Under these conditions, the activity of ANF can be reduced to acceptable levels (Van der Poel et al., 1990). Toasting under these conditions resulted in an increased protein digestibility in monogastrics, which could not be solely attributed to the inactivation of ANF's. For ruminants, toasting may result in a shift from degradation of protein in the rumen to digestion in the small intestine. An experiment, recently carried out in our laboratory (R. Zom, unpublished) showed, that pressurized toasting at higher temperatures (136°C) increases UIP of phaseolus beans much more than toasting at atmospheric conditions (Table 2). Based on these results, it was hypothesized that lower processing temperatures require longer processing times for achieving similar changes of UIP.

Table 2 Effect of toasting on rumen protein degradation characteristics of protein in phaseolus beans[1]

Temperature (°C)	time (min)	W (%)	D (%)	k_d (%/h)	UIP (%)
-	-	56	44	9.7	17
102	5	37	63	6.3	31
102	10	45	55	6.1	27
136	5	29	70	2.1	52
136	10	24	76	2.1	57

[1] R. Zom, 1991, unpublished. W = washable fraction, k_d = fractional rate of degradation; UIP = rumen undegraded intake protein fraction, calculated for a passage rate of 6 %/h.

Results of rumen protein degradation of legume seeds after autoclaving (Aguilera et al., 1992) were similar to the results after pressure toasting. Autoclaving for 30 min at 120°C decreased the soluble fraction of protein, as well as its degradation rate (Table 3). Consequently, UIP increased for all legume seeds, although there were differences in sensitivity against autoclaving among seeds.

Thomas et al. (1988) showed that autoclaving at 120°C increased the degradation rate of starch in corn and sorghum, although prolonged autoclaving sometimes resulted in a lower degradation rate compared to untreated sorghum.

Table 3 Effects of autoclaving on rumen protein degradation characteristics of legume seeds[1].

legume seed		S (%)	D (%)	k_d (%/h)	UIP (%)
Pea	C	39	61	8.6	25
	A	18	82	2.6	57
Lupin	C	36	63	9.8	25
	A	10	90	3.6	56
Field bean	C	33	66	8.6	28
	A	15	84	4.1	50
Vetch	C	22	78	4.3	46
	A	15	85	4.1	51
Bitter vetch	C	27	72	6.3	36
	A	15	85	4.5	48

[1] Aguilera et al. (1992). C = untreated control; A = autoclaved for 30 min at 120°C; S = soluble fraction; k_d = fractional rate of degradation; UIP = rumen undegraded intake protein fraction, calculated for a passage rate of 6 %/h.

In most other cases, treatments involve a combination of steam and shear treatment, such as steam-flaking, expander treatment and extrusion. Combinations of steam, pressure and

high temperature usually result in a decreased ruminal rate of degradation for protein (Broderick et al., 1991) and in an increased rate of ruminal degradation for starch (Nocek and Tamminga, 1991). Steamflaking is a combination of toasting for 15-30 min under atmospheric conditions (100-105°C), followed by flaking the heated seeds. Most reported studies with steamflaking are carried out with corn and sorghum, and are focussed on starch rather than protein degradability. It was generally concluded, that steam flaking of cereal grains increases the amount of starch fermented in the rumen, (Hale, 1973, Ørskov, 1976; Theurer, 1986) and increases its intestinal digestibility (Owens et al., 1986), resulting in a decrease of the amount digested in the small intestine. These effects are attributed to the decreased particle size, as well as to modifications in the starch granule. Similar effects on digestion of sorghum starch were found in a study by Prasad et al. (1975) after steam flaking, pressure cooking, expander treatment and extrusion. Xiong et al. (1990) found an inverse relation between in vitro starch availability and flake density after steamflaking, but a positive relation between rumen protein degradability and flake density.

Several studies have shown, that extrusion of horsebeans, peas, lupins, soybeans and rapeseed generally increases UIP (Table 4). It should be realized that these are in situ figures, a method for which no standardization yet exists. Results can therefore only be compared within experiments. The in situ results were however confirmed in vivo (Pena et al., 1986; Focant et al., 1990; Benchaar et al. 1991, 1994[ab]). Processing not only resulted in reduced rate of protein degradation, but also increased the in vivo duodenal flow of non-ammonia N (NAN). Whether such an increased NAN flow results in an improved animal performance depends on protein or energy being the most limiting factor. However, in other studies with processed protein sources no differences in duodenal protein flow were found (McMeniman and Armstrong, 1979; Annexstad et al., 1987; Focant et al., 1990; Scott et al., 1991; Ferlay et al., 1992).

Effects of expander treatment on protein degradability of legume seeds and rapeseed meal have not been studied very much. Sommer et al. (1994) showed, that expander treatment of rapeseed meal at 120, 130 and 150°C decrease protein degradability, while rumen degradability concomitantly increased. Regarding the similarities between single screw extrusion and expander treatment, it might be hypothesized that the effects on protein and starch degradability are similar.

Starch degradability in legume seeds is intermediate between slowly degradable corn and sorghum starch and the highly degradable starch in most other cereals (Herrera-Saldana et al., 1990; Tamminga et al., 1990). Results of extrusion on in situ starch degradability of legume seeds are hardly published. Walhain et al. (1992) reported a substantial decrease of in situ UIS after extrusion of peas, while Focant et al. (1990) reported increases of in vitro availability of starch after extrusion, but not after steamflaking.

Several studies have been carried out to study the effects of roasting on the digestive behaviour of soybeans (Tice et al., 1993) focussing on protein degradability (Table 5). In situ protein degradability usually decreases, resulting from a decreased protein solubility, as well as a smaller degradation rate (Stern et al., 1985; Faldet et al., 1991). The in situ results were also confirmed by in vivo studies. In a study by Tice et al. (1993), ruminal NH_3 N decreased, while duodenal feed N flows and apparent small intestinal N digestibility increased after roasting. Consequently, daily milk production increased, but milk composition remained unchanged. Knapp et al. (1991) found similar changes, but observed an increased in milk production and in milk fat percentage, while milk protein concentration decreased after inclusion of roasted soybeans. However, total tract N digestibility decreased after roasting, which resulted in impaired growth performance in lambs (Plegge et al., 1982; 1985). In another study no treatment differences were found (Scott et al., 1991).

Roasting was applied to increase UIP of lupins and compared with soybean meal in a diet for growing lambs (Kung et al., 1991) and dairy cows (Robinson and McNiven, 1993). Kung et al. (1991) observed a decreased in situ protein degradability, but did not observe an effect on growth of the lambs. Soybean meal and lupins resulted in a similar growth, which was related to the particle size of the lupins as fed. Supplementation of methionine to the roasted lupin diet did not affect growth response. Robinson and McNiven also found, that in situ UIP was increased from 7 to 33% after roasting at 115°C, while production of milk and milk components were similar for raw and treated lupins and soybean meal diets. However, milk protein concentration was lower for the lupin diets.

McNiven et al. (1994) studied effects of roasting on dry matter and protein degradability of barley, corn, oats and wheat. Roasting decreased the degradability of both dry matter and protein by reducing the soluble fraction. The fractional rate of degradation was increased in most cases, except for corn. For protein, solubility and the fractional rate of degradation decreased, resulting in an increased UIP. However, these results were not

confirmed in vivo (Robinson and McNiven, 1994). In situ incubation revealed that dry matter degradability of roasted barley was not very different from raw barley, although protein degradability was substantially reduced.

These results suggest, that, apart from optimization objectives, routine heat processing conditions will increase UIP, while UIS is usually increased.

Table 4 The effects of extrusion on in situ rumen protein degradation characteristics of legume seeds[1].

Feedstuff	Temperature (°C)	S (%)	k_d (%/h)	Undegraded protein (%)	DUP (%)	Reference
horsebeans	untreated	68	13.0	11	88	1
	120	39	6.6	30	90	1
horsebeans	untreated	56	-	9	78	2
	195	15	-	42	92	
lupins	untreated	81	22.0	5	63	3
	120	57	10.4	16	91	
	150	27	10.2	28	95	
	195	28	8.3	47	95	
lupins	untreated	-	-	2	50	4
	120	-	-	13	90	
	150	-	-	26	95	
lupins	untreated	75	19.2	7	84	5
	110	79	7.3	10	96	
	130	56	7.6	20	97	
	150	50	2.8	35	97	
	180	46	2.5	38	98	
lupins	untreated	30	-	5	56	6
	195	7	-	52	95	
peas	untreated	49	20.0	12	-	7
	140	13	18.0	46	99	
	180	13	15.0	45	-	
	220	18	16.0	29	-	
soybeans	untreated	-	-	2	-	8
	132	-	-	37	-	
	149	-	-	50	-	

[1] S= soluble fraction, k_d = fractional rate of degradation; Undegraded protein = rumen undegraded intake protein, calculated for a passage rate of 6 %/h, a result of multiple incubations, unless indicated otherwise; DUP = intestinal digestibility of UIP. 1: Cros et al., 1991[a]; 2: Benchaar et al. (1994[a]), 16 h rumen incubation; 3: Cros et al. (1991[b]), 16 h rumen degradation; 4: Cros et al., (1992), 16 rumen incubation; 5: Kibelolaud et al. (1993), DUP based on 16 h rumen incubation residue; 6: Benchaar et al. (1994[b]), 16 h rumen incubation; 7: Walhain et al. (1992); 8: Stern et al. (1985), 16 h rumen incubation.

Table 5 The effects of roasting on in situ protein degradation of soybeans in the rumen[1].

Feedstuff	Temperature/Time (°C/min)	S (%)	k_d (%/h)	Undegraded protein (%)	Reference
Soybeans	untreated			14	1
	102			32	
	128			41	
	144			65	
	159			74	
	185			86	
Soybean meal	untreated		11.3		2
	115		4.3		
	130		4.1		
	145		1.9		
Soybeans	untreated	42	17.4	28	3
	115/0	12	11.8	48	
	115/30	12	11.2	55	
	115/90	15	11.4	59	
	115/120	15	11.0	58	
	115/150	14	9.0	61	
Soybean meal	untreated	19	6.9	29	3
	113/0	13	3.6	41	
	130/30	10	1.8	54	
	130/90	11	1.9	58	
	130/150	9	1.2	63	
Soybeans	rolled, untreated			30	4
	rolled, 130/30			39	

[1]   S = soluble fraction, k_d = fractional rate of degradation; Undegraded protein = rumen undegraded intake protein, calculated for a passage rate of 6 %/h, a result of multiple incubations, unless indicated otherwise; 1: Plegge et al. (1982), 12 h rumen incubation; 2: Plegge et al. (1985), 3: Faldet et al. (1991); 4: Scott et al. (1991).

Apart from effects on physical quality and intake, pelleting also appears to influence degradative behaviour in the rumen. Published studies on digestion of concentrate feeds are limited to feeds for monogastric animals (Behnke, 1996). In a small number of studies

effects of pelleting (ground) roughages are described (Blaxter and Graham, 1956; Beardsley, 1964; Moore, 1964). Recent research in our laboratory (Houtmans, Kemp, Van der Velden, Hof and Tamminga, 1991, unpublished) with concentrates would suggest, that the size of the washable (and rapidly degraded) fractions of protein and starch increases due to pelleting, hence shifting the site of digestion to the rumen rather than to the intestines (Tables 6 and 7).

Table 6 Effect of pelleting on the rumen degradability of crude protein (CP) in concentrates[1].

concentrate	CP (g/kg DM)	mash				pellet[2]			
		W (%)	U (%)	k_d (%/h)	UIP (%)	W (%)	U (%)	k_d (%/h)	UIP (%)
A-standard	185	40	4.2	5.1	35	54	2.5	4.7	31
A-select	179	35	3.8	4.5	39	50	0.0	3.3	32
A-meadow	185	34	2.2	4.7	38	43	2.7	5.3	34
Corn-special	361	16	2.2	5.4	45	32	2.0	6.7	35
DVE-high	275	25	33.1	7.2	52	39	18.5	2.2	50
DVE-low	240	33	29.3	7.0	47	40	24.7	9.3	39

[1] J. Houtmans, G. Kemp, G. van der Velden, G. Hof and S. Tamminga, 1991; unpublished; W = washable fraction, U = rumen undegradable fraction, measured after 14 days of incubation; k_d = fractional rate of degradation; UIP = rumen undegraded intake protein, calculated for a passage rate of 6 %/h.

[2] Double pelleting: first compression in a fixed 6 x 60 mm die (bore hole x die thickness, Nordivan Mixcompress), subsequently in a rotating 5 x 85 mm die (bore hole x die thickness, Walter).

In the case of protein, the enlarged size of the soluble fraction is (at least partly) compensated by a decrease of the size of the U-fraction, resulting in more substrate available for microbial attack and therefore more microbial protein. The reason for the

changes in degradative behaviour due to pelleting is not entirely clear. Possible causes are changes in the structure of protein or changes in the particle size distribution. Pelleting also changed the digestive behaviour of starch (Table 7). The size of the washable fraction and/or the fractional degradation rate k_d increased. As a result, UIS is reduced by some 15 % units.

Table 7 Effect of pelleting on the rumen degradability of starch in concentrates[1].

Concentrate	Starch (g/kg DM)	Mash			Pellet[2]			reference[3]
		W (%)	k_d (%/h)	UIS (%)	W (%)	k_d (%/h)	UIS (%)	
Corn	358	13	5.0	49	21	5.2	44	1
Corn, barley, tapioca	373	51	7.7	27	59	9.3	22	1
A-standard	76	31	12.0	26	44	12.4	23	2
A-select	55	35	12.6	24	56	10.6	21	2
DVE-high	307	44	12.5	23	61	11.4	20	2
DVE-low	249	42	12.02	24	60	17.8	16	2

[1] W = washable fraction, k_d = fractional rate of degradation; UIS = rumen undegraded intake starch, calculated for a passage rate of 6 %/h.

[2] Double pelleting: first compression in a fix 6 x 60 mm die (bore hole x die thickness, Nordivan Mixcompress), subsequently in a rotating 5 x 85 mm die (bore hole x die thickness, Walter).

[3] 1: Tamminga et al. (1989). 2: J. Houtmans, G. Kemp, G. van der Velden, G. Hof and S. Tamminga, 1991; unpublished.

Aim and outline of this thesis

This thesis describes the effects of processing of legume seeds on digestive behaviour of protein and starch in the rumen and intestines of dairy cows. The information described in this thesis may give better insight in the mechanisms involved and the changes occurring upon processing of single feeds and mixtures of concentrate ingredients. This information enables feed manufacturers and nutritionists to formulate concentrates and rations which:

1. Optimize rumen fermentation processes
2. Influence the site of digestion, by shifting it from the rumen to the intestines, which qualitatively alters the supply of nutrients
3. Contribute to the reduction of unnecessary losses from the rumen.

The available literature shows that several physical treatments can be applied as effective means to alter ruminal digestion of protein and starch. In general, treatments such as (pressurized) toasting, roasting, expander treatment and extrusion will decrease rumen protein degradability and increase starch degradability. A decreased rumen protein degradability can be due to crosslinking between and within proteins and irreversible binding between aldehyde groups of carbohydrates and amino groups, especially the ϵ-amino groups of lysine. An increased starch degradability after processing can be attributed to gelatinization, while particle size reduction may play a role as well.

Grinding and pelleting as routine processes appear to increase both protein and starch degradability. The reasons for the observed changes after pelleting are not completely understood yet. It is hypothesized that particle size reduction may play an important role here too.

For studying effects of processing, feedstuffs that contain protein and starch in the native state are preferred, rather than feedstuffs like byproducts, that have undergone several treatments, of which the processing conditions are usually unknown. Raw legume seeds are available in the untreated form, which makes them suitable ingredients to study effects of processing. Another attractive characteristic of some of these seeds is that they contain both protein and starch in reasonable amounts. Moreover, the relatively high rumen degradability of the protein, gives good opportunities for studying effects of processing, which are usually carried out to decrease the rumen degradability. The starch degradability of legume seeds can be considered as intermediate, which makes them

suitable for studying effects of (heat) processing.

In the following chapters, the results of three experiments are described. In these experiments effects of 3 methods of processing are studied:

A. Pressure toasting
B. Conditioning/expander treatment
C. Conditioning/pelleting

Depending on the legume seeds studied, effects on protein and/or starch degradability and digestibility were studied. Treatments are sometimes carried out with single feedstuffs, while others are applied to mixtures of feedstuffs. Although it is generally assumed that mixing feedstuffs does not lead to interactions with respect to the nutritive value of the mixture, it unknown whether this also holds for processed mixtures.

The studies were set up, based on the following hypotheses:

1. Pressure toasting decreases protein degradability and increases starch degradability.
2. Expander treatment decreases protein degradability and increases starch degradability.
3. Pelleting increases protein degradability and starch degradability.
4. The effects of processing treatments, measured for single feedstuffs are additive. In other words, the effects are applicable to mixtures of feed mashes as well.

In all studies, rumen degradability and intestinal digestibility are measured with nylon bag incubations, the so-called *in situ* methods. These methods give quantitative estimates of degradability and digestibility. Although the absolute values of degradability may vary, for instance between feeding situations, the estimates can be used in a relative way. This makes them useful methods for the screening of processing effects on digestive behaviour.

On the other hand, the methods require the use of cannulated animals, and are labour intensive due to long incubations periods. Therefore, most experiments included various laboratory measurements which were assumed to mimic (part of) the digestive behaviour, and/or the effects of processing on digestion characteristics, which could be used in future research on processing.

In the study outlined in Chapter 2, effects of pressure toasting on peas, lupins and faba

beans and a mixture of these legume seeds are described. Chapter 3 describes the second experiment, in which the effects of pressure toasting, expander treatment and pelleting were studied, using the same mixture of legume seeds as in Chapter 2. The treatments were carried out separately, as well as in combination with each other.

The results of the third experiment are described in the Chapters 4, 5, 6 and 7. In this experiment, the effects of several conditions during pressure toasting on digestive behaviour of peas, faba beans, lupins, and soybeans were studied. The effects on the rumen degradability and intestinal digestibility of protein are described in Chapter 4, whereas the results of the laboratory measurements are shown in Chapter 5. For lupins, the results of toasting were also studied for the individual amino acids. These results are given in Chapter 6. In Chapter 7, the results of the in situ incubations and laboratory analyses for starch in peas and faba beans are presented.

In the General Discussion, the results reported in the Chapters 2-7 are discussed and evaluated. Suggestions for further research, as well as practical implications are presented.

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CHAPTER 1

EFFECT OF PRESSURE TOASTING ON THE RUMEN DEGRADABILITY AND INTESTINAL DIGESTIBILITY OF WHOLE AND BROKEN PEAS, LUPINS AND FABA BEANS AND A MIXTURE OF THESE FEEDSTUFFS

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Effect of Pressure Toasting on the Rumen Degradability and Intestinal Digestibility of Whole and Broken Peas, Lupins and Faba Beans and a Mixture of these Feedstuffs

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Abstract

The effects of pressure toasting of whole and broken peas, lupins and faba beans on *in situ* degradability of protein and starch and intestinal digestibility of protein were studied. To test for associative effects on rumen degradability and intestinal digestibility after toasting, a mixture of peas, lupins and faba beans was examined and results were compared with weighted averages of separately processed feedstuffs.

Pressure toasting for 3 min at 132°C decreased *in situ* protein degradability of peas, lupins and faba beans and *in situ* starch degradability of peas and faba beans, especially when broken vs. whole seeds were processed. Undegraded intake protein (%UIP) increased after toasting whole or broken seeds from 25% to 44% and 52% for peas, from 22% to 47% and 51% for lupins and from 20% to 48% and 57% for faba beans, respectively. Undegraded intake starch (%UIS) increased from 39% to 50% and 53% after toasting whole and broken peas and from 33% to 53% and 60% for toasted whole and broken faba beans, respectively. Total tract protein digestibility, measured after 12 h rumen and subsequent intestinal incubation, remained unchanged for peas and faba beans, but decreased from 99% to 98% for toasted whole lupins and to 97% for toasted broken lupins. For toasted whole and broken faba beans, pressure toasting increased %UIS from 33% to 53% and 60%, respectively. After pressure toasting, washable fractions (W) of all legume seeds decreased for both constituents, the fractional rate of degradation (k_d) of protein decreased, while the k_d of starch increased. It was concluded that protein degradability decreased after pressure toasting, without seriously affecting its total tract protein digestibility.

Toasting a mixture of peas, faba beans and lupins resulted in higher starch degradabilities than expected, based on the separately treated feedstuffs. The k_d's of the mixtures were higher than expected: 5.49 vs. 4.29%/h for whole seeds and 5.01 vs. 4.18%/h for broken seeds, respectively. Consequently, %UIS was lower than expected (47% vs. 51% for whole seeds and 50% vs. 57% for broken seeds).

Key words   Peas, Lupins, Faba beans, Pressure toasting, Rumen degradability, Intestinal digestibility, Protein, Starch

Introduction

Because of their high protein content, peas (*Pisum sativum*), lupins (*Lupinus spp.*) and faba beans (*Vicia faba*) may be of use in ruminant diets to balance other dietary ingredients low in protein (Dixon and Hosking, 1992). Recently, there has been renewed interest in these seeds because they can be grown in the European community (Aguilera et al., 1992). This makes them possible substitutes for imported protein sources such as soybean meal, although the protein content of these seeds is generally lower than in soybean meal.

The protein fraction in these leguminous seeds consists of 85 to 100% albumins and globulins (Van Straalen and Tamminga, 1990), which are highly soluble and easily degradable (up to 75%) in the rumen. Starch is the major storage carbohydrate in peas and faba beans, while for lupins it is ß- (1,4) galactan (Hill, 1977). Literature on the rumen degradability of starch from legume seeds is rare. Recent data shows that starch from peas (Walhain et al., 1992) and faba beans is highly soluble and easily degradable (Tamminga et al., 1990; Nocek and Tamminga, 1991).

Although legume seeds provide rumen degradable protein and starch, supplies of rumen undegraded protein and starch are of special importance for high producing dairy cows (Chalupa, 1974; Satter 1986; Nocek and Tamminga, 1991; Klopfenstein, 1996). Therefore, several treatments have been proposed to increase rumen escape protein through denaturation and Maillard reactions (Satter, 1986). Extrusion has been used to decrease protein degradability of horse beans (Cros et al., 1991), lupins (Cros et al., 1992; Kibelolaud et al., 1993) and peas (Benchaar et al., 1994) while protein degradability of lupins was reduced after roasting (Murphy and McNiven, 1994; Singh et al., 1995). Aguilera et al. (1992) showed that autoclaving was an effective method to reduce rumen protein degradability of peas, lupins, faba beans, vetch and bitter vetch. Differences among legumes were observed in response to the treatment.

Most treatments, where heat is involved, will increase starch degradability. This can be due to gelatinization and to changes in the physical structure of the feedstuff, caused by the physical processes that are associated with the heat treatments. For example, Walhain et al. (1992) reported a decreased rumen protein degradability from 88 to 66%, after extruding peas at 140°C while starch degradability, calculated from their data, increased from 87% to 96%.

Structural changes of protein and starch after heat treatment affects rumen degradation

depending on the temperature reached, the processing time, and the moisture content during processing (Lund, 1984; Stern et al., 1985; Cleale IV et al., 1987). In addition, seed particle size and the presence of hulls will influence the transfer of heat and moisture during processing (Goelema et al., 1997). Excessive heat treatment generally reduces intestinal digestibility of dietary amino acids through the formation of indigestible Maillard products (Van Soest, 1994). Optimal conditions of treatments are generally defined as those which decrease rumen degradability without negatively altering postruminal digestion (Stern et al., 1985; Satter, 1986).

Additivity of nutritive characteristics is often assumed in calculating the nutritional value of diets or concentrates from the values of the individual ingredients. This may be correct after mixing individual (treated) feedstuffs only, but treatments which are applied to the mixture of feedstuffs, like for instance extrusion, expander treatment or pelleting, may involve interactions between ingredients (Vik-Mo and Lindberg, 1985; Murphy and Kennelly, 1987; Chapoutot et al., 1990; De Boever et al., 1995; Van Straalen et al., 1997). The digestive properties after processing a complete feed may therefore differ from the weighted average of its constituents.

This experiment was conducted with the following objectives:

1) to measure the effect of pressure toasting on the rumen protein and starch degradability and the intestinal protein digestibility of peas, lupins, and faba beans.

2) to determine if the effects of pressure toasting on degradability and digestibility of these legume seeds are different for broken versus whole seeds.

3) to compare the rumen degradation and intestinal digestion characteristics of the toasted mixture of peas, lupins and faba beans with the weighted average of the individual seeds.

Materials and methods

Samples and treatments

Lupins (*Lupinus angustifolius*), peas (*Pisum sativum*) and faba beans (*Vicia faba*) were obtained from a commercial supplier (ACM, Meppel, The Netherlands). Each batch of seeds was randomly divided into two equal parts; one of which was coarsely broken with a roller mill (Roskamp TP 900-36). The roller mill had 3 roller pairs and a capacity of 20 ton h^{-1}. The gap width between the rollers was adjusted for each feedstuff to achieve maximum dehulling and minimal particle size reduction. The gap widths of the roller pairs

1, 2 and 3 were 4.15, 3.20, and 3.90 mm for peas, 4.20, 4.20 and 3.90 mm for lupins and 4.25, 4.50 and 5.20 mm for faba beans, respectively.

Two mixtures (1:1:1, weight basis) were made, consisting of either whole or broken seeds (Table 1).

The whole and broken seeds as well as the mixtures were processed for 3 min at 132° C in a laboratory scale pressurised toaster, as described by Van der Poel et al. (1990). Toasting treatments were repeated on two consecutive days. After toasting, the samples were oven-dried at 35°C for 15 h. Untreated samples of the single feedstuffs were used as controls.

Table 1 Chemical composition of untreated peas, lupins, faba beans and their mixture.

	Peas	Lupins	Faba beans	Mixture[1] measured	calculated
Dry matter (g/kg)	939.5	950.3	948.0	948.6	945.9
In dry matter (g/kg DM)					
Organic matter	966.3	970.9	958.9	962.4	965.4
Crude protein	261.4	349.5	306.2	308.9	305.7
Crude fat	8.7	56.0	11.6	24.6	25.4
Crude fibre	63.8	152.9	89.0	-[2]	-
Starch	404.3	-	413.1	-	-
NDF	118.5	248.7	151.9	-	-
ADF	78.0	196.6	111.6	-	-
ADL	2.2	8.5	3.2	-	-

[1] The mixture consisted of peas, lupins and faba beans on a 1:1:1 weight basis.

[2] -: Not determined

Rumen incubations

Rumen incubations were carried out according to Dutch standard methods (CVB, 1996) in which four rumen-cannulated, lactating Holstein cross Friesian cows were used to

measure ruminal crude protein and starch degradation. The cows received about 17 kg
of dry matter (DM) daily of a ration consisting of grass silage (48% of DM intake) and a
commercial concentrate (90 g intestinal absorbable protein and 6.5 MJ NEL per kg).
Nylon with a pore size of 40 μm (PA 40/30, Nybolt, Switzerland) was used to prepare
bags with an inner size of 10 x 19 cm. Feed samples were ground through a 3 mm sieve
(Retsch ZM1 centrifugal mill). The bags were filled with about 5 g DM of the ground
sample and incubated in the rumen for 0 (blank), 2, 4, 8, 12, 24 and 48 h. Treatments
were randomly divided over cows.
After incubation, bags were immediately placed in cold water and rinsed with tap water
to stop fermentation. Then the bags were washed in a domestic washing machine for 50
min with 70 l of cold water, without centrifugating. After washing, the bags were dried in
an forced air oven at 60°C for 24 h, air equilibrated and weighed. Residues from the bags
were pooled within time and treatment and ground through a 0.5 mm sieve (Retsch ZM1
centrifugal mill).

Intestinal incubations
Two non-lactating Holstein cross Friesian cows, fitted with a cannula in the proximal
duodenum, were used to measure intestinal protein digestibility. The cows received a daily
ration of approximately 11 kg DM, consisting of maize silage (74% of DM intake) and
grass silage.
Nylon with a pore size of 40 μm (PA 40/30, Nybolt, Switzerland) was used to prepare
bags with an inner size of 3 x 7 cm. The bags were filled with approximately 0.5 g DM of
the 12 h rumen incubation residue. Prior to incubation, the rumen incubation residue was
prepared and handled as described above, but with freeze-drying instead of oven-drying.
Prior to incubation in the proximal duodenum the bags were incubated in a solution
containing 1 g (2000 FIP U/g) pepsin in 1 l of 0.1 M HCL at 37°C for 1 h. Three bags
were inserted into the proximal duodenum trough the cannula of each cow every 20 min.
After insertion of 12 bags, a 20 min break was taken after which the procedure continued.
Bags were retrieved from the faeces every 2 h and stored at -20°C until all the bags had
been recovered. After thawing, the bags were rinsed, washed and freeze dried as
described above. Residues were pooled within treatment and ground through a 0.5 mm
sieve (Retsch ZM1 centrifugal mill).

Chemical analysis

Feeds were analysed for DM, inorganic matter (ASH), crude protein (CP, 6.25 x N), crude fat (CFAT), crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and starch. Acid detergent insoluble nitrogen (ADIN) in the feed samples was determined by analysing the N content of ADF residues. Pooled rumen incubation residues were analysed for DM, ASH, CP and starch. Intestinal incubation samples were analysed for DM, ASH and CP.

DM was determined by drying to a constant weight at 103°C following ISO method 6496, ASH by combustion at 550°C according to ISO method 5984. N was determined with the Kjeldahl method with $CuSO_4$ as the catalyst, following ISO method 5983. CF was analysed according to NEN method 5417 (1988). Crude fat was analyzed according to ISO/DIS method 6492 (1996). ADF and ADL were analysed according to Van Soest (1973). NDF was analysed according to the VVR/protocol NSP analyses (Anonymous, 1992). 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 of a phosphate buffer (pH 7). This incubation is carried out for 15 min at 40 °C, after boiling and removal of the ND.

Starch was analysed 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 h at 130°C and enzymatic breakdown (1 h at 60°C, pH 5) to glucose, using an enzyme cocktail containing amyloglucosidase, *a*-amylase and pullulanase (A). Glucose was subsequently determined using hexokinase and G6P-dehydrogenase. The degree of gelatinization (SGD) of starch was determined with two additional analysis where starch was analysed as above, but without the ethanol extraction (B) to quantify the amount of starch and lower sugars. Finally, the sample was hydrolyzed with amyloglucosidase (60 U/g sample) for 75 min at 50°C (pH 4.8) to determine gelatinized starch and soluble sugars (C).

The SGD was calculated as a percentage of total starch after correcting for lower sugars according to equation 1.

$$\text{SGD} = 100 \times \{ C - (B - A)\} / A \qquad (1)$$

Protein dispersibility index (PDI) was determined according to a modified AACC 46-24

procedure, as described by Thomas et al. (1997). N-solubility index (NSI) was determined according to a modified AOCS procedure (1986), as described by Thomas et al. (1997).

Calculation of degradability and digestibility
Both CP and starch were classified into three fractions: a readily available fraction (W), measured as the fraction disappearing after washing (0 h incubation); an undegradable fraction (U), measured as the asymptote of the degradation curve at infinite incubation time; and a potentially degradable fraction (D = 1 - 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). Undegraded intake crude protein (%UIP) and undegraded intake starch (%UIS) were calculated for the Dutch standard outflow rate (k_p) of 6%/h, equations 2 and 3, according to Tamminga et al. (1994). For starch, it was assumed that 10% of W escapes rumen fermentation and U is 0 (Tamminga et al., 1994).

$$\%UIP \qquad = U + D * (k_p / (k_p + k_d)) \qquad\qquad (2)$$
$$\%UIS \qquad = 0.1 * W + D * (k_p / (k_p + k_d)) \qquad\qquad (3)$$

The CP residue remaining after intestinal incubations (IUP) was used to calculate protein digestibility as a fraction of intake crude protein (%TDP) and as a fraction of the undegraded intake protein (%DUP).

Statistical analysis
Analysis of variance was conducted using the General Linear Models (GLM) procedure of SAS (SAS, 1989) with the following model:

$$Y_{ijk} \qquad = \quad \mu + D_i + F_j + T_k + (F \times T)_{jk} + \epsilon_{ijk}, \qquad\qquad (4)$$

where, Y_{ijk} is the dependent variable under examination (residues, W, k_d, %UIP, %UIS, %TDP, %DUP), μ is the overall mean, D_i is the treatment day effect (i= 1, 2), F_j is the feed effect (j= 1-4), T_k is th treatment effect (k= 1-3), $(F \times T)_{jk}$ is the interaction of feed and treatment, and ϵ_{ijk} is the residual error term.
The incubation residues and degradability characteristics of the treated mixtures were compared with the calculated weighted average of the single treated feedstuffs to test for

additivity. When calculated means were outside the 95% confidence limits of the measured values, it was concluded that there was interaction. Treatment effects were compared by contrast statements, using the GLM procedure of SAS (SAS, 1989).

Results

Chemical composition
The chemical composition of the untreated seeds, as well as the measured and calculated OM, CP and CFAT contents of the mixture are in Table 1. The values agree with tabular values (CVB, 1994) and those reported in other studies (Dixon and Hosking, 1992). The agreement between the measured and calculated values indicated that mixing was correct. A significant decrease of CP content was found for peas and lupins comparing BT and T (Table 2). Total starch content in peas and faba beans decreased after toasting (Table 3).

Protein degradability and digestibility
Untreated peas, lupins and faba beans were highly degradable in the rumen. UIP was 25% for peas, 22% for lupins and 20% for faba beans (Table 2).
Pressure toasting significantly increased %UIP by decreasing W and k_d (Table 2). Total protein digestibility (%TDP) was high and only slightly reduced after pressure toasting, although this reduction was significant for lupins. Compared to toasted whole seeds, a decreased W was observed and this caused higher values of UIP for toasted broken lupins and faba beans, while for broken peas this was caused by a decreased k_d. Toasting broken seeds increased UIP without altering %TDP or %DUP.
Relative to peas and faba beans, lupins had the highest ADIN contents (Table 4). Toasting increased ADIN for lupins and faba beans (P≤0.05) to 1.4% of total N for toasted broken lupins and 0.64% for broken toasted faba beans. ADIN correlated positively with UIP (r=0.47, P=0.0264), but negatively with %TDP and %DUP (r=-0.74, P= 0.0001 and r=-0.47, P=0.0281, respectively).
PDI and NSI values (Table 4) were higher than W (Table 2), but all three parameters responded similarly to the toasting treatment. PDI and NSI correlated positively with W of protein (correlation coefficients (r) were 0.92 and 0.91, respectively; P=0.0001) and negatively with %UIP (r= -0.79 and -0.77; P=0.0001, respectively).

Table 2 Effects of breaking and/or pressure toasting on the rumen degradation characteristics and intestinal digestion of crude protein[1].

Treatment[1]	C	T	BT	sem	P-values of contrasts[2] TREATMENT	BREAKING
Peas						
CP[3] (g/kg DM)	268.0	268.8	256.7	3.15	NS	0.0274
W (%)	55.9	23.2	19.4	7.37	0.0001	NS
D (%)	44.1	76.9	80.6	7.37	0.0001	NS
k_d (%/h)	4.52	4.39	3.27	0.30	0.1096	0.0318
%UIP	25.2	44.4	52.3	5.19	0.0001	0.0128
UIP (g/kg DM)	74.9	132.6	149.2	14.71	0.0001	0.0798
%DUP	96.1	97.6	97.1	0.37	0.0525	NS
%TDP	98.9	98.8	98.3	0.17	NS	NS
Lupins						
CP (g/kg DM)	349.6	354.4	332.5	4.25	NS	0.0009
W (%)	44.3	27.6	22.5	4.34	0.0001	0.0894
D (%)	55.7	72.4	77.5	4.34	0.0001	0.0894
k_d (%/h)	9.34	3.20	3.10	1.33	0.0001	NS
%UIP	21.9	47.2	51.2	5.91	0.0001	NS
UIP (g/kg DM)	84.9	185.7	188.9	21.96	0.0001	NS
%DUP	94.0	96.2	95.5	0.43	0.0070	NS
%TDP	98.5	98.0	97.4	0.21	0.0158	NS
Faba beans						
CP (g/kg DM)	314.0	315.9	321.1	2.40	NS	NS
W (%)	67.1	25.7	18.6	9.59	0.0001	0.0259
D (%)	33.0	74.3	81.4	9.59	0.0001	0.0259
k_d (%/h)	3.90	3.26	2.63	0.24	0.0340	NS
%UIP	20.0	48.2	56.6	7.06	0.0001	0.0091
UIP (g/kg DM)	69.6	169.1	201.7	25.31	0.0001	0.0033
%DUP	96.0	97.9	98.2	0.45	0.0032	NS
%TDP	99.1	98.9	98.9	0.08	NS	NS

[1] Treatments C: untreated; T: toasted; BT: broken seeds and subsequently toasted.

[2] Contrasts TREATMENT: T and BT vs. C and BREAKING: BT vs. T; NS: P>0.10).

[3] CP: crude protein in g/kg DM, W: washable fraction, D: potentially degradable fraction, k_d: fractional rate of degradation of D, %UIP: undegraded intake protein, as percentage of CP, UIP: undegraded intake protein (g/kg DM), %DUP: intestinal digestibility of UIP, %TDP: total protein digestibility as percentage of CP.

Starch degradability

Undegraded intake starch (%UIS) of untreated peas and faba beans was 39 and 33% (Table 3), versus %UIP of 25 and 20%, respectively (Table 2). This indicated that starch was less degradable in the rumen than protein.

Pressure toasting decreased W, and increased the k_d of starch. As a result, %UIS increased by 29% for whole peas and 58% for whole faba beans (Table 3). Toasting broken faba beans increased %UIS compared to whole beans due to a further reduction of W.

The decreased *in situ* starch degradability observed after pressure toasting was not consistent with the increased SGD after toasting (Table 4). SGD of untreated peas and faba beans was 11 and 5%, respectively, but exceeded 70% and 50% after toasting broken peas and faba beans, respectively.

Table 3 Effects of breaking and/or pressure toasting on the rumen degradation characteristics of starch[1].

| Treatment[1] | C | T | BT | sem | P-values of contrasts[2] | |
					TREATMENT	BREAKING
Peas						
Starch (g/kg DM)	472.9	458.7	438.1	6.64	0.0111	0.0422
W[3] (%)	45.9	15.7	4.8	7.83	0.0001	0.0060
k_d (%/h)	3.47	4.44	4.87	0.29	0.0091	NS
%UIS	38.9	50.1	53.2	2.94	0.0011	NS
UIS (g/kg DM)	183.9	229.8	233.4	11.49	0.0009	NS
Faba beans						
Starch (g/kg DM)	404.3	397.2	366.8	8.34	0.0168	0.0078
W (%)	58.9	12.8	4.9	10.69	0.0001	0.0254
k_d (%/h)	2.96	4.15	3.55	0.24	0.0313	NS
%UIS	33.4	52.8	60.3	5.12	0.0001	0.0308
UIS (g/kg DM)	135.1	209.9	220.8	17.06	0.0001	NS

[1] Treatments C: untreated; T: toasted; BT: broken seeds and subsequently toasted.

[2] Contrasts TREATMENT: T and BT vs. C and BREAKING: BT vs. T; NS: P>0.10).

[3] W: washable fraction, k_d: fractional rate of degradation of the potentially degradable fraction, %UIS: undegraded intake starch as percentage of starch, UIS: undegraded intake starch in g/kg DM.

Table 4 Effects of breaking and/or pressure toasting on degree of starch gelatinization (SGD, as % of total starch), protein dispersibility index (PDI, as % of total protein), nitrogen solubility index in water (NSI_{H2O}, as % of total N)[1] and acid detergent insoluble nitrogen (ADIN, as % of total N).

| Treatment[1] | C | T | BT | sem | P-values of contrasts[2] | |
					TREATMENT	BREAKING
Peas						
SGD	11.3	28.6	71.5	11.68	0.0001	0.0001
PDI	91.3	24.3	19.5	14.67	0.0001	0.0245
NSI_{H2O}	83.1	20.7	18.8	13.36	0.0001	NS
ADIN	0.36	0.39	0.53	0.04	NS	0.0929
Lupins						
PDI	31.8	13.0	12.9	3.97	0.0001	NS
NSI_{H2O}	24.4	12.6	13.1	2.46	0.0001	NS
ADIN	0.99	1.25	1.42	0.08	0.0003	0.0466
Faba beans						
SGD	4.7	18.1	53.5	9.38	0.0002	0.0002
PDI	91.7	33.5	18.0	14.21	0.0001	0.0001
NSI_{H2O}	85.7	28.0	17.0	13.50	0.0001	0.0001
ADIN	0.45	0.59	0.64	0.04	0.0255	NS

[1] Treatments C: untreated; T: toasted; BT: broken seeds and subsequently toasted.
[2] Contrasts TREATMENT: T and BT vs. C and BREAKING: BT vs. T; NS: P>0.10).

Additivity
No differences were found between the measured and calculated CP *in situ* incubation residues, expressed as a percentage of the non-washable CP fraction, except for the 8 h residue of the toasted mixture. Hence, no differences were found between measured and calculated k_d of CP and %UIP. The calculated starch residues, expressed as a percentage of the non-washable starch fraction, were mostly higher than measured values, indicating a higher actual starch degradation. The differences increased with time, especially after 8 and 24 h incubation. Due to the large variation, the difference was only significant for the 2 h incubation of toasted broken seeds. As a resultant, measured k_d and %UIS were different from the calculated values. The k_d's were higher and, consequently,

%UIS was lower than calculated.

Table 5 Calculated and measured values of the in situ degradation characteristics and laboratory parameters after breaking and/or pressure toasting.

Treatment[1]	C	T	BT	sem	P-values[2] INTERACTION	BREAKING
Mixture						
CP[3] (g/kg DM)	-	313.1	312.9	1.41	NS	NS
calculated	(310.54)	(313.1)	(303.4)			
W (%)	-	24.7	20.1	1.58	NS	NS
calculated	(55.2)	(25.7)	(20.3)			
D (%)	-	75.4	79.9	1.58	NS	NS
calculated	(44.8)	(74.3)	(79.7)			
k_d (%/h)	-	3.99	3.13	0.30	NS	0.0852
calculated	(5.80)	(3.54)	(2.95)			
%UIP	-	47.4	52.5	2.59	NS	0.0765
calculated	(22.8)	(46.8)	(53.5)			
UIP (g/kg DM)	-	164.7	182.4	8.92	NS	0.0647
calculated	(78.6)	(162.6)	(180.1)			
%DUP	-	96.8	96.3	0.31	NS	NS
calculated	(95.4)	(97.2)	(97.0)			
%TDP	-	98.3	97.8	0.25	NS	NS
calculated	(98.8)	(98.5)	(98.2)			
Starch (g/kg DM)	-	280.5	277.0	1.86	NS	NS
calculated	(292.4)	(285.3)	(268.3)			
W (%)	-	11.8	9.8	1.22	NS	NS
calculated	(51.9)	(14.3)	(4.9)			
k_d (%/h)	-	5.49	5.01	0.21	≤ 0.05	NS
calculated	(3.59)	(4.29)	(4.18)			
%UIS	-	47.2	50.2	1.37	≤ 0.05	NS
calculated	(35.3)	(51.4)	(56.5)			
UIS (g/kg DM)	-	132.5	139.0	3.05	≤ 0.05	NS
calculated	(103.3)	(146.6)	(151.7)			
SGD	-	19.8	55.6	10.62	NS	0.0002
calculated	(8.2)	(23.7)	(63.3)			
PDI	-	24.6	18.4	1.86	NS	0.0070
calculated	(68.6)	(23.1)	(16.6)			
NSI_{H2O}	-	21.5	18.1	1.04	NS	0.0669
calculated	(61.4)	(20.1)	(16.1)			
ADIN	-	0.79	0.82	0.03	NS	NS
calculated	(0.63)	(0.78)	(0.89)			

[1] Treatments C: untreated; T: toasted; BT: broken seeds and subsequently toasted.

[2] INTERACTION: measured values vs. calculated values; Contrast: BREAKING: BT vs. T; NS: P>0.10).

[3] For abbreviations see Tables 2, 3 and 4.

Discussion

Chemical composition

Toasting decreased the starch contents of peas and faba beans, which could be attributed to an increase of the fraction of soluble sugars. This, however, accounted for only about 30% of the decrease. Formation of atypical glycosidic bonds (Siljeström et al., 1989) was proposed by Tovar and Melito (1996) as the reason for the decreased starch contents of black beans and lima beans after autoclaving for 15 min at 120°C, and might have occurred also after toasting. Finally, the effect of steam treatment on the protein matrix embedding the starch (Holm et al., 1985) may have rendered the starch inaccessible for hydrolyzing enzymes during analysis.

Protein degradability and digestibility

Several treatments have been used to decrease protein degradability of legume seeds. Decreased protein degradability after heat treatment has been attributed to formation of Maillard products from reducing sugars and amino acids and to cross-linking between and within proteins (Hurrel et al., 1976).

Literature on effects of pressurised steam treatments without additional shear forces on protein degradability of peas, lupins and faba beans is limited to results of Aguilera et al. (1992) for autoclaving. Although the treatment is similar to pressure toasting, temperature and processing time were 120°C and 30 min in the study of Aguilera et al. (1992) versus 132°C and 1.5 min in our study.

Protein degradabilities of raw peas and lupins, reported by Aguilera et al. (1992) were consistent with our results, but %UIP of faba beans was higher than in our study. Their reported sensitivity to autoclaving varied between seeds, which was also observed in our study. For peas, lupins and faba beans, Aguilera et al. (1992) found increases of %UIP of 128%, 124% and 79%, while in our study the increases were 83%, 116% and 141%, respectively. Pressure toasting for 3 min at 132°C increased %UIP of faba beans more than autoclaving for 30 min at 120°C but due to the higher degradability of untreated beans in our study, %UIP of toasted beans (48%) was similar for the autoclaved beans (50%). The discrepancy between our results and those of Aguilera et al. (1992) could be due to the different processing conditions (temperature, but in particular processing time), differences in sample preparation (screen size) and in the procedure of *in situ*

measurements (soluble fraction, diet, animals), apart from differences between batches and species of legume seeds.

Compared to toasting whole seeds, the toasting of broken seeds resulted in a higher %UIP, but this was only significant for peas and faba beans. During processing, transfer of heat and moisture to the seed kernel is delayed by the presence of the covering hull. Breaking seeds removes the hull, breaks the seeds into halves and thus facilitates the diffusion of heat to the kernel. This increases the effective processing time (the time in which the temperature of the whole seed equals the processing temperature) which was confirmed by the decreased PDI. Since the rate of the Maillard reaction decreases with lower moisture contents (Lea and Hannan, 1949; Cleale IV et al., 1987), diffusion of water may have played an additional role. Since ADIN is an indicator of Maillard polymerization (Van Soest, 1994), the increased ADIN after the toasting of broken seeds compared to whole seeds is consistent with a more intensive heat treatment of broken seeds.

ADIN values of untreated lupins in the present study were lower than reported by Kung et al. (1991) and Murphy and McNiven (1994), but are similar to values of 1.6%, reported by Singh et al. (1995). Grinding fineness affects ADIN (Murphy and McNiven, 1994; Hussein et al., 1995) and may explain the discrepancy between our results and those reported by Kung et al. (1991), but not those of Murphy and McNiven (1994) and Singh et al. (1995). In the latter two papers, however, the reported ADF values were approximately 50% lower than found in our study and by Kung et al. (1991), which may have influenced ADIN. Based on the low ADIN values after toasting, it can also be concluded that denaturation rather than Maillard polymerization is largely responsible for decreased protein degradability after toasting.

Starch degradability

To our knowledge, there are no studies reported of pressurised steam treatments on *in situ* starch degradability. In reported studies, application of heat is combined with shear forces, such as in expander treatment (Arieli et al., 1995), extrusion (Focant et al., 1990; Walhain et al., 1992; Arieli et al., 1995) or steam-flaking (Theurer, 1986). Therefore, the effects of heat and shear are confounded and effects on starch degradability cannot be solely attributed to either.

In our experiment W of starch decreased and k_d slightly increased, which resulted in a decreased *in situ* starch degradability after pressure toasting. The application of moisture,

heat and shear during processing may induce several processes in starchy feedstuffs, such as swelling and gelatinization (Theurer, 1986; Nocek and Tamminga, 1991). Swelling is not a likely cause for the decreased W, since heat-moisture treatments like pressure toasting decrease swelling power and solubility of starch (Hoover et al, 1993; Eliasson and Gudmundsson, 1996).

Sieve analysis of the material used for the rumen incubations, on the other hand, revealed that the ground toasted seeds contained a smaller fraction of particles <71 μm compared to the untoasted seeds (Goelema et al., unpublished results). This may explain the decrease of W.

An increase in particle size decreased the k_d (Michalet-Doreau and Ould-Bah, 1992). This contrasts with our findings, but may result from a shift of small particles from the W of untreated samples to the D fraction after treatment. On the other hand, gelatinization changes the starch structure from a semi-crystalline to an amorphous state and may have contributed to a higher degradation rate as well. This is in agreement with the positive correlation between SGD and the k_d of starch (r= 0.61, P=0.06).

The effects of toasting on starch degradability were very much affected by the change of W. Although it did not alter the conclusions in this study, it was assumed that 10% of W escapes fermentation. This factor was introduced to correct *in situ* result to *in vivo* values (Nock and Tamminga, 1991) and is similar to the fraction of soluble sugars in cereal grains (Sutton, 1971) and dried grasses (Weston and Hogan, 1968) escaping microbial fermentation. It seems resonable to assume that W falls apart in two fractions, a really soluble fraction and a fraction of particles small enough to be washed out. Hungate (1968) assumed a degradation rate for soluble sugars of 2.0/h. For an outflow rate of 0.15, this would mean that 7% of the soluble sugars escape fermentation. Starch, on the other hand, may additionally leave the rumen incorporated in microbial cells (Nocek and Tamminga, 1991). Given a k_d of 2.0/h and a k_p of 0.15/h for the soluble fraction, and assuming that W consists for 60% of particle loss (Chamberlain and Choung, 1995), the required k_d for complete degradation of the remaining 90% of the washable starch fraction can be calculated. Entrapment in the rumen mat may prevent small particles from being flushed out of the rumen with the fluid resulting in a particle outflow rate of 0.06/h. This would require a k_d of 0.40/h, only slightly higher than that of the most easily degradable starches (Tamminga et al., 1990).

Breaking seeds increases the effective processing time but, for starch, improved water

diffusion may even be more important (Lund, 1984), since gelatinization temperature increases dramatically as moisture content decreases below 35% (Colonna et al., 1992; Keetels et al., 1995). The thick and mechanically resistant nature of the cotyledon cell wall of pulses constitutes a physical barrier, preventing complete swelling of starch granules during processing (Tovar et al., 1992), which confirms the marked increase of SGD after breaking the seeds.

The W of CP and %UIP correlated positively with W of starch and %UIS (r=0.99 and 0.94 respectively, P=0.0001).

Other autors also stressed the effects of the protein matrix on the starch degradability after processing (Trei, 1970; Holm et al., 1985; Theurer, 1986). Only steam treatments at higher temperatures, or in combination with mechanical treatments like for instance flaking, enhance starch degradability (Trei et al., 1970).

Heat treated, gelatinized, starches may recrystallize upon cooling (Siljeström et al., 1989; Van Soest, 1994). This increased the amount of resistant starch (starch, indigestible in the small intestine) after autoclaving (Tovar and Melito, 1996). In the present study, all feedstuffs were oven-dried (at 35°C, for 16 h) after toasting, which may also have lead to the formation of resistant starch. Preliminary results from another study showed, that starch digestibility was not decreased after toasting (Goelema et al., unpublished).

When sufficient amylolytic capacity is provided in the duodenum, an increase of rumen undegraded intake starch after toasting improves the nutritive value (Van Soest, 1994) of the feedstuffs. However, if the increased %UIS is (partly) due to the formation of resistant starch, it may be broken down by less efficient fermentation in the lower tract. In that case, the benefit of the toasting treatment is only related to the increased protein value.

Additivity

CP degradability of the treated mixtures can be calculated from the values of the individual constituents. Literature (*e.g.* Murphy and Kennelly, 1987; De Boever et al., 1995) on associative effects for *in situ* studies is unambiguous in showing that mixing has no influence on the degradability of protein sources or concentrate mixtures. Our results are consistent with these data. Conversely, Vik-Mo and Lindberg (1985) and Chapoutot et al. (1990) observed a higher DM degradability for short time *in situ* incubation of compound feeds consisting of feedstuffs with different carbohydrate and protein degradability. The differences were more pronounced for less degradable feeds, which

may be indicative of an improved microclimate for fermentation in the bags after combining feedstuffs of variable degradability.

Our data may be consistent with these data in demonstrating that differences between expected and actually measured values of starch k_d and %UIS were significant and could not be ascribed to a single residue. It could be hypothesized that starch degradation was hampered due to a shortage of N in peas and faba beans, as in these seeds the nitrogen/starch ratio were lower than in the mixtures. However, based on calculated ratios of fermented nitrogen and nitrogen free organic matter this possibility was ruled out as it appeared that there was no nitrogen deficiency in the bags.

Conclusions

Pressure toasting for 3 min at 132°C decreased rumen protein degradability of peas, faba beans and lupins and rumen starch degradability of peas and faba beans. This effect was enhanced when broken seeds were processed, instead of whole seeds. The washable fraction of all legume seeds decreased for both constituents, the k_d of protein decreased, while the k_d of starch increased. It was concluded that protein degradability was decreased after pressure toasting with this procedure, without seriously affecting total tract protein digestibility. Changes in starch crystallinity and a less degradable protein matrix that encapsulates the starch granules could be responsible for the reduction in rumen starch degradability.

Toasting a mixture of peas, lupins and faba beans resulted in higher starch degradabilities than expected, based on the separately treated feedstuffs. This could not be related to a N-deficiency in the nylon bags or to a changed SGD after toasting.

Further research will concentrate on the relation between thermal processing time and temperature and the effects on rumen degradability and on the intestinal digestibility of rumen undegraded intake starch.

Acknowledgements

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CHAPTER 2

EFFECTS OF PRESSURE TOASTING, EXPANDER TREATMENT AND PELLETING ON IN VITRO AND IN SITU PARAMETERS OF PROTEIN AND STARCH IN A MIXTURE OF BROKEN PEAS, LUPINS AND FABA BEANS

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Effects of Pressure Toasting, Expander treatment and Pelleting on In Vitro and In situ Parameters of Protein and Starch in a Mixture of Broken Peas, Lupins and Faba Beans

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Abstract

The effects of several technological treatments on the rumen degradability and intestinal digestibility of a mixture of broken peas, lupins and faba beans were studied. The treatments included pressure toasting (132°C, 3 min), expander treatment (115°C, 8 s) and pelleting (80°C, 10 s). Toasting was the most effective treatment in altering rumen protein degradability, as it decreased rumen protein degradability, mainly by reducing its fractional degradation rate (k_d). Expander treatment and pelleting both increased the washable protein fraction (W), whereas pelleting also increased k_d resulting in a decreased amount of rumen undegraded intake protein (UIP). Toasting slightly decreased both total protein digestibility (TDP) and intestinal digestibility of rumen undegraded intake protein (DUP). Expander treatment had no significant effect on TDP or DUP, whereas pelleting generally increased TDP and DUP. The observed in situ effects of both expander treatment and pelleting could be explained by particle size reduction during processing. Toasting hardly affected rumen undegraded intake starch (UIS), which contrasts with previous studies in which ground samples were used for in situ incubations, compared to incubations with broken samples in the current study. Total starch digestibility (TDS) and intestinal digestibility of starch (DUS) were not affected by toasting or expander treatment. However, pelleting significantly increased TDS and, in some cases, also DUS. Combinations of toasting, expander treatment and pelleting sometimes resulted in interactions, but the order of application of the treatments hardly affected their effects on protein and starch degradability. Results of this study, as well as other published studies, show that the effects on starch and protein degradability are very much dependent on the conditions applied during processing. A concept was proposed, describing the effects of heat, moisture level, shear and pressure during steam treatment on in situ starch degradability.

Key words   Processing, Degradability, Starch, Protein, Denaturation, Gelatinization, Particle size.

Introduction

In ruminant nutrition, the rate and site of digestion are important factors determining the nutritive value of feedstuffs. Rate of rumen degradation influences the VFA/microbial biomass ratio and the VFA pattern, whereas site of digestion has an influence on the supply of amino acids and glucose. During feed manufacturing, different processes can

alter the physico-chemical properties of feedstuffs, thereby affecting their digestive behaviour. Thus, different ways of feed processing may result in variation in animal performance.

Heat treatments affect rumen protein degradability by denaturation and Maillard reactions (Satter, 1986) depending on the temperature reached, the processing time and the moisture content during processing (Stern et al., 1985; Cleale IV et al., 1987). The application of heat, moisture, pressure and shear also affects degradability of starch (Hale, 1973; McAllister et al., 1990; Nocek and Tamminga, 1991), but the underlying physico-chemical changes are still poorly understood (Kotarski et al., 1992). Starch gelatinization usually increases rumen starch degradability, especially in combination with mechanical damage, for instance after steam flaking, expander treatment or extrusion (Focant et al., 1990; Cros et al., 1991; 1992; Walhain et al., 1992).

Pressure toasting is a treatment in which pressurized steam is used to heat feedstuffs. It reduces rumen protein degradability of legume seeds (Goelema et al., 1998). This may be of interest for feedstuffs with a relatively small fraction of rumen undegraded intake protein (UIP), for instance lupins, peas and faba beans.

Expander treatment is a relatively new technique in the manufacturing of feed, and involves treatment with heat, pressure and shear. The treatment may improve the nutritive value for ruminants (Nielsen, 1994; Prestløkken, 1994), although effects on degradability of protein and starch are not always consistent (Arieli et al., 1995; Goelema et al., 1996). Although pelleted concentrates are fed to dairy cattle in some parts of the world, data on effects of pelleting is limited to the effects of pelleting forages (Blaxter and Graham, 1956; Beardsley, 1964; Moore, 1964) and the effects on physical quality of animal feed (Thomas et al., 1998). In the feed industry, it is often assumed that pelleting of concentrate mixtures decreases protein degradability due to the heat increment during conditioning and pelleting. However, Goelema et al. (1996) showed that pelleting increases in situ protein and starch degradability of two dairy concentrates, which may be due to the effects of pressure and shear. In the Netherlands, starch degradability of pelleted feedstuffs is corrected by 12.5% for the pelleting effect (CVB, 1998).

The objective of this experiment was to compare the effects of pressure toasting, expander treatment and pelleting on rumen degradability and intestinal digestibility of protein and starch in a legume seed mixture. An additional objective was to determine if the effects of pressure toasting, expander treatment and pelleting on degradability and

digestibility of this mixture were additive.

Materials and methods

Samples and treatments

Lupins (*Lupinus angustifolius*), peas (*Pisum sativum*) and faba beans (*Vicia faba*) were obtained from a commercial supplier (ACM, Meppel, The Netherlands). In order to increase the effectiveness of pressure toasting, and possibly those of the other treatments, each batch of seeds was coarsely broken with a roller mill (Roskamp TP 900-36) with three vertical roller pairs and a capacity of 20 ton h^{-1}. To achieve maximal dehulling and minimal particle size reduction, the minimal gap width was adjusted for each feedstuff and set at 3.20 mm for peas, at 3.90 mm for lupins and at 4.25 mm for faba beans, respectively. The seeds were from the same batches used in a previous experiment (Goelema et al., 1998).

Table 1 Chemical composition of untreated peas, lupins, faba beans and the mixture.

	Peas	Lupins	Faba beans	Mixture[1]
Dry matter (g/kg)	939.5	950.3	948.0	939.6
In dry matter (g/kg DM)				
Organic matter	966.3	970.9	958.9	963.6
Crude protein	261.4	349.5	306.2	295.8
Crude fat	8.7	56.0	11.6	27.5
Crude fibre	63.8	152.9	89.0	121.7
Starch	404.3	-[2]	413.1	295.6
NDF	118.5	248.7	151.9	174.3
ADF	78.0	196.6	111.6	153.0
ADL	2.2	8.5	3.2	5.2

[1] The mixture consisted of peas, lupins and faba beans (1:1:1, dry matter basis).
[2] -: Not determined

The broken seeds (Table 1) were mixed (1:1:1, dry matter basis) and the mixture was subjected to the following treatment or combinations of treatments. In the abbreviations for the treatments, the codes also denote the sequence of treatments, the first letter being the treatment carried out first:

untreated control (U), expander treatment (E), pressure toasting (T), pelleting (P), expander treated and subsequently pelleted (EP), expander treated and subsequently pressure toasted (ET), pressure toasted and subsequently expander treated (TE), pressure toasted and subsequently pelleted (TP), expander treated, pressure toasted and subsequently pelleted (ETP), pressure toasted, expander treated and subsequently pelleted (TEP).

Processing was carried out at the Wageningen Feed Processing Centre (WFPC). Pressure toasting was carried out for 3 min at 132°C in a laboratory scale pressurized toaster, as described by Van der Poel et al. (1990). After toasting, the samples were dried in a forced air oven for 16 h at 35°C. When toasting was followed by expander treatment and/or pelleting, the toasted material was handled as described above, but when expanded feed was pelleted, this was done without previous drying or cooling. An Almex expander (150 mm Φ, Almex BV Zutphen, The Netherlands), fitted with a 22 kW engine was used for the expander treatment. Pelleting was carried out with a 5 x 45 mm (bore x hole) die, using a V2-30 pelletizer (Robinson milling systems B.V., Boxtel, The Netherlands). A detailed description of the expander/pelleting line is presented by Thomas et al. (1997). Processing temperatures were determined using thermocouples (Tempcontrol, Voorburg, The Netherlands) in the pressure toaster and in the mixing bolts of the expander. The temperature of the first mixing bolt in the expander was regarded as the temperature after conditioning, whereas the temperature of the last mixing bolt just before the exit of the expander was taken as the exit temperature of the expander. Product temperatures after pelleting were determined using thermos flasks.

Specific mechanical energy (SME) of conditioning, expander treatment and pelleting were corrected for the power consumption of the engines when running idle and expressed as the energy dissipated in 1 kg feed mash during the treatment. The mash was conditioned at 90°C for 35 sec (126 kJ SME/kg) before expander treatment or pelleting. The residence time in the expander was 8 sec, at 114°C (59 kJ SME/kg). Residence time in the die was 10 s and pellet temperature was 80°C (24 kJ SME/kg).

After expander treatment or pelleting as final treatments, the expandate or pellets were

cooled in a two-deck counterflow bunkercooler (Robinson milling systems B.V., Boxtel, The Netherlands), and subsequently dried in a forced air oven for 16 h at 35°C. This dried material was also used for the treatment combinations where toasting was preceded by expander treatment or pelleting.

Rumen incubations

Rumen incubations were carried out according to Dutch standard methods (CVB, 1996) in which four rumen-cannulated, lactating Holstein cross Frisian cows were used to measure ruminal crude protein and starch degradation. The cows received about 19 kg of dry matter (DM) daily of a ration consisting of grass silage (65% of DM intake) and a commercial concentrate (90 g intestinal absorbable protein and 6.5 MJ Net Energy for Lactation (NEL) per kg.

Nylon bags (40 μm pore size, Nybolt, Switzerland) were prepared as described by Goelema et al. (1998). Bags were filled (25 mg sample/cm^2) with the test samples without an additional grinding step to preserve the particle size after the treatments. Incubations in the rumen were carried out for 0 (blank), 2, 4, 8, 12, 24 and 48 h, as described by Goelema et al., (1998). Incubation residues were pooled within time and treatment and ground through a 0.5 mm sieve (Retsch ZM1 centrifugal mill).

Intestinal incubations

Three lactating Holstein cross Frisian cows, fitted with a cannula in the proximal duodenum, were used to measure intestinal protein digestibility. The cows received a totally mixed ration consisting (on dry matter basis) of 27% grass silage, 27% maize silage, 11% hominy chop, 25% of a commercial concentrate (115 g intestinal absorbable protein and 6.5 MJ NEL per kg) and 9% of soybean meal. The ration was fed in three portions, at 6:00, at 12:00 and at 16:00. Average daily intakes were 24, 24 and 18 kg DM. Small nylon bags (40 μm pore size, Nybolt, Switzerland) were filled (25 mg/cm^2) with the 12 h rumen incubation residue. This rumen incubation residue was prepared and handled as described above, but with freeze-drying instead of oven-drying. Details about the procedure for intestinal incubations were described previously (Goelema et al., 1998).

Chemical analysis

The untreated mixture was analysed for DM, inorganic matter (ASH), crude protein (CP, 6.25 x N), crude fat (CFAT), crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and starch. Prior to analyses, the samples were ground through a 1 mm sieve. Feed samples, pooled rumen incubation and intestinal incubation residues were analysed for DM, ASH, CP and starch, after grinding through a 0.5 mm sieve (Retsch ZM1 centrifugal mill) to increase homogeneity of the samples. All chemical analyses were carried out following procedures as described by Goelema et al. (1998).

Sieve analysis

Particle size distribution was determined by wet sieve analysis. A 25-30 g sample was sieved (Fritsch analysette 3) using six sieves with mesh sizes of 2.5, 1.25, 0.63, 0.315, 0.160 and 0.071 mm, respectively. Above the 2.5 mm sieve a sprinkler set was placed, to add water to the set of sieves. A second sprinkler set was placed between the 0.160 and 0.071 mm sieve, to prevent blockage of the smallest sieve. To reach complete disintegration of the pelleted feeds, all samples were soaked in 500 ml demineralized water at room temperature for 45 min.

Sieving was carried out in duplicate using the following procedure of first, adding tap water which was allowed to flow through the set of sieves. After 5 min, the outflow was stopped by placing the outflow tube at the level of the upper sieve and by stopping the water supply. Sieving was continued for 10 min after stopping water outflow. The water was then removed by lowering the tube. After removal of the water, the same procedure was repeated twice. After the last water removal, sieving was carried out with free flowing water for 5 min. Sieve fractions were quantitatively transferred to glass filter crucibles (Scott Duran 2: f40 mm) of known dry weight, and dried for 2 x 2 h at 103°C, placed in a desiccator for 1 h and weighed to determine the DM fraction retained on the sieves. The fraction < 0.071 mm was calculated by subtracting the total weight of the sieve fractions from the dried sample weight and expressing it as a fraction of the dried sample weight. Modulus of fineness (MF) and modulus of uniformity (MU) were calculated after Pfost and Headly (1976). For the MU, the coarse, medium and fine fractions were calculated by pooling the material retained on sieve 1 and 2 (2500 and 1250 μm), sieve 3 and 4 (630 and 315 μm) and the last two sieves, and the pan (160 and 71 and < 71 μm).

Calculation of degradability and digestibility

CP and starch were classified into three fractions: a washable fraction (W), measured as the fraction disappearing after washing (0 h incubation); an undegradable fraction (U), measured as the asymptote of the degradation curve at infinite incubation time, and a potentially degradable fraction (D = 1 - W - U). The fractional degradation rate of the D fraction (k_d), was calculated using a first order degradation model, without a lag time, as described by Robinson et al. (1986). Undegraded intake crude protein (UIP) and undegraded intake starch (UIS) were calculated according to equations 2 and 3, using a passage rate (k_p) of 0.06/h (Tamminga et al., 1994). For starch, it was assumed that 10% of W escapes rumen fermentation, as discussed by Goelema et al. (1998).

$$\%UIP \qquad = U + D * (k_p / (k_p + k_d)) \qquad\qquad (2)$$
$$\%UIS \qquad = 0.1 * W + D * (k_p / (k_p + k_d)) \qquad\qquad (3)$$

The CP and starch residues after intestinal incubations were used to calculate the digestibility as fractions of feed crude protein (TDP) and starch (TDS) and as fractions of undegraded intake protein (DUP) and undegraded intake starch (DUS).

Statistical analysis

Analysis of variance was conducted using the General Linear Models (GLM) procedure of SAS (SAS, 1989) with the following model:

$$Y_{ij} \qquad\qquad = \mu + T_i + D_j + \epsilon_{ij}, \qquad\qquad (4)$$

where, Y_{ij} is the dependent variable under examination (W, k_d, %UIP, %UIS, %DUP, %DP), μ the overall mean, T_i the treatment effect (i= 1-10), D_j the treatment day effect (j= 1, 2), and ϵ_{ij} the residual error term.

Effects of treatments were compared by contrast statements, after correcting for treatment day effects, using the GLM procedure of SAS (SAS, 1989).

Results

Laboratory measurements
Compared to untreated samples, expander treatment and pelleting significantly increased CP content (Table 2). Protein dispersibility (PDI) and N solubility in water (NSI_{H2O}) showed similar effects of processing. PDI and NSI_{H2O} significantly decreased after expander treatment (14.4 and 14.2% units), pelleting (10.1 and 11.1% units), and especially after toasting (43.5 and 43.1% units). After combinations of treatments, toasting dominates the effect on PDI and NSI_{H2O}, irrespective of the combination.

A similar, but reverse trend was observed for SGD. Expander treatment and pelleting increased SGD with 9 and 7% units, respectively, while toasting resulted in a 47% units increase. Combinations of toasting, expander treatment or pelleting resulted in similar values of SGD after toasting only. For PDI, NSI_{H2O} and SGD, the order of toasting and expander treatment had no effect on the results.

Expander treatment and pelleting reduced the fraction of large (>2.5 mm) particles (Table 3). This mainly resulted in an increased fraction of very small particles (<0.071 mm). Toasting slightly changed particle size distribution compared to untreated samples, but decreased the fraction of particles < 0.071 mm when applied after expander treatment. The results of the combination of toasting and expander treatment depended on the order of the treatments, with toasting as the final treatment resulting in the coarser mash.

The MF and MU also illustrated these results. Processing decreased MF, while for MU, the proportion of coarse particles decreased. The order of the toasting and expander treatment significantly affected MF. Toasting after expander treatment resulted in a coarser mash, which was maintained after pelleting.

Table 2 Effects of processing on protein dispersibility index (PDI), nitrogen solubility index (NSI_{H2O}) and starch degree of gelatinization (SGD)[1].

Parameter	Protein parameters			Starch parameters	
	CP (g/kg DM)	PDI (%)	NSI_{H2O} (%)	Starch (g/kg DM)	SGD (%)
Treatments					
Untreated (U)	286.9	62.7	61.8	295.6	11.3
Expanded (E)	311.3	48.3	47.6	297.7	20.0
Toasted (T)	300.7	19.2	18.7	284.1	58.5
Pelleted (P)	319.4	52.6	50.7	303.2	18.5
EP[2]	320.9	51.0	48.7	316.4	22.0
ET	308.0	18.1	16.8	294.4	57.3
TE	309.8	18.8	17.4	298.3	58.4
TP	293.8	19.4	18.3	284.1	58.9
ETP	309.8	22.1	21.3	286.3	56.6
TEP	293.6	18.8	17.8	274.9	59.2
SEM	2.9	4.0	3.9	3.2	4.6
CONTRASTS[3] :					
Toasting					
T vs. C	NS	***	***	NS	***
ET vs. E	NS	***	***	NS	***
Expander treatment					
E vs. C	*	***	***	NS	*
TE vs. T	NS	NS	NS	NS	NS
Pelleting					
P vs. C	**	***	***	NS	+
EP vs. E	NS	NS	NS	NS	NS
TP vs. T	NS	NS	NS	NS	NS
ETP vs. ET	NS	+	*	NS	NS
TEP vs. TE	NS	NS	NS	+	NS

[1] CP: crude protein in g/kg DM; Protein dispersibility index (PDI), as % of total CP; nitrogen solubility index (NSI_{H2O}), as percentage of total N; Starch: starch in feed in g/kg DM, starch degree of gelatinization (SGD), as percentage of total starch.

[2] EP: expanded and pelleted; ET: expanded and toasted; TE: toasted and expanded; TP: toasted and pelleted; ETP: expanded, toasted and pelleted; TEP: toasted, expanded and pelleted.

[3] Significance level: NS, not significant; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; +, $P < 0.10$.

Table 3 Effects of processing on particle size distribution (fractions as % of total DM), modulus of fineness MF) and modulus of uniformity (MF).

Particle size (mm)	<0.071	0.071 - 0.160	0.160 - 0.315	0.315 - 0.630	0.630 - 1.250	1.250 - 2.5	>2.5	MF	MU[1] (C:M:F)
Treatments									
Untreated (U)	7.4	0.1	0.1	0.1	0.4	2.6	89.4	5.5	9 : 0 : 1
Expanded (E)	23.6	2.3	1.5	2.2	2.8	6.4	61.3	4.2	7 : 0 : 3
Toasted (T)	10.3	0.7	0.3	0.4	0.7	3.4	84.2	5.3	9 : 0 : 1
Pelleted (P)	30.3	3.2	1.6	1.4	2.1	4.8	56.6	3.8	6 : 0 : 4
EP[2]	32.1	4.5	2.9	2.1	2.5	4.7	51.3	3.6	6 : 0 : 4
ET	12.8	0.6	0.5	0.9	1.7	5.0	78.4	5.1	8 : 0 : 2
TE	18.0	7.1	2.4	1.9	2.4	8.7	59.5	4.3	7 : 0 : 3
TP	26.1	13.8	1.8	3.3	6.7	17.0	31.3	3.3	5 : 1 : 4
ETP	23.4	9.1	1.9	3.6	6.9	17.7	37.4	3.6	6 : 1 : 3
TEP	25.5	12.9	2.3	3.1	5.3	16.4	34.6	3.4	5 : 1 : 4
SEM	1.3	0.8	0.2	0.2	0.4	0.9	3.1	0.2	-
CONTRASTS[3] :									
Toasting									
T vs. C	+	NS	NS	NS	NS	NS	**	**	-
ET vs. E	***	**	**	**	*	NS	***	***	-
Expander treatment									
E vs. C	***	***	***	***	***	**	***	***	-
TE vs. T	***	***	***	***	**	***	***	***	-
Pelleting									
P vs. C	***	***	***	**	**	*	***	***	-
EP vs. E	***	***	***	NS	NS	+	***	***	-
TP vs. T	***	***	***	***	***	***	***	***	-
ETP vs. ET	***	***	***	***	***	***	***	***	-
TEP vs. TE	***	***	NS	*	***	***	***	***	-

[1] Coarse: medium: fine.

[2] EP: expanded and pelleted; ET: expanded and toasted; TE: toasted and expanded; TP: toasted and pelleted; ETP: expanded, toasted and pelleted; TEP: toasted, expanded and pelleted.

[3] For significance levels see Table 2.

In situ degradability and digestibility of protein

Compared to untreated samples, expander treatment and pelleting decreased UIP by increasing W (Table 4). Pelleting additionally increased the k_d, resulting in a 55% decrease of UIP. In contrast, toasting did not affect W, but decreased k_d and consequently, UIP increased.

The results of combinations of the expander treatment and toasting emphasized the greater impact of toasting on k_d: when applied after expander treatment, toasting decreased k_d significantly, but expander treatment did not affect k_d when preceded by toasting. When applied after expander treatment and toasting, the effect of pelleting on k_d diminished.

Toasting did not change W compared of untreated samples but, relative to expander treatment and pelleting, it decreased W. The decreases of k_d and W after toasting dominated the effects of processing on UIP. The lowest UIP was observed after pelleting, while toasting resulted in the highest UIP. Combinations of treatments which included toasting resulted in a much higher UIP than after expander treatment and pelleting only. Toasting significantly decreased DUP and TDP of untreated or expanded feed. Pelleting, on the other hand, increased in all cases TDP, and DUP when applied after toasting or expander treatment/toasting. The order of toasting and expander treatment had no effect on protein degradation and digestion characteristics.

In situ degradability and digestibility of starch

Expander treatment and pelleting significantly decreased UIS due a larger W and a higher k_d (Table 5). Compared to untreated samples, toasting tended to decrease W and to increase k_d, but UIS did not change. On the other hand, toasting decreased W and increased UIS of expanded feed. In combination with other treatments, UIS decreased after expander treatment and pelleting. For the expander treatment, this was due to an increase of W, while after pelleting, both W and k_d increased.

Expander treatment and toasting hardly affected DUS and TDS, but pelleting significantly increased TDS. The sequence of the treatments did not affect any of the starch degradation or digestion characteristics, except for UIS, which tended to be lower for toasting/expander treatment, compared to expander treatment/toasting ($P < 0.10$).

Table 4 Effects of toasting, expander treatment and pelleting on in situ rumen degradation characteristics and intestinal digestion of crude protein[1].

Parameter	W (%)	D (%)	k_d (%/h)	UIP (%)	DUP (%)	TDP (%)
Treatments						
Untreated (U)	-3.3	103.3	3.6	64.7	94.2	95.9
Expanded (E)	14.5	85.5	3.5	54.1	94.7	96.9
Toasted (T)	-0.4	100.4	1.7	78.0	90.1	91.4
Pelleted (P)	22.8	77.2	10.0	28.9	94.1	98.1
EP[2]	22.5	77.5	8.3	32.6	95.5	98.4
ET	4.6	95.4	1.7	74.8	90.6	92.2
TE	5.4	94.6	1.8	72.4	91.5	93.2
TP	15.6	84.4	2.8	57.9	93.4	95.7
ETP	16.3	83.8	2.4	59.8	95.0	96.7
TEP	12.9	87.1	2.7	60.4	93.5	95.7
SEM	2.0	2.0	0.6	3.6	0.5	0.6
CONTRASTS[3]:						
Toasting						
T vs. C	NS	NS	***	***	**	**
ET vs. E	**	**	***	***	**	***
Expander treatment						
E vs. C	***	***	NS	**	NS	NS
TE vs. T	+	+	NS	*	NS	+
Pelleting						
P vs. C	***	***	***	***	NS	*
EP vs. E	*	*	***	***	NS	NS
TP vs. T	***	***	**	***	*	**
ETP vs. ET	**	**	*	***	**	**
TEP vs. TE	*	*	*	***	NS	*

[1] W: washable fraction; D: potentially degradable fraction; k_d: rate of degradation of D (%/h); UIP: undegraded intake protein, as percentage of CP; DUP: intestinal digestibility as percentage of UIP; TDP: total digestibility of protein, as percentage of feed CP.

[2] EP: expanded and pelleted; ET: expanded and toasted; TE: toasted and expanded; TP: toasted and pelleted; ETP: expanded, toasted and pelleted; TEP: toasted, expanded and pelleted.

[3] For significance levels see Table 2.

Correlations of laboratory measurements and in situ measurements

There was a strong positive correlation (r=0.99), between PDI and NSI_{H2O}, while SGD negatively correlated with both PDI and NSI_{H2O} (r=-0.99). PDI, NSI_{H2O} and SGD did not correlate with MF (Table 6).

PDI and NSI_{H2O} also positively correlated with k_d of CP and, consequently, negatively with UIP. No correlations were found between PDI or NSI_{H2O} and W of CP. Although SGD negatively correlated with W of starch (r=-0.53, P=0.016), it was not associated with other degradability or digestibility characteristics.

MF positively correlated with UIP and UIS (r=0.57, P=0.0085 and r=0.84, P=0.0001; respectively), due to negative correlations with the W of CP and with the W and k_d of starch, respectively.

Rumen degradation characteristics of CP and starch are also highly correlated (P < 0.001). W and k_d of CP and starch positively correlate (r=0.79 and r=0.81, respectively), as well as UIP and UIS (r=0.87). Similar associations were found for digestion characteristics: both intestinal digestibility and total tract digestibility of protein and starch significantly correlated (r=0.52 and r=0.67, respectively).Correlations of laboratory measurements and in situ measurements

Table 5 Effects of toasting, expander treatment and pelleting on in situ rumen degradation characteristics and intestinal digestion of starch[1].

Parameter	W (%)	D (%)	k_d (%/h)	UIS (%)	DUS (%)	TDS (%)
Treatments						
Untreated (U)	6.3	93.7	1.2	78.4	74.1	79.7
Expanded (E)	18.1	81.9	2.4	60.2	75.4	85.2
Toasted (T)	-2.0	102.0	2.1	75.2	75.5	81.6
Pelleted (P)	19.7	80.3	7.4	37.9	73.5	89.9
EP[2]	24.1	75.9	7.2	36.8	83.7	94.0
ET	1.3	98.7	2.5	69.6	75.3	82.7
TE	7.6	92.4	2.5	65.7	78.0	85.6
TP	10.3	89.7	4.3	53.3	82.4	90.6
ETP	9.9	90.1	4.4	53.2	87.5	93.3
TEP	16.0	84.0	4.5	49.7	84.9	92.5
SEM	1.9	1.9	0.5	3.2	1.3	1.2
CONTRASTS[3] :						
Toasting						
T vs. C	+	+	+	NS	NS	NS
ET vs. E	**	**	NS	**	NS	NS
Expander treatment						
E vs. C	*	*	*	***	NS	+
TE vs. T	*	*	NS	**	NS	NS
Pelleting						
P vs. C	**	**	***	***	NS	**
EP vs. E	NS	NS	***	***	+	**
TP vs. T	**	**	***	***	NS	**
ETP vs. ET	*	*	**	***	*	**
TEP vs. TE	*	*	***	***	NS	*

[1] W: washable fraction; D: potentially degradable fraction; k_d: rate of degradation of D (%/h); UIS: undegraded intake starch, as percentage of starch in feed, DUS: intestinal digestibility, as percentage of UIS; TDS: total digestibility of starch, as percentage of starch in feed.

[2] EP: expanded and pelleted; ET: expanded and toasted; TE: toasted and expanded; TP: toasted and pelleted; ETP: expanded, toasted and pelleted; TEP: toasted, expanded and pelleted.

[3] For significance levels see Table 2.

Table 6 Pearson correlation coefficients and significance levels of laboratory parameters and in situ results.

	Laboratory parameters			In situ parameters for protein				
	NSI$_{H2O}$ (%)	SGD (%)	MF	W (%)	k$_d$ (%/h)	UIP (%)	DUP (%)	TDP (%)
Laboratory parameters:								
PDI	0.99 ***	-0.99 ***	NS	NS	0.69 ***	-0.62 **	0.55 *	0.62 **
NSI$_{H2O}$		-0.99 ***	NS	NS	0.67 **	-0.61 **	0.55 *	0.62 **
MF				-0.83 ***	NS	0.57 **	-0.48 *	-0.58 **
In situ parameters for starch:								
W		-0.53 *	-0.67 **	0.79 ***	0.70 ***	-0.85 ***		
k$_d$		NS	-0.71 ***	0.87 ***	0.81 ***	-0.87 ***		
UIS		NS	0.84 ***	-0.95 ***	-0.73 ***	0.87 ***		
DUS		NS	-0.63 **				0.52 *	0.38 +
TDS		NS	-0.88 ***				0.64 **	0.67 **

[1] For abbreviations see Table 2-5.

[2] For significance levels see Table 2.

Discussion

Laboratory measurements

Degradability and particle size measurements were completed using unground samples, and thus are influenced by the coarseness of the incubated material. In contrast, PDI,

NSI_{H2O} and SGD were determined in finely ground (1 mm screen) samples. Results for the latter parameters therefore reflect the physico-chemical changes due to toasting, expander treatment and pelleting. Although screen size (1 mm vs. 0.5 mm) significantly affected PDI and NSI of peas, lupins and faba beans (Goelema, unpublished) and PDI (2 mm vs. 1 mm) of heat roasted soybeans (Hsu and Satter, 1995), there was no correlation between MF and PDI, NSI_{H2O} and SGD. Thus, it was concluded that the observed particle size reduction after processing had no effect on these parameters.

PDI and NSI_{H2O} were highly correlated, with PDI always showing slightly higher values than NSI_{H2O}. This is the result of a more rapid stirring during dispersing, and is consistent with the results of Horan (1974) for soy protein products. Both parameters are indicators of protein denaturation (Horan, 1974).

The marked decrease of PDI and NSI_{H2O} after toasting, compared to expander treatment and pelleting, is consistent with the shorter processing times and the decreasing input of thermal energy of these treatments. Conversely, PDI and NSI_{H2O} may be less suitable indicators for denaturation below a PDI or NSI level of 20%, which is in line with results of Hsu and Satter (1995) and Marsman et al. (1995). A decrease of PDI and NSI_{H2O} has been observed previously after toasting (Marsman et al., 1993; Goelema et al., 1998) and autoclaving (Araba and Dale, 1990; Parsons et al., 1991). In the latter three studies NSI was determined with KOH. Protein dispersibility, as measured in water suggests values which are lower than N solubility in KOH (Thomas et al., 1997), but changes in the same manner after processing. Thomas et al. (1997) concluded that PDI is a better discriminator than NSI_{KOH} between technological treatments under the conditions of a low to moderate level of SME (110 kJ/kg), as was the case in the present experiment. However, NSI_{KOH} is more suitable for conditions where very high specific mechanical energy is used, such as extrusion (Marsman et al., 1995). The present study shows that differences between PDI and NSI are more related to the medium used, rather than to the differences between the methods of dispersion and solubilization.

SGD increased after processing and showed the same, but opposite changes as PDI and NSI_{H2O}. Toasting was the treatment with the longest processing time and the highest temperature, and significantly increased SGD, while expander treatment and pelleting only slightly increased the SGD of untreated samples, but not after toasting.

Under conditions of low moisture content (<30%), gelatinization temperature of starches rapidly increases (Lund, 1984; Eliasson and Gudmundsson, 1996; Keetels, 1995). The

longer processing time during toasting facilitated water diffusion and, in combination with a processing temperature of 132°C, resulted in 58% gelatinization (Table 2). This is consistent with a previous study (Goelema et al., 1998) which showed that, especially for broken seeds, toasting increased SGD of peas and faba beans to a large extent. Upon cooling and drying after toasting, gelatinized starch may have retrograded (Eliasson and Gudmundsson, 1996). Subsequent expander treatment or pelleting may have induced renewed gelatinization of the retrograded starch. Since SGD was determined 4 months after production, it is not known whether retrogradation took place or not. Longterm storage (> 1 year) at 4°C, however had no effect on SGD of toasted peas and faba beans (Goelema, unpublished) compared to analyses 1 month after treatment.

Since the energy input of expander treatment and pelleting was insufficient for a significant increase of SGD of native starch (C) it is questionable whether expander treatment and pelleting could increase SGD after toasting. This was consistent with results of Thomas et al. (1998), who showed that after expander treatment of tapioca, its SGD did not exceed 60%. This is consistent with a study by Van Zuilichem and Van der Poel (1986), who reported that between 350 and 700 kJ SME per kg was needed for complete gelatinization of starch in single screw extruders.

In situ degradability and digestibility of protein and starch

In situ rumen degradability of protein and starch is affected by physico-chemical changes such as solubility, denaturation and gelatinization, as well as by the physical characteristics such as particle size. Standard procedures for in situ measurements (Michalet-Doreau and Ould-Bah, 1992; Huntington and Givens, 1995; CVB, 1996) include grinding, to mimic the effect of mastication as well as to obtain a representative sample. Concentrates are usually ground before the manufacturing of animal feeds and therefore, quantifying the effect of grinding fineness on in situ measurements is an important issue. Shear forces during processing may alter particle size distribution and subsequently degradability. Grinding was therefore not applied before incubation to be able to quantify this effect. Consequently, the degradability of the untreated mixture was lower than what is usually observed for these feedstuffs. In a previous study, Goelema et al. (1998) used 3 mm ground samples of the same batches of feedstuffs for rumen incubations. In their study, the washable fraction and degradation rate of protein and starch were higher (55.2 and 51.9%, and 5.80 and 3.59%/h, respectively) than in the present study (-3.3 and 6.3%,

and 3.6 and 1.2%/h, respectively. Consequently, UIP and UIS of the ground mixture (3 mm screen) in the previous study (Goelema et al., 1998) were much smaller (23 and 35%, respectively), than in the present study (65 and 78%, respectively).

The high correlation between the MF and the W of CP in the present study confirms that the increase of W (Table 4) was related to the decreased particle size after expander treatment and pelleting. For nearly all combinations of treatments, the reduction in MF was consistent with the increase of k_d. This agrees with previous studies where decreased particle size after grinding increased protein degradability (Michalet-Doreau and Cerneau, 1991; Michalet-Doreau and Ould-Bah, 1992). On the other hand, Nocek (1985) reported effects on protein solubility, but not on k_d. In contrast, Ehle et al. (1982) did not find a consistent influence of particle size on the k_d of CP. For starch, changes in MF after processing correlated with W, k_d and UIS, which is in line with effects of grinding on starch degradation (Cerneau and Michalet-Doreau, 1991). The relatively low CP and starch degradability of the untreated mixture in the present study also explains the small effect of toasting on degradability, as compared to previous results (Goelema et al., 1998).

The low k_d of CP after toasting suggests that this treatment has much more impact on k_d than subsequent expander treatment or pelleting. It should also be noted that, compared to untreated samples, expander treatment and pelleting decreased PDI and NSI_{H2O}, without increasing UIP. Other authors reported a decrease of the soluble fraction and the k_d of CP after expander treatment and extrusion (Cros et al., 1991, 1992; Walhain et al., 1992; Nielsen, 1994; Prestløkken, 1994; Arieli et al., 1995). These authors generally used temperatures ranging between 120°C and 135°C during expander treatment and extrusion, which is higher than those used in the present experiment. Walhain et al. (1992) extruded peas at 140, 180 and 220 °C, and Arieli et al. (1995) expanded feedstuffs at 115°C. However, these authors used ground samples for incubation. Since incubation in the unground form decreased the effect of toasting on UIP from 30% units (Goelema et al., 1998) to 10% units in the present experiment, this may also have affected the difference in UIP after expander treatment and pelleting. For ground concentrate mixtures, Goelema et al. (1996) and Tamminga and Goelema (1995) reported that pelleting decreased UIP and UIS by about 12% and 19%, respectively, while expander treatment decreased UIP and UIS by 14% and 36%, respectively.

The strong correlation of PDI and both k_d and UIP was also observed by Hsu and Satter (1995) after roasting soybeans. For soybeans, these authors found an optimum protection

of protein which corresponded to a PDI between 9 to 11%, which is lower than the lowest values for PDI observed in our study.

In agreement with other studies (Cros et al., 1991; 1992; Kibelolaud et al., 1993), toasting only slightly decreased DUP and TDP in our prior study (Goelema et al., 1998). In the present study, toasting decreased DUP and TDP by about 4% units, which is closely related to the coarseness of the incubated samples. Despite these small decreases, the higher UIP results in an increased supply of intestinal digestible undegraded protein. Expander treatment and pelleting decreased UIP when applied after toasting, but the combined treatments which involved toasting always resulted in a considerable larger UIP and amount of DUP compared to expander treatment and/or pelleting.

It is often assumed that gelatinization of starch after heat treatment of starches is the reason for an increased ruminal starch degradability (Walhain et al., 1992). However, the present experiment shows that toasting increases SGD considerably, without affecting UIS. Moreover, the lowest values of UIS (38% after pelleting and 37% after expander treatment/pelleting) corresponded with the lowest values of SGD of the processed feeds (19 and 22%). Thus, it is questionable whether this measure only represents gelatinization.

The correlation between the degradability of starch and protein (Theurer, 1986; Goelema et al., 1998) was maintained after processing, which agrees with results of Arieli et al. (1995). This suggests that the seed protein matrix influences the degradability of starch. Thus, the conditions during the treatments resulted in identical changes with respect to protein and starch degradability. Conditions such as higher shear rates and higher temperatures may result in opposite changes, as for instance was the case after extrusion of peas (Walhain et al., 1992) and steam flaking of corn and sorghum (Theurer, 1986). Denaturation of the protein matrix may have also reduced degradability of starch, by limiting access for starch hydrolyzing enzymes, as proposed by Holm et al. (1985) and McAllister et al. (1994). Retrograded starch, formed upon cooling the gelatinized starch in the toasted product (Keetels, 1995; Eliasson and Gudmundsson, 1996), is extremely difficult to solubilize (Greenwood, 1970) which resulted in a decreased W after combinations of treatments involving toasting. Moreover, complexes between protein and starch may be formed upon heat-moisture treatments (Thorne and Jenkins, 1983; Franco et al., 1995) and may have led to the reduced degradability after toasting, although others have questioned this explanation (McAllister et al., 1990). Retrogradation sometimes

results in the formation of small amounts of intestinal indigestible starch (Englyst et al., 1992). Although DUS was not decreased after processing, increased hindgut fermentation may have compensated for the reduced small intestinal digestibility of UIS, as processing generally leads to an increased total tract digestibility of starch (Owens et al., 1986).

Conclusions

Based on these results, the following hypothesis may explain the effects on starch degradability of steam treatment. When starchy feedstuffs are heated by steam, its condensation on the feedstuffs increases the moisture content of the feedstuff. If the temperature of the seeds during processing exceeds the gelatinization temperature at the actual moisture content then gelatinization starts. Depending on the moisture content, temperature, and processing time, this gelatinization will vary from a local and partial one to a complete gelatinization which increases starch degradability in the rumen. Upon cooling and drying, recrystallization can take place. Since gelatinization and retrogradation offset each other, different processing conditions lead to different outcomes. Shear forces during or after heat processing, for instance during flaking, expander treatment, extrusion, or pelleting, further disrupt the granular structure of starch and interact with the two afore mentioned processes. Since the chemical composition of feedstuffs and the structure and gelatinization properties of starches in different feedstuffs greatly vary, notably when shear is involved, differences among feedstuffs due to processing are to be expected. In addition, processing can alter the protein matrix (Thorne and Jenkins, 1983) and/or particle size distribution (Cerneau and Michalet-Doreau, 1991), which also influence starch degradability.

Overall, the present results show that toasting is an effective way to decrease in situ rumen protein degradability and in combinations with other treatments, rumen starch degradability. Expander treatment and pelleting increased rumen degradability of protein and starch. Processing hardly affected total tract digestibility of starch and protein, except for toasting, which decreased protein digestibility. Due to the large increase of UIP and UIS, toasting resulted in an increased amount of intestinal digestible protein and starch, which was only slightly decreased by subsequent expander treatment or pelleting. In dairy cow nutrition, the increased degradability of protein and starch after pelleting should be taken into account during diet formulation. Since expander treatment can result in an increased, as well as in a decreased degradability of protein and starch, the conditions

used during processing are of essential importance for a proper judgement of the
treatment with respect to degradability.

In this study, the effects of expander treatment and pelleting on in situ degradability were
closely related to the effects on particle size, whereas the effect of toasting directly related
to chemical changes. Comparison with results from the literature emphasizes the need for
a further evaluation of the specific effect of processing conditions on rumen degradability
of protein and starch. This should lead to a better understanding of the underlying
structural changes of protein and starch, and their interaction, which are responsible for
the observed changes in the digestive behaviour of protein and starch after processing.

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CHAPTER 3

Effect of various conditions during pressure toasting on rumen degradability and intestinal digestibility of faba beans, peas, lupins and soybeans

1. IN SITU DEGRADABILITY AND DIGESTIBILITY OF PROTEIN

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Effect of Various Conditions during Pressure Toasting on Rumen Degradability and Intestinal Digestibility of Faba Beans, Peas, Lupins and Soybeans

1. In situ degradability and digestibility of protein

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Abstract

Pressure toasting was used to increase the intestinal absorbable protein (DVE) content of faba beans, peas, lupins and soybeans. Whole seeds were toasted at 100, 118 or 136°C for 3, 7, 15 or 30 min. Ground samples were incubated in the rumen and intestines of dairy cows to measure *in situ* rumen undegraded intake protein (%UIP) and intestinal digestible rumen undegraded protein, respectively.

Toasting significantly increased %UIP by decreasing both the washable fraction (W) and the fractional rate of degradation (k_d) of crude protein (CP). Total tract protein digestibility of faba beans, peas and soybeans was not affected by the treatment, but for lupins it was slightly decreased.

Faba beans and peas show great resemblance in chemical composition, and were also similar with respect to the rumen degradability and the sensitivity to the different processing conditions. Likewise, lupins and soybeans showed a similar behaviour after pressure toasting.

For faba beans and peas, W consistently decreased when temperature or time of processing were increased. For lupins and soybeans, W decreased after toasting at 100°C, but slightly increased after toasting at higher temperatures. The k_d was strongly decreased after toasting at 136°C. Generally, higher processing temperature resulted in a higher %UIP and protein value (DVE). The largest increases of %UIP and DVE were found after pressure toasting for 15 min at 136°C. The %UIP increased by 152%, 156%, 142% and 56%, while for DVE, the increases were 91%, 80%, 76% and 71% for faba beans, peas, lupins and soybeans, respectively.

Taking into account the loss of small particles from the nylon bags increased the levels of UIP, as well as the effect of pressure toasting on UIP and DVE. Based on the results of this study, it was concluded that pressure toasting is an economical method to increase the protein value of faba beans, peas, lupins and soybeans.

Key words: Legume seeds, Pressure toasting, Processing conditions, Rumen undegraded intake protein

Introduction

In the Netherlands, protein in ruminant diets consists mainly of imported feedstuffs and byproducts from the food industry, such as soybeans and soybean meal, expeller and extracted forms of palm kernels, coconut, sunflower seeds and cottonseed. Possible

substitutes for these protein sources which can be grown in the European Community are legume seeds such as lupins, peas and faba beans. Most legume seeds contain significant amounts of protein, and some contain starch as well.

Heat treatments have been used successfully to increase the amount of UIP in legume seeds as measured *in situ* (Cros et al., 1991, 1992; Aguilera et al., 1992; Robinson and McNiven, 1993; Benchaar et al., 1994; Schroeder et al., 1995) and *in vivo* (Pena et al., 1986; Benchaar et al., 1991; Moshtaghi Nia and Ingalls, 1995), although an increased supply of rumen undegraded protein did not always show a positive production response (Focant et al., 1990; Singh et al., 1995).

Heat treatment converts protein from an ordered to a disordered (denatured) state. During the endothermic process of denaturation, intramolecular bonds are disrupted and polypeptide chains unfolded (Wright, 1982). Mild heat treatments mainly affect hydrogen bonds resulting in a loss of the tertiary and secondary structure. But when treatments become more severe, new intermolecular disulfide bridges and isopeptides are formed, this can be associated with some loss of amino acids (Hurrel et al., 1976). Moreover, heat treatments may result in Maillard reactions between reducing sugars and free amino groups of amino acids, peptides and proteins, which reduces rumen degradability (Cleale et al., 1987) of protein, and sometimes even its digestibility and subsequent absorption in the small intestine (Van Soest, 1994).

The effect of heat treatment depends on the temperature and the duration of the treatment. Little systematic research has been done on the combination of these two factors. An experiment was conducted to study the effects of temperature and residence time during toasting of faba beans, peas, lupins, and soybeans on the rumen degradability and the intestinal digestibility. In the present paper, the effects of processing on *in situ* protein degradability and digestibility are described.

Materials and methods

Samples and treatments

Faba beans (*Vicia faba* cv. Alfred), peas (*Pisum sativum*), lupins (*Lupinus angustifolius*) and soybeans (*Glycine max.*) were obtained from a commercial supplier. The whole seeds were pressure toasted (Van der Poel et al., 1990) at 100°C for 7, 15 and 30 min; at 118°C for 3, 7, 15 or 30 min, and at 136°C for 3, 7 or 15 min. Treatments were repeated

on two consecutive days. After toasting, the samples were oven-dried at 35°C for 16 h. Untreated samples of the feedstuffs were used as controls.

In situ incubations
Rumen incubations were carried out according to Dutch standard methods (CVB, 1996) with four rumen-cannulated, lactating Holstein cross Friesian cows. The cows received daily about 16 kg of dry matter (DM) of a ration consisting of hay (55% of total DM intake; 853 g DM/kg, 5.4 MJ NEL and 62 g intestinal absorbable protein (DVE)/kg DM) and a commercial pelleted concentrate (6.5 MJ NEL and 120 g DVE/kg). Average daily milk productions were 13.6, 18.8, 21.2 and 27.6 kg. Nylon cloth with a pore size of 40 μm and an effective surface of 30% (PA 40/30, Nybolt, Switzerland) was used to prepare bags with an internal surface of approximately 250 cm^2. Feed samples were ground through a 3 mm sieve (Retsch centrifugal mill). The bags were filled with about 5 g DM of the ground sample, weighed, and incubated in the rumen for 0 (blank), 2, 4, 8, 12, 24, 48 and 336 h. Treatments were randomly divided over cows.
After incubation, bags were immediately placed in ice water and rinsed with tap water to stop fermentation. Then the bags were washed in a domestic washing machine for 50 min with 70 l of cold water, without centrifugating. After washing, the bags were dried in a forced air oven at 60°C for 24 h, air equilibrated and weighed. Residues from the bags were pooled within time and treatment and ground through a 1 mm sieve (Retsch centrifugal mill).

Two lactating Holstein cross Friesian cows, fitted with a cannula in the proximal duodenum, were used to measure intestinal protein digestibility. The cows received approximately 23 kg DM of a totally mixed ration. The ration consisted of artificially dried grass (50%), maize silage (20%), hominy feed (7%), a commercial pelleted concentrate (9%) and soybean meal (2%) and contained 520 g/kg DM, 6.7 MJ NEL and 104 g DVE per kg DM. Average daily milk productions were 32 and 22 kg.
Nylon cloth with a pore size of 40 μm and an effective surface of 30% (PA 40/30, Nybolt, Switzerland) was used to prepare bags with an internal surface of approximately 25 cm^2. The bags were filled with approximately 0.5 g DM of the 12 h rumen incubation residue. Prior to incubation, the rumen incubation residue was prepared and handled as described above, but with freeze-drying instead of oven-drying.

Prior to incubation in the proximal duodenum the bags were incubated in a solution containing 1 g (2000 FIP U/g) pepsin in 1 l 0.1 M HCL at 37°C for 1 h. Three bags were inserted into the cannula of each cow every 20 min. After insertion of 12 bags, a 20 min break was taken after which the procedure continued. Bags were retrieved from the faeces every 2 h and stored at -20°C until all the bags had been recovered. After thawing, the bags were rinsed, washed and freeze dried as described above. Residues were pooled within treatment and ground through a 0.5 mm sieve (Retsch centrifugal mill).

Physico-chemical analyses
The seeds were analyzed for DM, inorganic matter (ASH), crude protein (CP, 6.25 x N), crude fat, crude fibre (CF), neutral detergent fibre, acid detergent fibre, acid detergent lignin and starch (Table 1). Pooled rumen and intestinal incubation residues were analyzed for DM, ASH, CP and starch.
DM was determined by drying to a constant weight at 103°C following ISO method 6496 (1983), ASH by combustion at 550°C according to ISO method 5984 (1978). N was determined with the Kjeldahl method with $CuSO_4$ as the catalyst, following ISO method 5983 (1979). CF was analyzed according to NEN method 5417 (1988). Crude fat was analyzed according to ISO/DIS method 6492 (1996) Acid detergent fibre and lignin were analyzed according to Van Soest (1973). Neutral detergent fibre was analyzed according to a modified method of Van Soest et al. (1991), as described by Goelema et al. (1998[a]). Total starch and was determined as described by Goelema et al. (1998[a]).

The water soluble fraction of the 3 mm ground samples used for *in situ* incubations was measured by filtration over filter papers (A.G. Frisenette & Sønner Aps, Ebelftoft, Denmark, nr. 607-90 mm), as described by Weisbjerg et al. (1990).

Table 1 Chemical composition of untreated faba beans, peas, lupins and soybeans.

	Faba beans	Peas	Lupins	Soybeans
Dry matter (g/kg)	887.3	883.6	909.0	925.4
In dry matter (g/kg DM)				
Organic matter	959.8	966.5	969.3	945.1
Crude protein	312.1	249.8	324.4	393.6
Crude fat	11.4	11.8	66.4	198.3
Crude fibre	82.1	53.6	143.8	67.4
Starch	417.2	491.2	-[1]	-
Neutral detergent fibre	119.9	97.8	230.4	126.1
Acid detergent fibre	116.7	71.9	196.0	90.1
Acid detergent lignin	13.9	7.6	10.4	10.5

[1] -: Not determined

Calculation of degradability and digestibility

CP (N x 6.25) was classified into three fractions: the washable fraction (W), a truly undegradable fraction (U), measured as the asymptote of the degradation curve at infinite incubation time; and a potentially degradable fraction (D) (D = 1 - 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). Rumen undegraded intake protein (%UIP) was calculated for an outflow rate (k_p) of 6%/h (equations 1 and 2), according to Tamminga et al. (1994).

$$\%UIP = U + D * (k_p / (k_p + k_d)) \tag{1}$$

$$UIP \text{ (g/kg DM)} = CP * 1.11 * \%UIP / 100 \tag{2}$$

The crude protein residue remaining after intestinal incubations (IP, in g/kg DM) was used to calculate total protein digestibility (%TDP) as a fraction of intake crude protein (CP, in g/kg DM) and as a fraction of the undegraded intake protein (%DUP), using equations 3 to 5.

%TDP	$= 100 * (CP - IP) / CP$	(3)
%DUP	$= 100 * (UIP - IP) / UIP$	(4)
DUP (g/kg DM)	$= UIP * \%DUP / 100$	(5)

The protein value, *i.e.* the amount of intestinal absorbable protein (DVE, g/kg DM) and the rumen degradable protein balance (OEB, g/kg DM) were calculated according to standard procedure (Tamminga et al. 1994). For the untoasted seeds, tabular values (CVB, 1998) were used for the faecal digestibility of organic matter (%DOM). For the toasted seeds, tabular values of DOM of untoasted seeds were corrected for the difference in total digestibility of organic matter (%TDOM) (equation 6). The %TDOM was calculated similarly as explained above for %TDP (equation 3).

$$\%DOM_{toasted} = \%DOM_{CVB,\ untoasted} - (\%TDOM_{untoasted} - \%TDOM_{toasted}) \qquad (6)$$

For example, when $\%DOM_{CVB,\ untoasted} = 80$, $\%TDOM_{untoasted} = 90$ and $\%TDOM_{toasted} = 85$, then $\%DOM_{toasted} = 80 - (90 - 85) = 75$.

Statistical analysis

Analysis of variance was conducted using the General Linear Models (GLM) procedure of SAS (SAS, 1989) with equation 7. Treatment means were compared pairwise using Tukey's HSD-test ($P \leq 0.05$).

$$Y_{ij} = \mu + Treatment_i + D_j + \epsilon_{ij}, \qquad (7)$$

where, Y_{ij} is the dependent variable under examination (W, D, k_d, %UIP, %TDP, %DUP, DUP, DVE, OEB), μ the overall mean, $treatment_i$ the treatment effect (i= 1 - 11), D_j the treatment day effect (j= 1, 2).

Linear correlations were analyses with proc CORR (SAS, 1989). Regression analysis was carried out with proc GLM, using equation 8 (SAS, 1989), after omitting the untreated controls from the dataset. For regression, processing temperature was linearly transformed into increments from 100°C (*i.e.* 0, 18 or 36). If possible, models were reduced in complexity by excluding non-significant quadratic effects ($P > 0.10$). When the remaining interaction model only contained non-significant effects ($P > 0.10$), it was tested

whether excluding the interaction term resulted in significant effects of toasting time and temperature. When this was the case, the remaining additive model was presented.

$$Y_{ij} = \alpha + D_i + \beta_1 X_1 + \beta_2 X_2 + \beta_3 (X_1 X_2) + \beta_4 X_1^2 + \beta_5 X_2^2 + \epsilon_{ij}, \tag{8}$$

where, Y_{ij} is the dependent variable under examination (W, D, k_d, %UIP, %TDP, %DUP, DUP, DVE, OEB), α the intercept, D_i the treatment day effect (i= 1, 2), X_1 the temperature effect (in °C), X_2 the time effect (in min), β_1 the linear effect of temperature, β_2 the linear effect of time, β_3 the interaction of time and treatment
β_4 the quadratic effect of temperature, β_5 the quadratic effect of time and ϵ_{ij} the residual error term.

Table 2 List of abbreviations used in the paper.

Abbreviation	parameter
CP	crude protein content (N x 6.25)
DOM	apparent (faecal) organic matter digestibility
D	potentially degradable fraction (%)
DM	dry matter content (g/kg)
DUP	intestinal digestible UIP, as % of UIP or in g/kg DM
DVE	intestinal absorbable true protein, in g/kg DM
FS	filer soluble fraction (in %)
IP	indigestible protein (g/kg DM)
k_d	fractional degradation rate (%/h)
k_p	fractional outflow rate (%/h)
OEB	rumen degradable protein balance (g/kg DM)
SEM	standard error of the mean
TDOM	total digestibility of organic matter (in %)
TDP	total digestibility of crude protein (in %)
U	truly rumen undegradable fraction (%)
UIP	rumen undegraded intake protein, as % of CP
$X_{50.N}$	apparent mean N particle size (μm)
W	washable fraction (%)

Results

Chemical composition

Table 1 lists the abbreviations used in the paper. Table 2 shows the chemical composition of the untreated legume seeds. Faba beans and peas both contain considerable amounts of starch compared to the other seeds. Lupins had the highest crude fibre, neutral detergent fibre and acid detergent fibre content. Soybeans were richest in CP and crude fat.

Protein degradability and digestibility

In tables 3, 4, 5 and 6 the rumen protein degradation characteristics are presented for faba bean, peas, lupins and soybeans, respectively. In all legume seeds toasting increased %UIP by decreasing the W and the k_d, but there were differences in sensitivity to the processing conditions. Effects of day of processing were never significant (P > 0.10).

W of CP was higher for untreated faba beans and peas, than for lupins and for soybeans. The W of faba beans (Table 3) and peas (Table 4) responded similarly to an incremental temperature and time during processing. For faba beans, the treatment time effect on W was linear (P < 0.10), whereas for peas, the smaller response after prolonged toasting resulted in quadratic effect for peas.

The sensitivity of W to pressure toasting for lupins (Table 5) corresponded to that for soybeans (Table 6). For lupins, processing conditions exceeding a temperature of 118°C and a duration of 7 min only slightly affected W. A minimum was found after 30 min toasting at 118°C. For soybeans, a minimum W of 32% was found after toasting for 7 min at 118°C. A further increase of processing temperature or time hardly affected W.

Untreated faba beans, peas and soybeans had comparable k_d's (4.03, 4.84 and 5.15 %/h, respectively), but the k_d of lupins was much higher (9.32%/h). After treatment, differences in k_d of CP between seeds diminished.

An increase of the toasting time slowed down the rate of degradation. Especially at 136°C, prolonged toasting resulted in a drastic decrease of the k_d. Again, faba beans and peas showed similarities with respect to the effects of the processing conditions. Lupins and soybeans also reacted similarly.

Soybeans had a higher %UIP than the other seeds (28% vs. 21%). Toasting at 136°C for

15 min resulted in the highest %UIP for all legume seeds (54, 51, 51 and 43%, respectively). The effect of the different processing conditions were numerically larger for faba beans and for peas than for lupins and soybeans. The quadratic effect of both time (for all legume seeds) and temperature (only for faba beans and soybeans) suggests that %UIP will approach a maximum close to the tested conditions.

Table 3 Effects of pressure toasting on CP degradation and digestion characteristics of faba beans[1].

Treatments[2]:	W (%)	D (%)	k_d (%/h)	UIP (%)	TDP (%)	DUP (%)	DUP g/kg DM
untreated	64.2^a	35.8^f	4.03^a	21.4^h	96.4	85.0^e	63.2^i
100/7	61.4^{ab}	38.6^{ef}	3.45^{ab}	24.5^{gh}	96.7	87.8^{cde}	73.4^{hi}
100/15	58.2^b	41.8^e	3.66^a	26.0^{fgh}	96.4	87.5^{de}	78.2^{gh}
100/30	54.4^c	45.6^d	3.66^{ab}	28.4^{efg}	96.7	89.6^{bcd}	87.8^{fg}
118/3	51.8^{cd}	48.2^{cd}	3.39^{ab}	30.8^{ef}	96.2	89.0^{bcd}	93.6^{ef}
118/7	48.6^d	51.4^c	3.36^{ab}	33.0^{de}	96.5	90.4^{abcd}	102.7^e
118/15	41.2^e	58.8^b	3.26^{ab}	38.1^{cd}	96.3	91.3^{abc}	118.7^d
118/30	38.1^e	61.9^b	3.34^{ab}	39.8^c	96.2	91.4^{abc}	124.6^{cd}
136/3	34.2^f	65.8^a	3.11^{ab}	43.3^{bc}	96.4	92.4^{ab}	135.5^c
136/7	31.0^f	68.7^a	2.53^{bc}	48.6^{ab}	96.5	93.4^a	154.7^b
136/15	31.3^f	69.0^a	1.65^c	53.9^a	96.4	94.0^a	175.9^a
SEM	2.54	2.54	0.14	2.18	0.06	0.58	7.42
Treatment day[3]	NS	NS	NS	NS	NS	NS	NS
Temperature	***	***	***	*	NS	***	*
Time	***	***	NS	**	NS	**	**
Time x temp.	NS	NS	-	+	NS	NS	*
Temp. x temp.	-	-	-	**	-	-	**
Time x time	-	-	-	*	-	-	**

[1] For abbreviations see Table 1. Within columns, different superscripts indicate significant differences (P < 0.05, Tukey's HSD-test).

[2] Codes denote the processing temperature (in °C) and the processing time (in min) e.g. the 100/7 treated seeds have been toasted for 7 min at 100°C.

[3] NS = P > 0.1; + = P < 0.1; * = P < 0.05; ** = P < 0.01; *** = P < 0.001, - = excluded from the model.

Table 4 Effects of pressure toasting on CP degradation and digestion characteristics of peas[1].

	W (%)	D (%)	k_d (%/h)	UIP (%)	TDP (%)	DUP (%)	DUP g/kg DM
Treatments[2]:							
untreated	62.1[a]	37.9[g]	4.84[ab]	21.0[g]	98.3	92.5[b]	53.8[g]
100/7	55.2[b]	44.8[f]	5.07[a]	24.3[fg]	98.6	94.8[ab]	63.6[fg]
100/15	-	-	-	-	-	-	-
100/30	49.2[c]	50.8[e]	4.58[abc]	28.8[efg]	98.6	95.6[a]	76.4[efg]
118/3	47.6[c]	52.4[e]	3.87[abc]	31.9[def]	98.3	95.2[ab]	83.7[def]
118/7	40.8[d]	59.2[d]	3.83[abc]	36.3[cde]	98.3	95.9[a]	95.7[cde]
118/15	35.3[e]	64.7[c]	3.82[abc]	39.7[cd]	98.4	96.3[a]	107.7[cd]
118/30	33.0[e]	67.0[c]	3.36[abc]	43.1[bc]	98.5	96.9[a]	115.7[bc]
136/3	30.9[ef]	69.1[bc]	3.34[abc]	44.4[bc]	98.3	96.5[a]	120.0[bc]
136/7	27.2[fg]	72.8[ab]	2.82[bc]	49.6[b]	98.4	97.1[a]	135.2[ab]
136/15	24.6[g]	75.4[a]	2.44[c]	53.7[a]	98.6	97.6[a]	146.8[a]
SEM	2.67	2.67	0.20	2.31	0.04	0.32	6.56
Treatment day[3]	NS	NS	NS	NS	NS	NS	NS
Temperature	***	***	**	***	NS	**	***
Time	**	**	NS	**	NS	NS	**
Time x temp.	NS	NS	NS	NS	NS	NS	NS
Temp x temp.	-	-	-	-	-	-	-
Time x time	*	*	-	*	-	-	*

[1]   For abbreviations see Table 1. Within columns, different superscripts indicate significant differences (P < 0.05, Tukey's HSD-test).

[2]   For treatment codes see Table 3.

[3]   For probability levels see Table 3.

Table 5 Effects of pressure toasting on CP degradation and digestion characteristics of lupins[1].

	W (%)	D (%)	k_d (%/h)	UIP (%)	TDP (%)	DUP (%)	DUP g/kg DM
Treatments[2]:							
untreated	47.6[a]	51.4[b]	9.32[a]	21.2[f]	98.6[a]	93.8[b]	72.2[f]
100/7	39.7[ab]	60.3[ab]	5.64[b]	31.2[e]	98.4[a]	95.3[ab]	108.8[e]
100/15	37.0[ab]	63.0[ab]	4.03[bc]	37.7[de]	98.2[ab]	95.6[a]	131.3[de]
100/30	38.1[ab]	61.9[ab]	3.50[bc]	39.1[cde]	98.1[ab]	95.6[a]	136.0[cd]
118/3	34.0[ab]	64.5[ab]	4.49[bc]	38.4[de]	98.2[ab]	95.8[a]	134.5[cde]
118/7	32.0[b]	68.0[a]	4.02[bc]	40.7[bcd]	98.0[abc]	95.5[ab]	140.0[bcd]
118/15	30.0[b]	70.1[a]	3.08[bc]	46.3[abcd]	97.8[abc]	95.7[a]	161.4[abc]
118/30	28.4[b]	70.9[a]	3.02[bc]	47.9[ab]	97.3[bc]	94.9[ab]	165.3[ab]
136/3	28.7[b]	69.9[a]	2.85[c]	48.8[a]	97.9[abc]	96.2[a]	171.4[a]
136/7	31.9[ab]	66.7[ab]	2.73[c]	47.3[abc]	97.7[abc]	96.1[a]	166.8[ab]
136/15	31.4[b]	67.1[ab]	2.07[c]	51.4[a]	97.1[c]	95.0[ab]	179.9[a]
SEM	1.36	1.34	0.43	1.88	0.10	0.15	6.68
Treatment day[3]	NS	NS	NS	NS	NS	NS	NS
Temperature	*	*	***	***	NS	*	***
Time	NS	NS	***	**	NS	NS	**
Time x temp.	NS	NS	**	NS	**	**	NS
Temp. x temp.	+	+	-	-	-	-	-
Time x time	-	-	**	-	-	-	+

[1] For abbreviations see Table 1. Within columns, different superscripts indicate significant differences (P < 0.05, Tukey's HSD-test).

[2] For treatment codes see Table 3.

[3] For probability levels see Table 3.

Table 6 Effects of pressure toasting on CP degradation and digestion characteristics of soybeans[1].

	W (%)	D (%)	k_d (%/h)	UIP (%)	TDP (%)	DUP (%)	DUP g/kg DM
Treatments[2]:							
untreated	48.5^a	51.5^d	5.15^a	27.7^c	95.2^{bcd}	84.4^e	102.7^d
100/7	41.9^b	58.1^c	4.20^{ab}	34.2^b	92.2^f	79.4^f	120.2^{cd}
100/15	38.3^{bc}	61.7^{bc}	3.87^{bc}	37.5^{ab}	93.9^e	85.3^{de}	142.3^{bc}
100/30	35.1^{cd}	64.9^{ab}	3.41^{bcd}	41.4^a	94.7^{de}	88.5^{cd}	162.9^{ab}
118/3	34.7^{cd}	65.3^{ab}	3.62^{bcd}	40.8^a	95.3^{bcd}	89.6^{bc}	162.3^{ab}
118/7	31.7^d	68.3^a	3.50^{bcd}	43.2^a	95.1^{cd}	89.8^{bc}	171.1^a
118/15	34.6^{cd}	65.5^{ab}	3.25^{bcd}	42.5^a	96.5^a	92.6^{ab}	176.2^a
118/30	34.2^{cd}	65.5^{ab}	3.47^{bcd}	41.9^a	96.2^{abc}	91.8^{abc}	171.3^a
136/3	35.4^{cd}	64.2^{ab}	3.52^{bcd}	40.8^a	96.4^{ab}	91.9^{abc}	165.6^{ab}
136/7	35.7^{cd}	64.0^{ab}	3.02^{cd}	42.9^a	97.0^a	93.7^a	179.2^a
136/15	37.8^{bc}	61.8^{bc}	2.66^d	43.3^a	97.2^a	94.1^a	182.7^a
SEM	0.99	0.98	0.14	1.03	0.31	0.95	5.43
Treatment day[3]	+	NS	NS	NS	NS	NS	NS
Temperature	***	***	**	***	***	***	***
Time	***	***	*	**	***	***	***
Time x temp.	***	***	NS	**	***	***	**
Temp x temp	***	***	-	**	***	***	***
Time x time	-	-	*	+	***	***	**

[1] For abbreviations see Table 1. Within columns, different superscripts indicate significant differences (P < 0.05, Tukey's HSD-test).

[2] For treatment codes see Table 3.

[3] For probability levels see Table 3.

Total protein digestibility (%TDP) of faba beans and peas was not significantly affected by toasting. Only the %TDP of lupins slightly decreased with increasing processing temperature. For soybeans, there was no consistent pattern for %TDP after toasting with temperature or time during toasting.

By increasing the UIP without seriously affecting %TDP, toasting increased DUP. Goelema et al. (1998[b]) showed, that rumen degradability of faba beans and pea starch also decreased after toasting. As a results, the amount of rumen fermentable organic matter for microbial protein synthesis substantially decreased after toasting. Despite this reduction, the protein value of legume seeds, as denoted by the DVE (Table 7) increased. The maximal increments in DVE were found after toasting for 15 min at 136°C. The increases were 71% for soybeans, 76% for lupins, 80% for peas and 91% for faba beans. Concomitantly with the increase of DVE, the rumen degradable protein balance (OEB) decreased, which implies a shift from rumen degradation to intestinal digestion of protein. For faba beans, peas and soybeans, DVE was quadratically affected by temperature and time, while both parameters showed interaction as well, except for peas. For lupins, temperature related linearly to DVE, while for processing time the relation tended to be quadratic.

Solubility

Table 8 shows the results of the N filter solubility (FS) and the %UIP values based on FS and W. FS decreased after toasting for 15 min at 136°C, which is consistent with the change of W. FS was always lower than W (on average 31% for untreated and 40% for toasted seeds), which indicates that small, insoluble N containing particles have left the nylon bags during washing. Assuming that only FS (instead of W) was immediately available for fermentation resulted in a higher %UIP, especially for soybeans.

The particle loss also affected the effect of toasting on %UIP. Based on FS, the effect of toasting on %UIP remained the same for peas, but for faba beans, lupins and soybeans the effect on %UIP increased with 5.5, 6.8 and 12.1 % units, respectively.

Table 7 Effects of pressure toasting on the protein value of faba beans, peas, lupins and soybeans[1].

	Faba beans		Peas		Lupins		Soybeans	
	DVE	OEB	DVE	OEB	DVE	OEB	DVE	OEB
Treatments[2]:								
untreated	108.9[h]	147.3[a]	107.2[h]	95.0[a]	132.6[f]	141.7[a]	136.3[d]	201.9[a]
100/7	122.7[g]	130.4[ab]	119.4[gh]	83.2[ab]	168.9[e]	105.7[b]	167.3[c]	152.8[b]
100/15	124.2[fg]	130.7[ab]	-	-	189.8[de]	84.1[bc]	190.1[bc]	139.0[bc]
100/30	134.6[ef]	123.3[b]	129.9[fgh]	75.5[bc]	193.3[cd]	79.8[bcd]	209.2[ab]	124.1[c]
118/3	136.7[e]	116.7[bc]	134.0[efg]	70.3[bcd]	194.0[cd]	81.1[bcd]	211.3[ab]	123.9[c]
118/7	144.9[e]	113.0[bcd]	144.5[def]	61.1[cde]	198.4[bcd]	71.6[cde]	217.8[a]	115.1[c]
118/15	156.3[d]	100.5[cde]	155.0[cde]	57.2[de]	216.2[abc]	57.7[cde]	225.3[a]	118.9[c]
118/30	162.0[cd]	96.1[de]	161.1[bcd]	49.0[ef]	218.1[ab]	53.7[de]	219.2[a]	121.5[c]
136/3	173.2[c]	82.3[ef]	168.0[bc]	44.2[ef]	226.3[a]	49.8[e]	215.6[a]	121.6[c]
136/7	189.9[b]	70.8[fg]	180.4[ab]	34.1[fg]	220.7[ab]	57.5[cde]	228.8[a]	115.5[c]
136/15	208.2[a]	59.7[g]	193.1[a]	22.8[g]	232.8[a]	44.4[e]	232.4[a]	115.6[c]
SEM	6.34	5.73	5.84	4.76	6.13	6.05	6.19	5.47
Treatment day[3]	NS	NS	NS	NS	NS	NS	NS	NS
Temperature	NS	NS	+	NS	***	**	***	***
Time	*	NS	*	NS	*	*	***	**
Time x temp.	*	*	NS	**	NS	NS	**	**
Temp. x temp.	***	**	*	*	-	+	***	**
Time x time	*	-	+	-	+	+	**	+

[1] Intestinal absorbable protein (DVE, in g/kg DM) and rumen degradable protein balance (OEB, in g/kg DM). Within columns, different superscripts indicate significant differences ($P < 0.05$, Tukey's HSD-test).

[2] For treatment codes see Table 3.

[3] For probability levels see Table 3.

Table 8 Effects of pressure toasting on rumen degradation characteristics, filter solubility and corrected fraction of rumen undegraded intake protein of faba beans, peas, lupins and soybeans[1].

	Faba beans			Peas			Lupins			Soybeans		
	U (%)	T (%)	SEM	U (%)	T (%)	SEM	U (%)	T (%)	SEM	U (%)	T (%)	SEM
W (%)	64.2[a]	31.3[b]	9.51	62.1[a]	24.6[b]	10.38	47.6[a]	31.4[b]	4.73	48.5[a]	37.8[b]	3.16
FS (%)	51.1[a]	14.3[b]	10.76	49.4[a]	15.0[b]	9.93	24.0[a]	9.6[b]	4.29	33.2[a]	8.1[b]	7.23
k_d (%/h)	4.03[a]	1.65[b]	0.69	4.84[a]	2.44[b]	0.70	9.32[a]	2.07[b]	2.15	5.15[a]	2.66[b]	0.73
%UIP	21.4[a]	53.9[b]	9.38	21.0[a]	53.7[b]	9.44	21.2[a]	51.4[b]	8.72	27.7[a]	43.3[b]	4.54
%UIP$_c$	29.2[a]	67.2[b]	10.99	28.0[a]	60.5[b]	9.41	30.6[a]	67.6[b]	10.75	36.0[a]	63.7[b]	8.06

[1] %UIP$_c$ = undegraded intake protein calculated using FS instead of standard procedures. Within type of sieve analysis, different superscripts indicate significant ($P \leq 0.05$) differences between untreated (U) and toasted samples (T, pressure toasted for 15 min 136°C).

Discussion

Chemical composition and in situ degradability

The chemical composition of the untreated seeds (Table 1) was in agreement with tabular values (CVB, 1998) and those reported in other studies (Dixon and Hosking, 1992; Goelema et al., 1998[a]). Toasting affected neither protein nor starch content (results not shown) of the seeds.

The fraction UIP of untreated faba beans, peas, lupins and soybeans was low, and ranged from 21% (peas) to 29.4% (soybeans). The 100°C/15 min treatment for peas was not incubated, which is the reason for the missing results for this treatment. The total tract protein digestibility of these legume seeds was high, ranging from 95% for soybeans to 99% for lupins. These results were consistent with other studies (Sampath, 1987; Tamminga et al., 1990; Aguilera et al., 1992; Antoniewicz et al., 1992; Dixon and Hosking, 1992; Kandylis and Nikokyris, 1997; Goelema et al., 1998[a]). Some studies reported lower values for %UIP, such as 11% for faba beans (Cros et al., 1991; Yu et al., 1998), 5% and 12% for peas (Cros et al., 1991; Walhain et al., 1992) and 7% and 15% for lupins (Kibelolaud et al., 1993; Murphy et al., 1994). In nearly all cases, the differences could be attributed to a smaller grinding size (0.8 mm vs. 3.0 mm in our study) and a larger pore size (43-46 μm vs. 40 μm), or both, resulting in higher values for W and k_d than in our study. Higher literature values for %UIP of lupins, faba beans and peas were reported in studies where rumen incubations were carried out with a larger particle size (Dixon and Hosking, 1992; Singh et al., 1995).

The high rumen protein degradability of peas and faba beans is related to the composition of the protein in legume seeds (Van Straalen and Tamminga, 1990). Based on solubility, storage proteins can be classified into albumins, globulins, prolamins and glutelins which are soluble in water, salt solutions, aqueous ethanol solutions and in alkaline solutions, respectively.

In faba beans, peas, lupins and soybeans, albumins and globulins account for more than 85% of total protein (Kinsella, 1979; Casey et al., 1982; Carrouée and Gatel, 1995). In *Lupinus angustifolius*, the glutelin fraction accounts for 12-17% of the protein (Hill, 1977; Varasundharosoth and Barnes, 1985), which is higher than in other seeds. Several studies, however, showed that there is a large genetic, an environment-dependent and a seasonal variability in protein fractions (Casey et al., 1982; Sosulski et al., 1985), so

these figures are only indicative.

In our study, the W and FS of protein (Table 8) was very high, especially for peas and faba beans. This was in agreement with other results (Tamminga et al., 1990). Both W and FS (Table 8) of untreated legume seeds largely exceeded the contribution of the water-soluble albumin fraction in these seeds. The water-soluble fraction contains not only soluble protein, but also soluble non-protein N, such as free amino acids, citrulline, τ-amino butyric acid (Wiewiorowsky, 1958). Moreover, physical damage during grinding may have solubilized and washed out part of the protein which was initially enclosed by the starch or cell-wall fraction. Additionally, some globulins are soluble in water at pH of 7, as demonstrated for lupin conglutin-a by Bladgrove and Gillespie (1975).

The different sedimentation behaviour during ultracentrifugation (Millerd et al., 1975; Quillien et al., 1995) is also frequently used to discriminate between the different protein fractions. The sedimentation coefficients are expressed in Svedberg units (S). Most legume seed proteins consist of 7S and 11S globulins. The 7S globulin fractions of peas and soybean meal are more easily degraded than the 11S fraction (Aufrère et al., 1994). In a previous study Aufrère et al. (1992) showed that the basic subunits of the 11S protein fraction of soybean meal were not degraded, and the acid subunits only very slowly. For peas, the protein fraction most resistant to rumen degradation comprised both basic and acid 11S protein fractions. Pernollet and Mossé (1983) hypothesized that this was due to the different storage systems (reticular vs. vacuolar). The larger number of disulfide bridges and the higher molecular weight of the 11S fraction (Derbyshire et al., 1976) may be another important explanation for the lower *in situ* degradability compared to the 7S fraction. This agreed with results of an *in vitro* study by Mahadevan et al. (1980), who also concluded that the presence of disulfide bonds is an important factor explaining differences in resistance to rumen protein degradation.

Effects of pressure toasting on in situ disappearance
There was good agreement on the effects of pressure toasting on the W, k_d and %UIP between faba beans and peas and between lupins and soybeans. Differences in sensitivity to heat treatment of legume seeds was previously observed using autoclaving (Aguilera et al., 1992), dry and moist heat treatment (Sommer et al., 1994) and pressure

toasting (Goelema et al., 1998[a]).

In numerous studies, various heat treatments (oven-drying; autoclaving; roasting, extrusion, expander treatment, micronization) showed to be effective in decreasing the rumen degradability of several protein sources (see *e.g.* Arieli et al., 1989, Benchaar et al., 1994; Mostaghi Nia and Ingalls, 1995; Wang et al., 1997). However, only a few of them studied the effects of time and temperature during heat treatment on protein degradability to identify optimal conditions (Faldet et al., 1991). Optimal conditions are usually defined as those which result in the maximum supply of UIP (Arieli et al., 1989; Yu et al., 1998), and intestinal digestible undegraded intake protein or amino acids (Aldrich et al., 1995; Schroeder et al., 1995).

Pressure toasting decreased both the W and the k_d of CP in legume seeds. This was in agreement with results of studies after autoclaving (Aguilera et al., 1992), roasting (Robinson and McNiven, 1993, Hsu and Satter, 1995; Yu et al., 1998) and pressure toasting (Goelema et al., 1998[a]). Heat treatments differ in the way energy is transferred to the material. The latter occurs via electric heating in oven-drying, by using a flame in roasting, by steam at different pressure during autoclaving and pressure toasting or by combinations of heat, pressure and shear in for instance extrusion and expander processing. So, widely different chemical and physical processes are involved in the reduction of rumen protein degradability. Sommer et al. (1994) reported that heat treatment with steam was more effective in reducing N solubility than dry heat treatment. Information in literature is too variable on this particular subject to draw conclusions about the mechanisms and effectiveness of the different types of treatment.

An increasing temperature and/or time during processing usually leads to an incremental depression of rumen degradability (Satter, 1986). Some specific proteins, however, may give opposite effects, as shown by Aldrich et al. (1995). These authors found that roasting soybeans for 30 min at 141°C decreased rumen protein degradability compared to the untreated beans, but at 149° and 157°C it increased again. As shown in the present study, treatments at 100°C require longer processing times to obtain significant increases of %UIP or the amount of intestinal digestible UIP, compared to higher temperatures. McMeniman and Armstrong (1979) showed that heating faba beans for 24 h in a forced air oven at 105°C had no effect on postruminal delivery of amino acids, although low feed intake (4.5 kg DM/day, 37% hay) may also have played a role in the failure to obtain

significant *in vivo* effects. Roasting lupins for 1 min at 105°C on the other hand did not affect growth performance in beef steers in a study by Murphy and McNiven (1994). In dairy cows, however, it increased milk production and thereby protein, fat and lactose production in dairy cows (Singh et al., 1995).

Studies in literature on roasting of faba beans (Yu et al., 1998) and soybeans (Hsu and Satter, 1995) were in good agreement with our results. For faba beans, Yu et al. (1998) found that W slightly increased after roasting at 110°C, but subsequently decreased with increasing processing time or temperature. The k_d was especially affected by treatments at temperatures $\geq$ 130°C during toasting (in the present study) or roasting (Yu et al., 1998). In their study, UIP increased from 11.3% to 43% after roasting for 45 min at 150°C.

Denaturation and solubility of protein are not always inversely related. Denatured proteins can be soluble (Hermansson, 1979) and denaturation of 11S soy proteins may lead to the formation of soluble fractions (Wolf and Tamura, 1969; Kinsella, 1979). These authors showed that, upon heating at 100°C, the 11S soy protein fractions converts into a fast sedimenting fraction and a slow sedimenting fraction of 4S. Prolonged heating stabilized the soluble aggregate, followed by the formation of disulfide bonds which results in precipitation of the larger insoluble aggregate (Marsman, 1998). These results are in agreement with our results for soybeans. Although it has not been reported yet for other legumes, our data for lupins and data by Yu et al. (1998) for faba beans strongly supports the hypothesis that this might happen for other legume seeds as well.

In the present experiment a strong positive linear relation was found between the degradation characteristics of protein (as described in the present paper) and starch (Goelema et al., 1998[b]). For the W, the k_d and the rumen undegradable fraction of CP and starch, the correlation coefficients (r) were 0.97 (P $\leq$ 0.0001), 0.52 (P $\leq$ 0.0001) and 0.97 (P $\leq$ 0.0001), respectively. This was consistent with previous results for W and the rumen undegradable fraction after toasting for 3 min at 132°C (Goelema et al., 1998[a]). In that study, values for r were 0.99 and 0.94 (P $\leq$ 0.0001), respectively. These results suggest that heat processing results in the formation of complexes of protein and starch. Because of size difference, it may be more difficult to solubilize or wash out these complexes. Moreover, the decreased surface to volume ratio may have reduced accessibility for rumen microbes, which in turn contributes to the lower k_d.

For soybeans, the decrease in FS was more pronounced then the change in W after toasting. The *in situ* incubations and solubility measurements were carried out with fullfat soybeans. The high fat content of these seeds (19.8%) has probably interfered with level of W and, due to the mild nature of the method, especially of FS.

Using the soluble protein fraction instead of the washable protein fraction increased %UIP, especially for the toasted legume seeds. Consequently, the effect of the treatment (136°C/15 min) on %UIP increased.

Toasting did not negatively affect the %TDP, which means that the treatment did not result in the formation of indigestible products. Goelema et al. (1998[a]) showed, that after pressure toasting for 3 min at 132°C, the fraction of acid detergent insoluble N values for lupins increased from 1% to only 1.4% of total feed protein. This is consistent with the significant decrease of %TDP for lupins in the present study, which may be related to the relatively high neutral detergent fibre content of lupins. The higher intestinal disappearance of %UIP of extruded lupins (Benchaar et al., 1991) suggests, that during extrusion other mechanisms are involved in the structural changes of proteins than during toasting.

Qin et al. (1998) showed, that pressure toasting for 10 min at 136°C considerably decreased the 1-fluor-2,4-dinitrobenzene reactive (FDNB) lysine content of Argentine soybeans by 16% (from 19.3 to 16.2 g/kg), while in Chinese soybeans, a 7% decrease was found (from 17.4 to 16.2 g/kg). After treatment at 100°C or 118°C, no changes in FDNB lysine were observed. For lupins and rapeseed, toasting for 1.5 min at 130°C did not alter FDNB lysine (Goelema, unpublished). Grala et al. (1994), however, found drastic decreases of FDNB lysine after toasting defatted rapeseed cake for 10 min at 100 and 120°C. These results indicate that part of the protein has become nutritionally unavailable after treatment at 136°C, although this was not observed in %TDP. Hurrel et al. (1976), however, demonstrated that lysine isopeptides in heated chicken muscle were *in vivo* as digestible as total N, total lysine and FDNB reactive lysine in rats.

Protein values
Taking into account the discrepancy between W and FS as representation for the readily available fraction, increments of protein values may be even larger. When part of the protein has become nutritionally unavailable, a question which cannot be answered from the results of this study, the beneficial effects of toasting may be reduced.

Regarding the relatively low costs for processing (Melcion and Van der Poel, 1993), it was concluded that pressure toasting is an economical way to improve the protein value of these legume seeds.

Conclusions

Pressure toasting was effective in shifting the actual protein digestion from the rumen to the intestines, thus increased DVE values, ranging from 71% for soybeans to 91% for faba beans. The effects were due to decreases of W and k_d, without negatively affecting the intestinal digestibility of toasted seeds. Toasting for 15 min at 136°C resulted in the largest response in %UIP, DUP and DVE. For faba beans and peas, increasing processing time after toasting at 118°C or 136°C results in larger increments for %UIP, DUP and DVE than for lupins and soybeans. The latter seeds seemed to approach maximum for the parameters observed.

Acknowledgements

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CHAPTER 4

Effect of various conditions during pressure toasting on rumen degradability and intestinal digestibility of faba beans, peas, lupins and soybeans

2. LABORATORY MEASUREMENTS FOR PROTEIN

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Effect of Various Conditions during Pressure Toasting on Rumen Degradability and Intestinal Digestibility of Faba Beans, Peas, Lupins and Soybeans

2. Laboratory measurements for protein

J.O. Goelema, M.R. Roth, C. Steendam and H. Boer

Abstract

Effects of processing temperature and time of toasting were studied on in situ degradability and digestibility of protein in faba beans (*Vicia faba* cv. Alfred), peas (*Pisum sativum*), lupins (*Lupinus angustifolius*) and soybeans (*Glycine max.*) were studied. The results of in situ incubations were compared with laboratory measurements. Protein dispersibility index (PDI) and denaturation enthalpy according to differential scanning calorimetry (DSC) were used to determine effects of pressure toasting on protein denaturation. Dry and wet sieve analyses were used to characterize particle size distribution.

For all legume seeds, PDI significantly decreased after pressure toasting. Differences in PDI due to processing conditions, however, differed between legume seeds. The untreated legume seeds had a similar denaturation enthalpy (δH_{tot}). After toasting, the residual enthalpy decreased. For lupins and soybeans, two distinct endothermic transition peaks were observed, which were attributable to the 7S and 11S protein fractions in these seeds. For faba beans and peas, only one peak was observed. The first peak in lupins and soybeans was more sensitive to pressure toasting than the second one. The first peak disappeared after intermediate toasting conditions, whereas the second peak was only completely denatured after 15 min toasting at 136°C.

The lowest PDI and δH_{tot} values coincided with the highest values for the intestinally digestible undegraded protein (DUP) concentration. Based on linear regression, DUP could be predicted very well from PDI, except for lupins, where it failed to discriminate the differences in DUP due to processing conditions. The δH_{tot} was more discriminating than PDI at higher DUP values. For lupins and soybeans, differences between observed and predicted values were larger for untreated and mild toasting treatments, even though mean prediction errors for DUP were only 10%.

There were only small differences in in vitro protein digestibility (IVPD) due to pressure toasting. Only severe toasting decreased IVPD for peas and lupins. Correlations of IVPD and in situ intestinal digestibility and total tract protein digestibility (%TDP) were poor. A significant positive correlation of IVPD was only found with %TDP ($r = 0.80$, $P \le 0.0001$) for lupins.

Results of wet sieve analysis indicated that ground toasted legume seeds had a larger apparent mean N particle size than untoasted seeds, which coincided with a decreased rumen protein degradability.

It was concluded that PDI and δH_{tot} are useful indicators for protein denaturation, which can be used to evaluate effects on DUP after pressure toasting.

The IVPD, however, failed to discriminate between in situ digestibility of untreated and pressure toasted legume seeds in general, but could be used to evaluate effects on %TDP for lupins.

Introduction

Effects of heat treatments on rumen undegraded intake protein (UIP) are usually evaluated using the in situ nylon bag method (Ørskov and McDonald, 1979; Mehrez and Ørskov, 1977). The mobile nylon bag technique (Hvelplund et al., 1992) is generally used to measure intestinal digestion of undegraded feed protein (Erasmus et al., 1994). Both methods are labour-intensive and expensive because of long incubation periods and the requirement for cannulated animals. Therefore, development of rapid, cheap and reliable laboratory methods for these measurements is of great importance.

Laboratory methods, such as incubation with rumen fluid (Broderick, 1987) or proteolytic enzymes have been used to study rumen protein degradability (Poos-Floyd et al., 1985; Aufrère and Cartailler, 1988). The method of Aufrère and Cartailler (1988) involves the incubation of feed samples with a protease from *Streptomyces griseus*. This method was used successfully to evaluate the rumen protein degradability of various protein sources (Aufrère et al., 1991; Cone et al., 1996), but failed to identify differences in protein degradability of legume seeds after pressure toasting (Feil et al., 1996). Incubation of feed with pepsin and pancreatin can be used to measure in vitro protein digestibility (IVPD) of pig feeds (Babinszky et al., 1990), and the in vitro protein digestibility of rumen undegraded protein in dairy cows (Antoniewicz et al., 1992).

Processing may affect the particle size of processed feeds. This is obvious for treatments which involve shear forces, such as flaking, expander treatment, extrusion and pelleting. Hsu and Satter (1995) showed that the protein dispersibility index (PDI), which is an indicator for protein denaturation, was a useful parameter for evaluating heat damage of proteins. Goelema et al. (1998[a]) observed that the decreased in situ protein degradability after pressure toasting was associated with a decreased PDI.

Differential scanning calorimetry (DSC) has proved to be a suitable tool to study phase transitions, such as the denaturation of proteins (Arntfield and Murray, 1981; Wright, 1982). The technique provides the temperature of denaturation (T_p) and the enthalpy change associated with the transition (δH). The δH value represents a combination of

exothermic reactions such as those associated with disruption of hydrophobic interactions and aggregation of the molecule, and of the endothermic contributions of disruption of nitrogen bonds and unfolding of the polypeptide chains (Wright, 1982). No studies are known where effects of heat treatment on protein degradation are evaluated with DSC. An experiment was conducted to study the effects of temperature and residence time during pressure toasting on the rumen degradability and the intestinal digestibility of peas, lupins, and faba beans.

In a previous paper, effects of pressure toasting on protein degradability and digestibility of were described (Goelema et al., 1998[b]). In the present paper, effects on DSC, PDI, IVPD, and particle size are presented, and compared with in situ parameters for protein.

Materials and methods

Samples and treatments

Faba beans (*Vicia faba* cv. Alfred), peas (*Pisum sativum*), lupins (*Lupinus angustifolius*) and soybeans (*Glycine max.*) were obtained from a commercial supplier. The seeds were toasted at 100°C for 7, 15 or 30 min; at 118°C for 3, 7, 15 or 30 min, or at 136°C for 3, 7 or 15 min, as described by Goelema et al. (1998[b]). After toasting the samples were dried in a forced-air oven for 16 h at 35°C. Untreated seeds were used as controls. All samples were ground over a 3 mm screen (Retsch centrifugal mill) prior to rumen incubation.

In situ incubations and laboratory analyses

in situ rumen incubations and intestinal protein digestibility of rumen undegraded protein were measured as described by Goelema et al. (1998[b]).

Chemical analyses were carried out as described by Goelema et al. (1998[b]) using samples ground over a 1 mm screen (Retsch centrifugal mill). PDI was determined according to a modified AACC method 46-24 procedure, as described by Thomas et al. (1997).

The denaturation enthalpy (δH) is a measure for the amount of native protein present in the sample. Thus, a difference in δH between toasted and untreated samples represents the degree of denaturation due to the heat treatment. Denaturation enthalpy (δH) of the samples was determined by DSC in duplicate, using a Mettler-Toledo DSC12-E, as

described by Thomas et al. (1998). Onset (T_o), peak (T_p) and endset temperature (T_e) of the denaturation peaks were determined as described by Wright (1982) (Figure 1). Enthalpy was calculated by integration of the peak surface (from T_o to T_e), using the Mettler system TA89E software package.

IVPD was determined according to a modified procedure of Babinszky et al. (1990). Samples were incubated with pepsin-hydrochloric acid solution (pH 1.0) at 40°C for 1 h. After neutralization with sodium bicarbonate, incubation is continued (40°C, 1h) with potassium phosphate buffer (pH 6.8) containing pig pancreatin, α-amylase, lipase and bile salts. Following incubation, a hydrochloric acid/acetic acid solution was added to stop the reaction. The undissolved material was then filtered through a scintered-glass filter crucible, which was fitted with a layer of N-free ashless floc (Whatman 1704-010). After washing (3 x, demineralized water), the undigested residue and floc were brought into a Kjeldahl flask for determination of the indigestible N content by the Kjeldahl method. The digestible N-content was calculated by subtracting the indigestible N content from the N content of the sample before digestion.
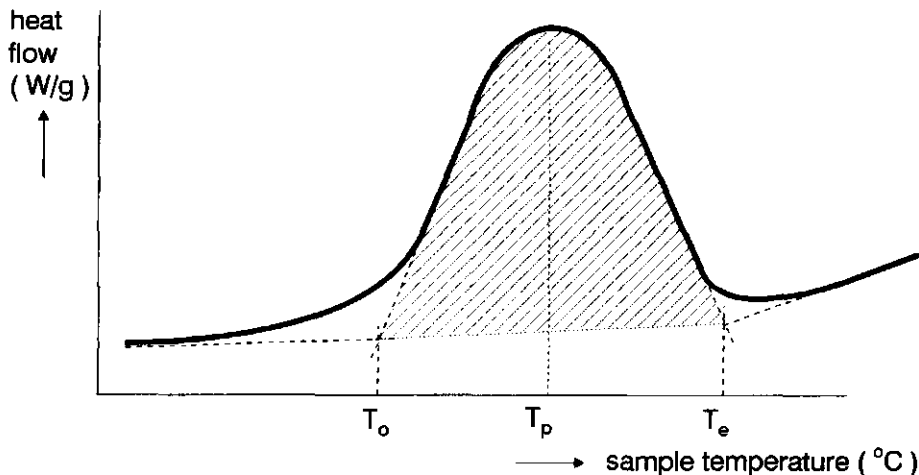
Figure 1 Differential scanning calorimetry thermogram with associated parameters (modified from D.J. Wright (1984), endothermic heat flow upwards).

Particle size distribution of the 3 mm ground samples, used for in situ incubations, was determined by wet and dry sieve analysis (Retsch AS 200), as described by Goelema et al. (1999). Only the untreated seeds and those toasted for 15 min at 136°C were analyzed. Sieve fractions were analyzed for DM and N and were expressed as a fraction of the sample N. If sieve fractions were insufficient for analysis, samples were pooled with the other fractions. The fraction < 71 μm after wet sieve analysis was calculated by subtraction. For wet sieve analysis, the filter solubility procedure (Weisbjerg et al., 1990) was used to divide the fraction < 71 μm into a ('soluble') fraction < 8 μm and a fraction of particles between 8 and 71 μm. The distribution of N over the (pooled) sieve fractions was used to calculate an apparent mean N particle size ($X_{50,N}$), using the logarithmic normal distribution as described by Waldo et al. (1971). Using the mean N content and the pooled fraction had very little effect on the $X_{50,N}$ compared to using the mean N content for the individual small fractions.

Statistical analysis

Analysis of variance was conducted using the General Linear Models (GLM) procedure of SAS (SAS, 1989) with equation 1. Treatment means were compared pairwise using Tukey's HSD-test (P $\leq$ 0.05).

$$Y_{ij} = \mu + \text{Treatment}_i + D_j + \epsilon_{ij}, \tag{1}$$

where, Y_{ij} is the dependent variable under examination (δH, T_o, T_p, T_e, PDI, IVPD), μ the overall mean, treatment$_i$ the treatment effect (i=1 - 11), D_j the treatment day effect (j= 1, 2) and ϵ_{ij} the residual error term. Regression analysis was carried out with Proc REG (SAS, 1989), after omitting the untreated controls from the dataset. Linear and quadratic effects of temperature and time were evaluated, as well as the interaction of time and temperature, as described by Goelema et al. (1999). For regression, processing temperature was linearly transformed into increments from 100°C (*i.e.* 0, 18 or 36). If possible, models were reduced in complexity by excluding non-significant quadratic effects (P > 0.10). Pearson correlation coefficients were calculated with Proc CORR (SAS, 1989). Linear regression (SAS, 1989) was used to evaluate the prediction of the amount of intestinal digestible rumen undegraded protein fraction (DUP) based on the denaturation parameters δH or PDI, and the digestibility of rumen undegraded protein (UIP) in the

intestines (%DUP), or the total tract protein digestibility (%TDP) from IVPD (equation 2).

$$PRED_{ij} = \alpha + \beta\ OBS_i + \epsilon_{ij} \tag{2}$$

where, PRED is the predicted value of the variable under examination, α the intercept, OBS the observed value, β the slope, and ϵ_{ij} the residual error term ($i = 1, 2, ... n$; $j = 1, 2$). Predictive values were compared with observed values using the mean squared prediction error (MSPE, equation 3) according to Bibby and Toutenburg (1977).

$$MSPE = \Sigma\ (\ OBS_i - PRED_i\ )^2\ /\ n \tag{3}$$

The square root of the MSPE was expressed as a proportion of the observed mean (MPE), and decomposed into errors in central tendency (bias, the difference between the predicted and the measured mean), errors due to regression (*i.e.* deviation of the regression slope from one), and errors due to disturbances.

Table 1 List of abbreviations used in the paper.

Abbreviation	Parameter
DSC	differential scanning calorimetry
DUP	intestinal digestible UIP, as % of UIP
DVE	intestinal absorbable true protein, in g/kg DM
δH	DSC denaturation enthalpy (J/g CP)
IVPD	in vitro protein digestibility (%)
k_o	fractional rate of rumen degradation (%/h)
PDI	protein dispersibility index (in %)
SEM	standard error of the mean
TDP	total tract digestibility of crude protein (in %)
T_o	onset temperature of the DSC denaturation peak (°C)
T_e	endset temperature of the DSC denaturation peak (°C)
T_p	DSC denaturation peak temperature (°C)
UIP	rumen undegraded intake protein, as % of CP
W	washable fraction (%)
$X_{50.N}$	apparent mean N particle size (μm)

Results

Protein dispersibility index (PDI) and in vitro protein digestibility (IVPD)
Table 1 gives an overview of the abbreviations used in the paper. Table 2 shows the PDI and IVPD for faba beans, peas, lupins and soybeans. The PDI of untreated faba beans and peas was similar (87.1% and 84.5%, respectively), but higher than for lupins (38.4%) and soybeans (69.4%). For faba beans and peas, PDI linearly decreased with increasing toasting time or temperature. Untreated faba beans and peas had a comparable PDI, but toasting at 100°C and 118°C resulted in a lower PDI for peas. After toasting at 136°C, however, values for PDI of faba beans and peas were similar. For faba beans and peas, the smallest PDI coincided with the highest values for %UIP, and was observed after toasting for 15 min at 136°C.

For lupins, toasting reduced the PDI, but the differences between treatment conditions were small compared with the other seeds. For lupins and soybeans, PDI showed a quadratic response to treatment temperature and time, as well as an interaction between these parameters.

The IVPD of peas, lupins and soybeans was significantly higher (95.0%, 95.1% and 93.2%, respectively) than for faba beans (83%, Table 2). For faba beans, pairwise comparisons of treatments on IVPD were never significant. Regression analysis revealed a quadratic response to temperature as well as a significant interaction between processing temperature and time. Severe toasting decreased the IVPD for peas and lupins. Processing temperature linearly decreased IVPD for peas, while for lupins there was a quadratic effect of temperature, as well as an interaction between toasting temperature and time. For soybeans, changes in IVPD due to different processing conditions were rather small.

Table 2 Effects of pressure toasting on protein dispersibility index (PDI) and in vitro protein digestibility (IVPD) of faba beans, peas, lupins, and soybeans[1].

Treatments[2]	PDI (%)				IVPD (%)			
	Faba beans	Peas	Lupins	Soybeans	Faba beans	Peas	Lupins	Soybeans
untreated	87.1^a	84.5^a	38.4^a	69.4^a	83.0	95.0^a	95.1^a	93.2^{bc}
100/7	74.2^b	65.5^b	17.4^b	45.4^b	90.0	94.0^{ab}	94.1^{ab}	92.6^c
100/15	71.7^{bc}	61.1^c	15.7^c	34.6^c	88.4	93.4^{abc}	93.6^{ab}	94.7^{ab}
100/30	70.5^c	55.9^d	15.0^{cde}	23.5^d	84.8	93.0^{abc}	93.6^{ab}	94.9^{ab}
118/3	67.1^d	52.0^d	15.3^{cd}	25.8^d	82.6	93.4^{abc}	94.8^a	94.5^{abc}
118/7	57.5^e	39.4^e	14.3^{de}	15.9^e	84.3	92.7^{bc}	94.1^a	94.8^{ab}
118/15	39.5^f	24.7^f	13.9^e	11.5^{ef}	85.1	92.2^{bc}	94.0^{ab}	93.6^{abc}
118/30	19.7^h	18.5^g	13.8^e	10.4^f	84.3	92.2^{bc}	92.6^{bc}	94.9^{ab}
136/3	24.4^g	19.9^g	14.6^{cde}	11.0^{ef}	83.0	92.3^{bc}	94.3^{ab}	93.4^{abc}
136/7	16.3^i	17.4^g	14.9^{cde}	11.4^{ef}	82.4	92.7^{bc}	93.5^{ab}	95.3^a
136/15	16.3^i	17.6^g	15.5^{cd}	12.5^{ef}	86.0	91.4^c	91.4^c	93.6^{abc}
SEM	5.57	4.91	1.48	3.99	0.76	0.22	0.24	0.20
Treatment day[3]	NS	NS	**	NS	NS	NS	**	NS
Temperature	***	***	***	***	**	*	**	NS
Time	*	*	**	***	*	NS	NS	+
Temp. x time	+	NS	***	***	*	NS	***	NS
Temp. x temp.	-	-	***	***	*	-	***	-
Time x time	-	-	*	**	-	-	-	-

[1] For abbreviations see Table 1. Within columns, different superscripts indicate significant differences (P < 0.05, Tukey's HSD-test).

[2] Codes denote the processing temperature (in °C) and the processing time (in min) *e.g.* the 100/7 treated seeds have been toasted for 7 min at 100°C.

[3] Probabilities: NS = P > 0.1; + = P ≤ 0.1; * = P ≤ 0.05; ** = P ≤ 0.01; *** = P ≤ 0.001; -: excluded from the model.

DSC denaturation characteristics

Toasting significantly affected the DSC denaturation characteristics of the legume seeds (Tables 3, 4, 5). For faba beans and peas, one protein denaturation peak was found (Table 3), while for lupins (Table 4) and soybeans (Table 5), two peaks were observed. Denaturation enthalpy (in case of lupins and soybeans the sum of the two peaks) ranged from 3.7 J/g CP (peas) to 4.9 J/g CP (soybeans). The enthalpy of the two endothermic peaks of lupins is similar, while the first peak of soybeans had a lower enthalpy than the second one.

After toasting, onset temperature (T_o) and peak temperature (T_p) (figure 1) of faba beans and peas increased (Table 3), while denaturation enthalpy (δH_{tot}) decreased. The endset temperature (T_e) of faba beans was not affect by toasting, but for peas T_e linearly decreased with increasing toasting temperature and tended to decrease with longer processing time. For faba beans, processing temperature was linearly related to δH_{tot}, while the relation with time was quadratic. For peas, both temperature and time during processing showed a tendency to a quadratic effect for δH.

The two protein fractions, represented by the two peaks in lupins and soybeans, showed a similar sensitivity to pressure toasting. The first peak of lupins and soybeans completely disappeared after toasting at 118°C for 15 and 7 min, respectively, while the size of the second peak decreased only after more intensive toasting treatments. For lupins, δH_2 decreased after toasting at 118°C for 15 min, and completely disappeared after toasting for 15 min at 136°C. For soybeans, δH_{tot} decreased particularly after toasting at 136°C, and similar to lupins, it (almost) completely disappeared after 15 min of toasting.

Changes in T_o and T_p after toasting were smaller for lupins and soybeans than for the other seeds. For lupins, T_{o1} and T_{o2} decreased after toasting, while T_{e1}, T_{p1} and T_{p2} were only slightly affected. For soybeans, increasing processing time increased T_{o1} and T_{o2} after toasting at 100°C, but at higher temperatures, T_{o2} decreased. The T_{e1} of soybeans showed a positive linear response to increasing toasting time and temperature, while T_{e2} of toasted soybeans was unaffected by the treatment conditions. For lupins, δH_1 showed a linear response to increased toasting temperature, and a tendency for linearity with time. The denaturation enthalpy of both endothermic peaks of soybeans was quadratically related to processing temperature and time.

Table 3 Effects of pressure toasting on DSC denaturation characteristics of faba beans and peas[1].

	Faba beans				Peas			
	T_o	T_p	T_e	δH_{tot}	T_o	T_p	T_e	δH_{tot}
	°C	°C	°C	J/g CP	°C	°C	°C	J/g CP
Treatments[2]								
untreated	81.1^d	91.6^{abc}	103.2	4.3^a	79.0^{de}	91.4^{ab}	101.7	3.7^a
100/7	80.7^d	90.6^{bc}	102.5	4.5^a	77.8^e	88.1^b	101.5	2.3^{ab}
100/15	81.4^d	90.7^{bc}	103.4	3.9^{ab}	78.5^{de}	87.9^b	102.6	1.9^{bc}
100/30	82.2^{cd}	90.1^c	103.3	3.0^{bc}	79.9^{de}	87.2^b	99.7	1.4^{cde}
118/3	81.4^d	91.1^{abc}	103.4	3.3^{bc}	81.4^{cd}	88.1^b	101.5	1.7^{bcd}
118/7	82.6^{bcd}	91.0^{abc}	103.1	2.5^c	84.4^{bc}	94.0^a	101.1	0.4^{cde}
118/15	84.6^{ab}	92.0^{abc}	103.7	1.6^d	85.3^b	94.4^a	99.8	0.4^{cde}
118/30	86.4^a	93.4^{ab}	101.9	1.0^d	86.2^{ab}	95.2^a	99.5	0.3^{de}
136/3	84.3^{abc}	93.2^{ab}	102.4	1.4^d	85.9^{ab}	95.4^a	99.5	0.4^{cde}
136/7	85.3^a	93.9^a	103.8	0.8^{de}	89.1^a	95.4^a	100.2	0.1^e
136/15	ND	ND	ND	0^e	ND	ND	ND	0^e
SEM	0.44	0.31	0.26	0.32	0.87	0.80	0.31	0.25
Treatment day[3]	NS	NS	NS	NS	+	NS	NS	NS
Temperature	***	NS	NS	***	***	*	*	**
Time	*	NS	NS	***	*	NS	+	*
Temp. x time	*	**	NS	NS	NS	*	NS	NS
Temp. x temp.	-	*	-	-	-	-	-	+
Time x time	+	-	-	**	*	-	-	+

[1] For abbreviations see Table 1. Within columns, different superscripts indicate significant differences (P < 0.05, Tukey's HSD-test). ND: not detected.

[2] For treatment codes see Table 2.

[3] For significance levels see Table 2.

Table 4 Effects of pressure toasting on denaturation characteristics of lupins[1].

	T_{o1}	T_{p1}	T_{e1}	δH_1	T_{o2}	T_{p2}	T_{e2}	δH_2	δH_{tot}
	°C	°C	°C	J/g CP	°C	°C	°C	J/g CP	J/g CP
Treatments[2]									
untreated	82.1[a]	90.5	98.1	2.1[a]	98.1[a]	106.6	115.2	1.9[a]	4.1[a]
100/7	81.6[a]	90.8	98.1	2.0[a]	98.1[a]	106.2	116.1	1.8[a]	3.9[ab]
100/15	80.5[ab]	91.2	97.5	1.9[a]	97.5[a]	106.0	114.9	1.8[a]	3.7[abc]
100/30	79.2[b]	91.0	97.2	1.5[ab]	97.2[a]	105.9	114.7	1.6[a]	3.0[bc]
118/3	81.7[a]	91.0	97.7	1.6[a]	97.7[a]	105.9	115.3	1.8[a]	3.3[abc]
118/7	81.5[a]	90.7	97.8	0.8[b]	97.8[a]	105.9	115.1	1.9[a]	2.7[cd]
118/15	ND	ND	ND	0[c]	96.5[ab]	105.8	114.7	1.9[a]	1.9[d]
118/30	ND	ND	ND	0[c]	94.0[b]	105.9	115.1	1.7[a]	1.7[de]
136/3	ND	ND	ND	0[c]	96.0[ab]	105.6	115.3	1.9[a]	1.9[d]
136/7	ND	ND	ND	0[c]	96.5[ab]	105.9	115.2	0.7[b]	0.7[de]
136/15	ND	ND	ND	0[c]	ND	ND	ND	0[c]	0[f]
SEM	0.31	0.08	0.13	0.20	0.29	0.08	0.12	0.13	0.28
Treatment day[3]	NS	NS	NS	NS	NS	NS	NS	NS	NS
Temperature	NS	+	NS	***	NS	NS	*	*	***
Time	**	+	+	+	+	NS	*	NS	***
Temp. x time	NS	+	NS	NS	*	NS	*	*	NS
Temp. x temp.	-	-	-	-	-	-	+	**	-
Time x time	-	+	-	-	-	-	+	-	**

[1] For abbreviations see Table 1. Within columns, different superscripts indicate significant differences (P < 0.05, Tukey's HSD-test). ND: not detected.

[2] For treatment codes see Table 2.

[3] For significance levels see Table 2.

Table 5 Effects of pressure toasting on denaturation characteristics of soybeans[1].

Treatments[2]	T_{o1} °C	T_{p1} °C	T_{e1} °C	δH_1 J/g CP	T_{o2} °C	T_{p2} °C	T_{e2} °C	δH_2 J/g CP	δH_{tot} J/g CP
untreated	70.3[b]	78.6	86.5[c]	1.2[a]	92.3[ab]	99.4[a]	101.7	3.8[a]	4.9[a]
100/7	71.9[ab]	78.4	86.0[c]	1.0[ab]	92.4[ab]	99.1[ab]	109.3	3.8[a]	4.8[ab]
100/15	71.8[ab]	78.6	87.0[bc]	0.8[ab]	92.3[ab]	98.9[ab]	109.6	3.8[a]	4.6[abc]
100/30	73.0[a]	78.7	88.5[ab]	0.8[b]	93.2[a]	99.0[ab]	110.0	3.6[a]	4.4[abcd]
118/3	72.9[a]	78.8	88.8[a]	0.3[c]	93.3[a]	99.4[ab]	110.1	3.7[a]	4.0[bcd]
118/7	ND	ND	ND	0[c]	91.3[bc]	99.2[ab]	109.1	3.9[a]	4.0[cd]
118/15	ND	ND	ND	0[c]	90.7[cd]	99.3[ab]	108.4	3.6[a]	3.6[d]
118/30	ND	ND	ND	0[c]	89.7[d]	99.9[a]	110.5	4.0[a]	4.0[bcd]
136/3	ND	ND	ND	0[c]	91.0[c]	99.6[a]	110.1	2.7[b]	2.7[e]
136/7	ND	ND	ND	0[c]	91.4[bc]	99.6[a]	112.0	2.1[b]	2.1[e]
136/15	ND	ND	ND	0[c]	86.5[e]	98.4[b]	111.0	0.6[c]	0.6[f]
SEM	0.33	0.06	0.37	0.10	0.41	0.09	0.25	0.25	0.27
Treatment day[3]	NS	NS	NS	NS	NS	NS	NS	NS	NS
Temperature	*	NS	***	***	NS	NS	NS	**	NS
Time	+	NS	***	*	NS	*	NS	*	+
Temp. x time	-	-	-	+	**	NS	NS	NS	NS
Temp. x temp.	-	-	-	***	+	-	-	-	*
Time x time	-	-	-	**	+	*	-	*	*

[1] For abbreviations see Table 1. Within columns, different superscripts indicate significant differences ($P < 0.05$, Tukey's HSD-test). ND: not detected.

[2] For treatment codes see Table 2.

[3] For significance levels see Table 2.

Sieve analysis

The N-distribution over the different particle size classes of the 3 mm ground samples was different between legume seeds (Tables 6, 7). For faba beans and peas $X_{50,N}$ was smallest, ground soybeans were intermediate and ground lupins had the largest $X_{50,N}$. The $X_{50,N}$ was smaller after wet sieve analysis than after dry sieve analysis. Compared to dry sieve analysis, the contribution of the fraction of particles $< 71\ \mu m$ was increased, which was mainly due to an increase of the soluble fraction, represented by the fraction

$< 8 \mu m$.

Ground toasted legume seeds had a larger $X_{50,N}$ than the untreated seeds (Tables 6, 7), except for soybeans after dry sieve analysis. The effect of toasting was caused by a reduction of the particle size fraction $< 71 \mu m$. Wet sieve analysis showed that there was a shift in particle size from $< 8 \mu m$ to particles between 8 and 71 μm. For faba beans and peas, differences in $X_{50,N}$ were only significant for the wet sieve analysis.

Table 6 Effects of pressure toasting of faba beans and peas on the N distribution (in %) over different particle size classes[1].

Type of sieve analysis	Faba beans				Peas			
	Dry		Wet		Dry		Wet	
	U (%)	T (%)	U (%)	T (%)	U (%)	T (%)	U (%)	T (%)
$< 8 \mu m$	-	-	51.1^a	14.3^b	-	-	49.4^a	15.0^b
8 - < 71 μm	-	-	15.3	27.1	-	-	13.8^b	22.0^a
$< 71 \mu m$	30.0^a	24.7^b	66.3^a	41.4^b	31.7^a	19.8^b	63.2^a	37.0^b
71 - < 160 μm	5.3	8.8	0.5^a	15.6^b	4.3^a	13.8^b	0.5^a	20.7^b
160 - < 315 μm	5.4	5.0	0.7^a	2.6^b	5.4	6.1	0.9	5.5
315 - < 630 μm	11.3^a	11.8^b	3.1^a	6.4^b	12.7	14.5	3.6^a	6.3^b
630 - < 1250 μm	33.9^a	37.0^b	12.5	16.4	32.9	34.1	13.4	14.7
1250 - < 2500 μm	14.1	12.7	16.6	17.4	13.1	11.6	18.2^a	15.8^b
≥ 2500	0.0	0.0	0.3	0.1	0.0	0.0	0.2	0.1
SEM	7.96	8.48	4.95	6.77	7.85	8.76	5.39	6.58
$X_{50,N}$ (μm)	311	338	6^a	123^b	289	333	12^a	137^b

[1] Within type of sieve analysis, different superscripts indicate significant (P $\leq$ 0.05) differences between untreated (U) and toasted samples (T, pressure toasted for 15 min 136°C).

Table 7 Effects of pressure toasting of lupins and soybeans on the N distribution (in %) over different particle size classes[1].

Type of sieve analysis	Lupins				Soybeans			
	Dry		Wet		Dry		Wet	
	U	T	U	T	U	T	U	T
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
< 8 μm	-	-	24.0^a	9.6^b	-	-	33.2^a	8.1^b
8 - < 71 μm	-	-	26.6	29.6	-	-	8.6^b	33.9^a
< 71 μm	4.9	3.5	50.5	39.2	21.9^a	24.6^b	41.8	42.0
71 - < 160 μm	7.9	6.1	1.1	0.8	5.1	6.4	5.9	3.5
160 - < 315 μm	5.5	4.1	1.2	1.2	5.8^a	7.6^b	2.1^a	4.9^b
315 - < 630 μm	9.0	7.1	2.7^a	3.3^b	12.6	14.2	5.4^a	9.0^b
630 - < 1250 μm	32.9	33.8	8.1	8.7	37.9^a	31.0^b	18.8	17.5
1250 - < 2500 μm	39.7^a	45.4^b	34.7	44.2	16.6	16.3	25.9	23.0
$\geq$ 2500	0.0	0.0	1.7	2.7	0.0	0.0	0.2	0.2
SEM	9.97	10.22	7.41	9.13	8.90	8.38	8.33	7.48
$X_{50 N}$ (μm)	1151^a	1545^b	112^b	268^a	439^a	362^b	93^b	145^a

[1] Within type of sieve analysis, different superscripts indicate significant (P $\leq$ 0.05) differences between untreated (U) and toasted samples (T, pressure toasted for 15 min 136°C).

Relations between IVPD, PDI, δH and degradation characteristics

Overall correlations between IVPD and in situ intestinal digestibility of rumen undegraded protein (%DUP) and total protein digestibility (%TDP) were rather poor (Table 8).

For lupins, IVPD was significantly correlated with %TDP (r = 0.80; P $\leq$ 0.0001, results not shown). For %DUP, significant correlations were found with IVPD of soybeans and peas (r = 0.43, P $\leq$ 0.05 and r = -0.83, P $\leq$ 0.0001, respectively).

Overall correlations between PDI and δH were significant, but correlation coefficients were only small (Table 8). Within legume seeds, correlations between PDI and δH$_{tot}$ were 0.96 and 0.93 for faba beans and peas, respectively. For lupins and soybeans, correlations

were only significant for δH_1, and correlations between PDI and δH_{tot} were smaller than for faba beans and peas. The δH for the two endothermic peaks of lupins and soybeans were significantly ($P \leq 0.0001$) correlated with the total enthalpy of the peaks (Table 8), with correlation coefficients of 0.53 and 0.84 for the respective peaks.

The inverse relation between the denaturation parameters δH and PDI and the protein degradation characteristics (%UIP, DUP and DVE) were strong, with coefficients of 0.8 or higher (Table 8). Since %UIP, DUP and DVE showed practically the same pattern, only results for DUP are presented.

Table 8 Correlations between laboratory parameters and in situ results[1].

	Laboratory parameters				
	PDI (%)	δH_1 (J/g CP)	δH_2 (J/g CP)	δH_{tot} (J/g CP)	IVPD (%)
Laboratory parameters					
PDI (%)		0.40[2] **[3]	0.42 **	0.39 ***	-0.31 **
δH_1 (J/g CP)			NS	0.53 ***	NS
δH_2 (J/g CP)				0.84 ***	0.34 *
δH_{tot} (J/g CP)					NS
In situ parameters for protein					
W (%)	0.93 ***	0.61 ***	NS	0.47 ***	
k_d (%/h)	0.34 **	0.74 ***	NS	0.49 ***	
%UIP (%)	-0.84 ***	-0.79 ***	-0.32 *	-0.60 ***	
%DUP	-0.51 ***	NS	-0.73 ***	-0.69 ***	0.23 *
%TDP	NS	NS	-0.73 ***	-0.50 ***	0.18 +
DUP (g/kg DM)	-0.85 ***	-0.87 ***	NS	-0.24 *	
DVE (g/kg DM)	-0.87 ***	-0.81 ***	NS	-0.21 +	

[1] For abbreviations see Table 1.
[2] Pearson correlation coefficient.
[3] For significance levels see Table 2.

Figure 2 shows the regression of DUP on PDI for the different seeds. The data showed a linear relationship for faba beans (Figure 2a), peas (Figure 2b) and soybeans (Figure 2d), but for lupins (Figure 2c), PDI could not discriminate between treatment conditions. Regression analysis of DUP on PDI showed that intercepts were similar for faba beans and peas but different from those of soybeans. The R^2 values for faba beans, peas and soybeans were 0.89, 0.89 and 0.90, respectively. For DUP (Figure 2) mean prediction errors (MPE, expressed as a proportion of the mean observed value) were almost completely attributable to random disturbance, and were 10.1, 10.0, 13.5 and 4.9% for faba beans, peas, lupins and soybeans, respectively.

a: FABA BEANS

DUP = -1.26 * PDI + 172.19, R-square = 0.89
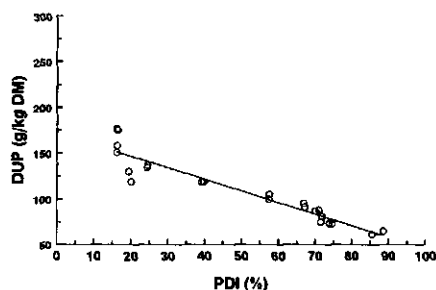
b: PEAS

DUP = -1.22 * PDI + 148.12, R-square = 0.89
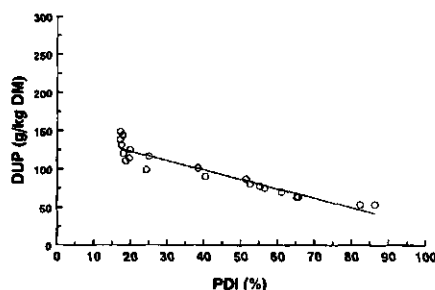
c: LUPINS

DUP = -3.51 * PDI + 202.70, R-square = 0.60
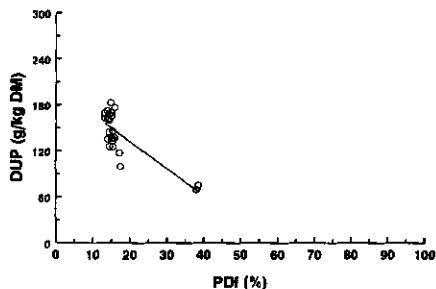
d: SOYBEANS

DUP = -1.32 * PDI + 190.51, R-square = 0.90
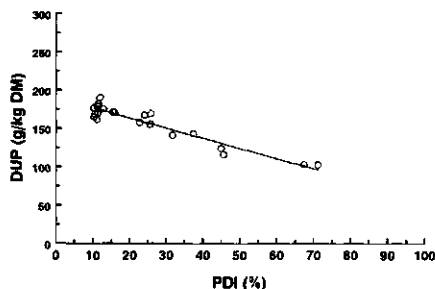
Figure 2   Regression equations of protein dispersibility index (PDI, in %) on intestinal digestible rumen undegraded protein (DUP, in g/kg DM) for faba beans (a), peas (b), lupins (c) and, soybeans (d).

a: FABA BEANS

DUP = -22.40 * dH + 163.44, R-square = 0.92
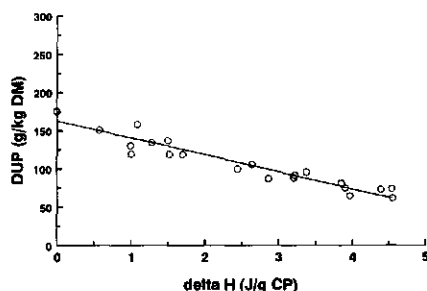
b: PEAS

DUP = -22.19 * dH + 123.27, R-square = 0.76
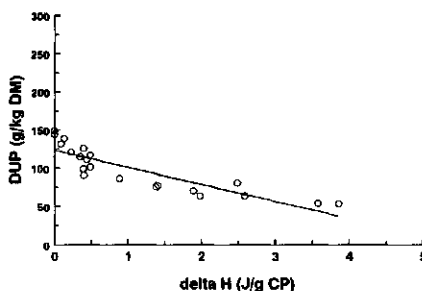
c: LUPINS

DUP = -20.58 * dH + 192.78, R-square = 0.74
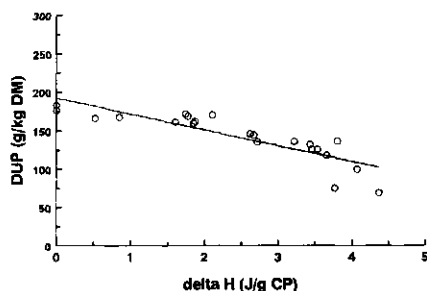
d: SOYBEANS

DUP = -13.46 * dH + 206.32, R-square = 0.46
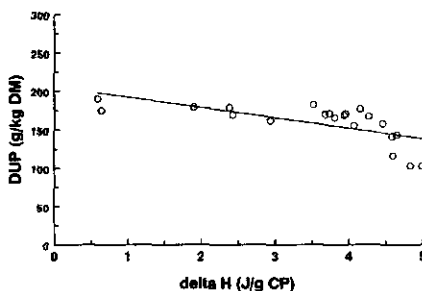
Figure 3   Regression equations of total DSC denaturation enthalpy (δH, in J/g CP) on intestinal digestible rumen undegraded protein (DUP, in g/kg DM) for faba beans (a), peas (b), lupins (c) and, soybeans (d).

Figure 3 shows the regression of DUP on δH for the different seeds. The intercepts of the regression equations were significantly different between legume seeds. Slopes were similar for faba beans (Figure 3a), peas (Figure 3b) and lupins (Figure 3c), but different for soybeans (Figure 3d).

Residual variation for lupins and soybean was higher at larger values for δH, while for

peas, smaller δH corresponded with larger variation. R^2 values ranged from 0.46 for soybeans to 0.92 for faba beans. Based on δH, MPE was 8.9, 14.6, 10.9 and 11.6% for DUP of faba beans, peas, lupins and soybeans, respectively. Decomposition of the prediction error showed, that more than 90% of the prediction errors was due to random disturbance.

Multiple linear regression, with both PDI and δH_{tot} as regressors, only improved the prediction of DUP in lupins. R^2 increased from 0.60 (for PDI) and 0.74 (for δH_{tot}) to 0.93, while MPE decreased from 13.5 and 10.9 to 5.6%, respectively.

Discussion

Protein composition and denaturation

Plant proteins consist of different fractions, which can be divided into albumins, globulins, glutelins and prolamins based on their solubility in different media. Differences in sedimentation behaviour are also frequently used to classify proteins in for instance 2S, 7S, and 11S fractions. The contribution of the protein fractions to total protein varies between plant proteins, between seeds of similar chemical composition, such as peas, faba beans and phaseolus beans (Sosulski et al., 1985), and within species, as was demonstrated for peas by Casey et al. (1982) and for lupins by Wright and Boulter (1980). The protein fractions can differ in terms of the conformation of the protein, the amino acid composition, molecular size, the charge distribution on the protein, the extent of inter- and intra-molecular bonding and the matrix surrounding the protein.

After heating, the protein structure changes from a native, ordered state to a denatured, disordered state due to disruption of intramolecular hydrogen bonds and unfolding of polypeptide chains, followed by aggregation. As a result, the PDI of proteins decreases upon heating, as does the δH. The more heat-labile the protein, the larger the decrease of PDI and δH_{tot}. Literature on the effects of heat treatments on PDI or denaturation enthalpy refers mainly to legume seeds, such as soybeans, peas, faba beans, phaseolus beans, lentils and lupins (Wright and Boulter, 1980; Sosulski et al., 1985; Hsu and Satter, 1995; Qin et al., 1998). In the latter studies, PDI is related to the total protein fraction, whereas in the DSC studies, the enthalpy usually refers to the globulin fractions. In DSC studies, albumins, glutelins and prolamins are hardly ever mentioned, probably because of the relatively small contribution of these fractions to total protein.

Protein dispersibility

The results for PDI of untreated faba beans, peas and lupins were consistent with previous results (Goelema et al., 1998[a]). The PDI of soybeans was lower than that observed by Hsu and Satter (1995) and Qin et al. (1998) for untoasted seeds (69 vs. 86%), and of toasted soybeans (Qin et al., 1998). The PDI of 100°C toasted soybeans in our study showed a similar pattern to those toasted at 100°C for 5, 10, 20 and 40 min in the study by Qin et al. (1998). The PDI level of toasted soybeans in our study was on average 12.5% and 18.7% units lower than the 100°C toasted Argentine and Chinese soybeans in the study by Qin et al. (1998). After toasting at 118°C and 136°C, differences in PDI between the two studies and between the two varieties of Qin et al. (1998) diminished, especially with longer processing times.

This shows that genetic variation or differences in growing conditions between batches of soybeans of different origin may play a role in differences in PDI of soybeans, especially after mild pressure toasting.

The relatively low PDI of untreated lupins may be related to the high glutelin fraction in this seed (Hill, 1977), which is in agreement with the negative correlation between the proportion of glutelins in feeds and the N solubility in McDougalls' buffer (Kandylis and Nikokyris, 1997). Moreover, the high cell wall fraction in lupins probably limits dispersion of lupin protein.

Increasing the processing time or processing temperature at a PDI level of about 20% only slightly affected PDI. This was in agreement with the curvi-linear decrease for PDI observed by Hsu and Satter (1995), and with the results of Marsman et al. (1995). Hsu and Satter (1955) found, that PDI was hardly affected after increasing temperature during roasting over 140°C. Marsman et al. (1995) concluded that when high amounts of energy were dissipated during extrusion, PDI failed to discriminate between treatments. Hsu and Satter (1995) observed differences between roasting and oven-drying at similar sample temperature, which were due to differences in the speed of heat transfer. Therefore, pressure toasting, oven-drying and roasting may give different results.

The small increase of PDI after prolonged toasting of lupins and soybeans at 136°C was consistent with results of Van der Poel et al. (1990) after toasting Phaseolus beans at 136°C. These authors attributed the observed increase in PDI to protein hydrolysis, although no evidence was obtained for that in their study. In conclusion, PDI is a suitable indicator for protein denaturation after heat treatment, but discriminatory power is reduced when treatments become very severe.

DSC denaturation characteristics

Differential scanning calorimetry has been used to study the denaturation behaviour of proteins in food and feed, as well as in purified fractions of proteins (Hermansson, 1978). Results of DSC studies, however, can be influenced by many factors. When purified protein fractions are studied, the processing steps carried out during purification, such as defatting, extraction and precipitation for example, influence the denaturation behaviour of the protein (Murray et al., 1985).

In addition to variations in methodology, differences between species and environmental conditions during seed development can also affect the thermal behaviour of protein (and starch) in legume seeds (Sosulski et al., 1985), which makes it difficult to compare our experimental results with literature results. Despite this variation, protein fractions sometimes do exhibit a similar denaturation behaviour. Sousa et al. (1995), for instance, showed that isolated 11S fractions of lupins and soybeans had similar denaturation enthalpies and temperatures, while the isolated 7S fraction showed large differences for both parameters. In our study, however, denaturation enthalpy and T_p of lupins and soybeans protein fractions were different for both peaks.

For all legume seeds in our study, two endothermic peaks were observed after DSC. For the starch-containing seeds, faba beans and peas, the first peak was observed at 71.6°C and 68.6°C, respectively (Goelema et al., 1998°). In agreement with results of Murray et al. (1985), Van der Poel et al. (unpublished) and Wright and Boulter (1980), the first endothermic transition of faba beans and peas was attributed to gelatinization of starch, whereas the second was related to protein denaturation.

Since lupins and soybeans hardly contain any starch, the two observed peaks undoubtedly represent protein denaturation. Based on the homology between the protein fractions of legume seeds (Derbyshire et al., 1976; Gilroy et al., 1979), one would expect two peaks for faba beans and peas, one representing the 7S fraction, the other representing the 11S fraction. It remains unclear why faba beans and peas showed only one protein peak. It might be related to a smaller contribution of the 7S and 11S fraction to the total protein in faba beans and peas. That distribution was not measured in the present experiment, and literature gives variable results.

The T_p of the faba beans, peas and soybeans in our study were close to results of whole seeds in the study by Murray et al. (1985). Our results for T_p of two peaks for lupins were consistent with results of Wright and Boulter (1980), although these authors could

distinguish a third peak. This was probably caused by dialysis of the sample against a NaCl and NaH_2PO_4 solution at pH 8, which changes the thermal stability of protein fractions, resulting in different denaturation behaviour (Wright, 1982). Other DSC studies were carried out with protein fractions, purified by air-classification, defatting, and various kinds of extractions (see *e.g.* Arntfield and Murray, 1981; Sosulski et al., 1985; Sousa et al., 1995). In these studies, the different protein fractions usually exhibited distinct peaks, which often resulted in other values for T_p.

The peak temperatures for peas observed by Casey et al. (1982), at 94°C and 102°C, were within the relatively broad denaturation temperature interval (from onset until completion of denaturation) in our study. The broad denaturation interval in our study, as well as the similar δH_{tot} for peas, faba beans on the one hand, and of lupins and soybeans on the other, suggest that the phase transitions of the 7S and the 11S protein fractions of peas and faba beans overlapped.

In our study, denaturation enthalpies were considerable lower than those reported in studies with purified protein fractions (Arntfield and Murray, 1981; Sosulski et al., 1985), but were close to those of Murray et al. (1985) for whole faba beans, peas and soybeans and Thomas et al. (1998) for soygrits. Murray et al. (1985) showed that differences in denaturation enthalpy between whole seeds and their purified protein fractions are due to differences in sample preparation. It seems contradictory that (native) whole seeds had lower values for denaturation enthalpy than did the purified samples, but the contribution of interactions between protein and non-proteinaceous components in the seeds can result in lower enthalpies. Murray et al. (1985) hypothesized that factors in the seed hull other than phenolic components, were responsible for the increased enthalpy after dehulling, while the higher enthalpy after defatting (which decreased phenolic contents) was attributed to a smaller contribution of hydrophobic interactions and to an overall effect of phenolic compounds on protein conformation. As disruption of hydrophobic interactions is exothermic in nature, a decrease or the removal of this interaction results in an increased denaturation enthalpy. Lipids (abundant in lupins and soybeans) as well as starch can interact with protein (Kinsella, 1979), and this has very likely affected the denaturation enthalpy in our study. Anionic polysaccharides are known for their destabilization effect during heat treatment (Wright, 1982), resulting in a broadening of the transition peak and a decreased denaturation enthalpy. At a neutral pH, starch is negatively charged and may therefore form complexes with protein (Dahle, 1971), especially after heat treatment (Thorne et al., 1983; Michniewicz and Jankiewicz, 1988).

Since there are several indications that this has occurred after pressure toasting (Goelema et al., 1998[a,d]), this may be another explanation for the low denaturation enthalpy in our study.

For practically all seeds, δH_{tot} progressively declined after toasting at 118°C for 7 min. The results for lupins and soybeans suggest that at that point, the 7S fraction was completely denatured.

At the same stage, the 11S fractions of faba beans and peas started to denature, resulting in a decline of δH_{tot}, similar to lupins and soybeans. This progressive disappearance of the first and second peak for lupins and soybeans reflects the differences in heat-lability of 7S protein compared to 11S proteins (Derbyshire et al., 1976; Hermansson, 1979). After 15 min toasting at 136°C, a small amount of native protein was detected only in soybeans. It was therefore concluded that for all legume seeds, this treatment resulted in near complete denaturation of the protein fractions.

Relations between laboratory parameters and in situ parameters

Correlations between PDI and δH_{tot} were higher for faba beans and peas (r = 0.96 and 0.93, P ≤ 0.0001) than for lupins (r = 0.45, P ≤ 0.05) and soybeans (r = 0.63, P ≤ 0.01 and P ≤ 0.01). The smaller correlation coefficient for the latter seeds was mainly due to the worse correlations with δH_{tot} (r = 0.19 and r = 0.38, respectively). This is in agreement with the lack of discriminatory power of PDI after intensive heat treatment and higher energy inputs (Hsu and Satter, 1995; Marsman et al., 1995).

The high correlations between δH_1 and the W in lupins and soybeans and the poor, non-significant correlations for δH_2 are in agreement with Hermansson (1979), who showed that denaturation and solubility are not always correlated. Denatured proteins can be soluble, and denaturation of 11S soy proteins may lead to the formation of soluble fractions (Wolf and Tamura, 1969). According to these authors, the 11S soy protein fraction is transformed upon heating at 100°C, into a fast-sedimenting fraction and a slow-sedimenting fraction of 4S, which increases solubility (Kinsella, 1979). Prolonged heating increased the size of the soluble aggregate to 7S proteins, eventually resulting in precipitation. This increased solubility was in agreement with the effect of toasting on W. For these seeds, a small but consistent increase of W was observed after prolonged toasting at 118°C, while δH_{tot} gradually decreased. Although this segregation/aggregation phenomenon has not been reported for lupins, the data in our study suggests that this may also occur for those seeds.

The increased $X_{50,N}$ for toasted legume seeds coincided with a higher %UIP, which confirms the results of Dixon and Hosking (1992), Michalet-Doreau and Cerneau (1991) and Michalet-Doreau and Ould-Bah (1992) who also reported an inverse relationship between N degradability and particle size. These studies, however, were carried out with untreated protein sources which were ground through screens of different sizes. Few studies reported effects of other methods of processing on changes in particle size distribution and %UIP. In two earlier studies, a decreased particle size was observed after pelleting and expander treatment of a concentrate (Goelema et al., 1996) and a mixture of dry rolled legume seeds (Goelema et al., 1999). In the latter study, a significant inverse relationship was found between particle size and W, k_d and %UIP. In the latter study, however, no effects on particle size were observed after pressure toasting. This was probably due to the use of samples that were not ground: %UIP of the untoasted mixture was 65% and toasting for 3 min at 132°C increased %UIP by only 13% units.

Hence, effects of heat treatments on in situ degradability also depend on the particle size distribution of the incubated samples.

The larger particles after grinding the toasted legume seeds may be the result of the formation of complexes of starch and protein, as suggested by the strong correlation of in situ protein and starch degradability (Goelema et al., 1998[a,c,d]) and by DSC, as described above. It seems that these complexes cause a different fracture behaviour, resulting in an increased $X_{50,N}$

Toasting decreased IVPD for peas and lupins, but for faba beans and soybeans, there was no consistent effect of toasting. The reason for these differences remains unclear. The similarities between faba beans and peas on the one hand, and between lupins and soybeans on the other, as observed for most degradation parameters and the denaturation parameters are inconsistent with the results for IVPD.

In the study by Van der Poel et al. (1992), IVPD of high tannin faba beans was 90.9% which is higher than in our study, but consistent with the results of Meijer et al. (1994), who reported an IVPD of 92%. Our value of 83%, however is in agreement with results of Carbonaro et al. (1996) which ranged from 83 to 86%. These authors, however, used a different multi-enzyme in vitro procedure.

The very similar %TDP (Goelema et al., 1998[b]) for untreated and treated legume seeds, as well as the inconsistent pattern for IVPD of faba beans and soybeans resulted in poor overall correlations with IVPD. For lupins, the significant positive correlation between IVPD and %TDP shows that IVPD is a useful predictor for effects of pressure toasting on %TDP

in this case.

For soybeans, %DUP significantly correlated with IVPD, but the correlation coefficient was too low (r = 0.43) to be useful for prediction purposes. For peas, the correlation was negative (r = -0.83), which means that the clear increase of %DUP could not be mimicked by in vitro incubation.

In general, IVPD was not a good predictor for the effects of pressure toasting on in situ protein digestibility. Moreover, the differences in nylon bag digestibility (%TDP and %DUP) of the untreated legume seeds were not in agreement with results for IVPD. These results are not consistent with results of Antoniewicz et al. (1992) who found significant correlations (R^2 = 0.90, P ≤ 0.0001) between %DUP and in vitro protein digestibility with pepsin and pancreatin. These authors, however, incubated the 12 h rumen incubation residue in vitro and filtered the undigested residue after incubation over a nylon cloth to determine protein digestibility, instead of using the total undigested residue of the incubated feed samples as in our study.

Apart from the fact that the in situ disappearance probably represents true digestibility rather than apparent protein digestibility, the method was designed to study differences between diets and feedstuffs (Babinszky et al., 1990). Effects of heat treatments are mainly due to the denaturation of protein, which involves very specific changes of the protein structure. Changes in digestibility due to heat treatment are therefore not necessarily related to changes in IVPD but to differences in other factors, such as anti-nutritional factors (Jansman et al., 1993). Our results are in agreement with results of Van der Poel et al. (1991) who found poor correlations in pigs between IVPD and faecal digestibility or mobile nylon bag digestibility of beans (Phaseolus vulgaris) after pressure toasting.

Prediction of intestinal digestible rumen undegraded protein (DUP)

For PDI, simple linear regression equations fitted the relationships with DUP for faba beans, peas and soybeans, but for lupins, it failed to discriminate between higher levels of DUP. Generally, residual errors were larger at PDI < 25%, since PDI could not discriminate the intensity of heat treatment below this level. Moreover, PDI could not mark differences between high levels of DUP, which is consistent with results of Hsu and Satter (1995). Hsu and Satter (1995) found optimal protection against rumen fermentation after roasting soybeans corresponding to PDI values ranging from 9 to 11%, which is close to our results.

Using linear relations, changes of δH_{tot} could discriminate between different DUP very

well, except for soybeans. At higher levels of DUP, δH_{tot} clearly marked the differences, where PDI failed. Overall, the small prediction errors indicated that PDI and δH_{tot} are useful predictors for DUP. Moreover, decomposition of the MPE showed, that the slope of the regression lines was not significantly different from 1, and that the mean predicted and observed values were very similar.

The discrepancy between the δH_{tot} in our study and results of purified protein fractions, as well as the large variations in δH_{tot} due to factors related to the actual analysis, stress the need for standard analytical procedures. Moreover, it shows that in studies on the relationship between degradability and δH_{tot}, DSC analysis should be performed with whole seeds, rather than with purified protein fractions.

Conclusions
Protein denaturation enthalpy (δH_{tot}), as measured by DSC, as well as PDI, are both useful indicators of protein denaturation. Changes of these parameters after heat treatment were strongly related to the observed effects of pressure toasting on DUP, although PDI was not discriminatory between higher levels of DUP. It was concluded that PDI and δH_{tot} are accurate predictors for DUP after toasting, although its relations were different amongst legume seeds.

The IVPD, however, failed to discriminate between in situ digestibility of untreated and toasted legume seeds. It was only suitable for the evaluation of effects of heat treatment on total tract protein digestibility of lupins.

Acknowledgements

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CHAPTER 5

Effect of various conditions during pressure toasting on rumen degradability and intestinal digestibility of faba beans, peas, lupins and soybeans

3. IN SITU DEGRADABILITY AND DIGESTIBILITY OF AMINO ACIDS IN LUPINS AS CORRECTED FOR MICROBIAL CONTAMINATION

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Effect of Various Conditions during Pressure Toasting on Rumen Degradability and Intestinal Digestibility of Faba Beans, Peas, Lupins and Soybeans

3. In situ degradability and digestibility of amino acids in lupins as corrected for microbial contamination

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Abstract

In this experiment, whole lupins were toasted at 100°C (7, 15 or 30 min), 118°C (3, 7, 15 or 30 min) and at 136°C (3, 7 or 15 min). Ground samples were incubated in the rumen and intestines of dairy cows to measure *in situ* the fraction of rumen undegraded intake amino acids (UIAA) and the intestinal digestible rumen undegraded amino acids (DUAA). Diaminopimelic acid (DAPA) was used to correct for microbial contamination of feedstuffs and incubation residues.

Toasting significantly decreased rumen degradability of amino acid N (AAN) by decreasing its washable fraction and its rate of degradation. Toasting for 15 min at 136°C slightly decreased the total tract digestibility of AA, but intestinal digestibility of rumen undegraded intake AA was increased after toasting. As a result, the amount of intestinal digestible rumen undegraded intake AA substantially increased. For most amino acids, toasting for 30 min at 118°C resulted in a maximal or similar response to toasting at 136°C. Toasting for 30 min at 118°C increased %UIAA of total AAN from 14.3 to 42.2%. For lysine and methionine, %UIAA increased from 9.3 to 36.1% and from 0.9 to 27.7%, respectively. Effects of processing time and temperature showed a curvi-linear response for rumen degradability.

Since processing conditions affected degradability of individual AA in a similar way, it was concluded that toasting predominantly resulted in denaturation of protein, rather than altering protein degradability of some specific AA preferentially, for instance through Maillard reactions.

The use of DAPA as a microbial marker revealed contamination of some dried toasted lupins and in *in situ* residues. The microbial contamination showed a positive relation with rumen incubation time. Based on the increased contamination in oven dried residues after washing, and in oven dried rumen incubation residues, compared to freeze dried residues, it was concluded that freeze-drying could overcome a substantial part of the contamination.

Correction for microbial contamination resulted in a higher AAN degradability compared to uncorrected N degradability. Moreover, correction affected the ranking of treatments with respect to degradability of individual amino acids.

Key words: Lupins; Pressure toasting; Amino acid degradability, Diaminopimelic acid

Introduction

In ruminant nutrition, heat treatments are often used to increase the supply of intestinal absorbable amino acids (Satter, 1986). Increased amounts of rumen undegraded intake protein in the diets, however, not always lead to an improved performance, which may be related to an imbalance between required and available amino acids (Ashes et al., 1984). Individual amino acids vary in degradability and digestion (Weisbjerg et al., 1996; Dakowski et al., 1996) and also exhibit a different sensitivity for heat treatments (Dakowski et al, 1996) which can lead to changes in the amino acid profile after rumen fermentation (Crooker et al., 1986). Since degradation of AAN can differ from that of total N (Dakowski et al., 1996), evaluation of heat treatments should be based on their potential to increase supply of intestinal absorbable amino acids.

Previously, it was shown that pressure toasting is an effective method to protect lupin protein from rumen degradation, without negatively affecting the intestinal protein digestibility (Goelema et al., 1998[a]).

In situ incubation with nylon bags are frequently used to measure ruminal degradation and intestinal disappearance of rumen undegraded amino acids (Erasmus et al., 1994; Weisbjerg et al., 1996). However, microbial contamination of nylon bag residues can be substantial, especially for low protein roughages (Varvikko and Lindberg, 1985). Several markers such as purines, DAPA and ^{15}N have been used to quantify microbial contamination and to correct feed samples, digesta and incubation residues for this (Broderick and Merchen, 1992).

In this experiment, the effects of processing conditions during pressure toasting on the in situ rumen degradability and intestinal digestibility of individual amino acids in lupins were studied, taking into account microbial contamination during incubation.

Materials and methods

Samples and treatments

Lupins (*Lupinus angustifolius*) were pressure toasted at 100°C for 7, 15 or 30 min; at 118°C for 3, 7, 15 or 30 min, or at 136°C for 3, 7 or 15 min, as described by Goelema et al. (1998[b]). For this study, only samples treated on the first day were used. After toasting, samples were dried in a forced-air oven for 16 h at 35°C. Untreated samples of the feedstuffs were used as controls.

In situ incubations
Ruminal and intestinal incubations were carried out as described by Goelema et al. (1998[b]). Samples were incubated for 0, 8, 12 and 24 h in the rumen. For measuring the intestinal digestibility, a 12 h rumen incubation residue was used. Rumen incubation residues were dried in a forced-air oven for 24 h at 60°C. The residue after 12 h rumen incubation which was used for intestinal digestion, as well as its residue after intestinal digestion, were freeze dried.

Chemical analysis
Feeds and incubation residues were analyzed for dry matter (DM), N and amino acids (AA). Prior to chemical analysis, samples were ground through a 0.5 mm screen (Retsch centrifugal mill). DM and N were determined as described by Goelema et al. (1998[b]). For amino acid (AA) analysis, samples were hydrolyzed for 22 h with 6 N HCL at 108°C (under reflux). Feed AA and DAPA were determined with an amino acid analysator (Biotronik LC 5001, Eppendorf-Biotronik, Maintal, Germany). For methionine and cystine samples were oxidized for 16 h at 0°C with performic acid (Moore, 1963) prior to acid hydrolyzis.

Calculation of degradability and digestibility
The AA content of the feed and the residues after ruminal and intestinal incubation were corrected for microbial contamination using DAPA as microbial marker. The amino acid profile of rumen microbes (Storm and Ørskov, 1983) was used to correct the individual residues according to equation 1, as described by Varvikko (1986).

AA feed origin   = sample AA - (sample DAPA * microbial AA)/ microbial DAPA   (1)

where, sample AA and DAPA are the AA and the DAPA content (g/kg DM) of the sample, and microbial AA and DAPA are the AA and DAPA content of rumen microbes (Storm and Ørskov, 1983).

For AA, two fractions were distinguished: the washable fraction (W) which is assumed to be readily available, a truly undegradable fraction (U), and a potentially degradable fraction (D) (D= 1 - W - U). Since lupins were nearly completely rumen degradable, it was

assumed that U = 0. The fractional rate of degradation of the D fraction (k_d, in %/h) was calculated using a first order degradation model, as described by Robinson et al. (1986).

%UIAA $= D * (k_p / (k_p + k_d)$ (2)

UIAA $=$ %UIAA * feed AA content   (3)

The amount of rumen undegraded intake amino acids (UIAA) was calculated with equations 2 and 3, for an outflow rate (k_p) of 6%/h. UIAA and AA content are expressed in g amino acid N/100 g feed AAN. The AA residue remaining after intestinal incubation (IAA) was used to calculate total tract digestibility (%TDAA) as a fraction of original feed AA content, and as a fraction of UIAA (%DUAA), as well as the amount of intestinal digestible amino acids (DUAA, in g AAN/100 g feed AAN), using equations 4 to 6 (Tamminga et al., 1994). Results for N were calculated in the same way as described for AA, without correcting for microbial contamination.

%TDAA $= 100 * (AA - IAA) / AA$ (4)

%DUAA $= 100 * (UIAA - IAA) / UIAA$ (5)

DUAA $= UIAA *$ %DUAA   (6)

Statistical analysis

To estimate the effects of the treatment conditions, regression analysis was carried out with proc GLM (SAS, 1989) using equation 7, omitting the untreated controls from the dataset. For regression, processing temperature (T) was linearly transformed into increments from 100°C (*i.e.* 0, 18 or 36).

$$Y_i = a + AA_i + ß_1 X_1 + ß_2 X_2 + ß_3 X_1^2 + ß_4 X_2^2 + ß_5 AA X_1 + ß_6 AA X_2 + ß_7 AA X_1 X_2 + ß_8 X_1 X_2 + \epsilon_i \quad (7)$$

where, Y_i is the dependent variable under examination, *a* the intercept, AA_i the amino acid effect (i = 1-17), X_1 the temperature (in min), X_2 the duration of the treatment (in min), $ß_1$ the linear effect of temperature, $ß_2$ the linear effect of processing time, $ß_3$ the quadratic effect of temperature, $ß_4$ the quadratic effect of processing time, $ß_5$ the interaction effect of AA and X_1, $ß_6$ the interaction effect of AA and X_2, $ß_7$ the interaction effect of AA, X_1 and X_2, $ß_8$ the interaction effect of X_1 and ϵ_i the residual error term.

When regression analysis (equation 7) revealed significant interactions with AA, regression was carried out for the individual AA, omitting the AA effect and the interaction effects with AA. If possible, non-significant interaction terms and quadratic effects (P > 0.15) were also removed from the model. Contrasts were used to evaluate the differences between the results for total corrected amino acid N (AAN) and uncorrected N, and between total essential (EAAN) and non-essential amino acid N (NEAAN). To evaluate differences between untreated and toasted samples, analysis of variance (proc GLM, SAS, 1989) was used, taking into account the differences between individual AA (equation 8).

$$Y_{ij} \qquad = \mu + AA_i + T_j + \epsilon_{ij}, \qquad\qquad (8)$$

where, Y_{ij} is the dependent variable under examination, μ is the intercept, AA_i is the amino acid effect (i = 1 - 17), T_j the treatment effect (j = 0 for untreated and 1 for toasted samples) and ϵ_{ij} is the residual error term.

Results

Amino acid profile and microbial contamination
The amino acid and N contents of the untreated and toasted lupins are given in Table 1. N content and amino acid N (AAN) as fraction of total N were not significantly affected by toasting. The individual amino acids showed very little variation, except for Lys, His and Glu. Lys content decreased after toasting at 118°C and 136°C, which resulted in a significant interaction between processing time and temperature, and a tendency (P ≤ 0.10) to a quadratic temperature effect (results not shown). Concomitantly, His and Glu slightly increased with increasing processing time after toasting at 136°C (not significant). Consequently, total essential AAN (EAAN) decreased, while non-essential AAN (NEAAN) increased. The latter parameters showed a tendency to a quadratic effect for time, and linear effects for time and temperature during processing (P ≤ 0.10, results not shown).

Table 2 gives the AAN content (after correction for microbial contamination) of total N and the microbial contribution to the AAN content in lupins and their incubation residues. The AAN was 96% of total N in untreated lupins, and ranged from 86.7% to 96.8% in toasted lupins. Lower values for %AAN (86.7, 89.3 and 90.4%, respectively) were found for feed samples that contained some microbial contamination (3.4, 1.7 and 5.5% of total AAN in lupins, toasted for 30 min at 118°C and for 3 and 15 min at 136°C, respectively).

Microbial AAN content significantly increased with rumen incubation times until 24 h (Table 2). Concomitantly, %AAN showed an inverse relation with rumen incubation time. The freeze dried 12 h rumen incubation residue had a larger microbial AAN content and a smaller %AAN compared to the unincubated feed. Compared to the oven dried 12 h rumen incubation residue, the freeze dried residue had a smaller microbial AAN content.

The residue after mobile nylon bag incubation had a smaller %AAN and microbial AAN content than the freeze dried residue after 12 h rumen incubation (Table 2). There was a tendency to a higher microbial AAN content for the untreated lupins (P ≤ 0.10) compared to toasted lupins, which was significant for the essential AA (EAAN) (P ≤ 0.05). Differences in %AAN or microbial AAN content among toasted lupins were not significant.

Table 1 Effect of pressure toasting[1] on amino acid profile of lupins (in g AAN/100 g total AAN).

Temperature	U	100°C			118°C				136°C			SEM
Duration (min)		7	15	30	3	7	15	30	3	7	15	
Essential amino acids												
Arg	25.1	25.2	25.2	25.3	25.3	25.2	25.2	25.6	25.3	25.0	25.2	0.05
Cys	1.0	1.0	1.0	1.0	1.0	1.0	0.9	0.9	0.9	1.0	0.9	0.02
His	5.4	5.4	5.4	5.4	5.5	5.4	5.5	5.7	5.7	5.4	6.1	0.07
Ile	3.4	3.5	3.5	3.5	3.5	3.5	3.5	3.4	3.5	3.5	3.5	0.01
Leu	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.4	5.5	5.5	0.01
Lys	6.6	6.6	6.6	6.6	6.6	6.6	6.5	6.2	6.4	6.4	5.6	0.09
Met	0.5	0.5	0.5	0.5	0.4	0.5	0.4	0.4	0.4	0.5	0.4	0.02
Phe	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.4	2.5	2.4	2.4	0.01
Thr	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.2	3.1	0.01
Tyr	2.2	2.2	2.1	2.1	2.2	2.1	2.1	2.1	2.1	2.1	2.0	0.02
Val	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	0.00
Non-essential amino acids												
Ala	4.0	4.0	4.0	4.0	4.0	4.0	4.0	3.9	3.9	4.1	3.8	0.02
Asp	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.8	7.9	7.9	8.0	0.02
Glu	14.6	14.6	14.6	14.6	14.7	14.7	14.7	14.8	14.7	14.8	15.2	0.05
Gly	5.9	5.9	5.9	5.8	5.8	5.8	5.9	5.8	5.9	5.9	5.9	0.02
Pro	3.4	3.3	3.3	3.4	3.3	3.4	3.4	3.4	3.2	3.3	3.1	0.03
Ser	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.4	5.3	5.4	5.5	0.02
AAN[1] (% of N)	96.0	92.9	95.3	96.1	95.2	96.8	95.9	90.4	89.3	96.8	86.7	0.10
EAAN (% of AAN)	58.9	59.1	59.0	59.0	59.1	59.0	58.9	58.9	59.0	58.6	58.5	0.06
NEAAN (% of AAN)	41.1	40.9	41.0	41.0	40.9	41.0	41.1	41.1	41.0	41.4	41.5	0.06
N (g/kg DM)	51.9	52.9	52.5	52.8	52.9	51.5	52.4	52.3	53.0	53.0	53.0	0.15

[1] U=untreated; AAN = amino acid N; EAAN = essential AAN; NEAAN = non-essential AAN; N = N content not corrected for microbial contamination; SEM = standard error of the mean.

Table 2 Amino acid N (in g/100 g N) and microbial contamination (in g microbial AAN/100 g AAN) in feed samples and residues after ruminal and intestinal incubation of lupins.

Treatment temperature	U[1]	100°C			118°C				136°C			SEM
Duration (min)		7	15	30	3	7	15	30	3	7	15	
				g AAN per 100 g N								
Oven dried												
Feed	96.0	92.9	95.3	96.1	95.2	96.8	95.9	90.4	89.3	96.8	86.7	1.04
Residue after washing	88.9	91.3	83.6	90.5	81.9	76.3	77.6	89.5	86.5	86.8	83.2	1.53
Rumen incubation, 8 h	55.0	66.8	67.2	64.1	66.9	67.1	70.6	73.1	73.2	64.6	67.3	1.50
Rumen incubation, 12 h	55.7	60.4	61.4	69.8	60.3	66.2	66.9	71.0	64.6	61.3	69.5	1.23
Rumen incubation, 24 h	33.5	56.1	65.8	66.7	63.9	68.6	68.3	72.1	74.4	73.3	75.0	3.58
Freeze dried												
Rumen incubation, 12 h[2]	85.1	92.2	90.8	90.3	92.1	91.3	88.6	84.4	83.0	82.5	82.3	1.23
Mobile bag residue	51.8	53.3	53.4	52.3	51.5	51.1	55.9	58.4	50.9	52.9	57.2	0.77
				g microbial AAN per 100 g AAN								
Oven dried												
Feed	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.4	1.7	0.0	5.5	0.56
Residue after washing	7.2	0.0	0.0	1.1	0.0	0.0	1.5	7.3	9.6	9.6	9.6	1.33
Rumen incubation, 8 h	43.1	25.9	27.7	25.7	23.8	21.9	22.1	22.0	23.8	35.6	34.5	2.09
Rumen incubation, 12 h	40.3	27.1	29.8	19.1	32.9	28.4	25.9	25.2	35.2	34.9	33.6	1.78
Rumen incubation, 24 h	59.8	30.9	25.3	24.3	26.1	21.7	25.3	23.8	22.2	22.3	26.1	3.27
Freeze dried												
Rumen incubation, 12 h[2]	9.6	5.2	5.4	5.6	5.7	6.3	9.8	8.3	9.7	12.0	10.0	0.72
Mobile bag residue	32.2	30.2	33.3	32.3	31.3	28.4	26.6	23.3	27.7	28.2	22.5	1.08

[1] For abbreviations see Table 1.
[2] Freeze dried residue after 12 h rumen incubation, used for mobile bag incubation.

In situ degradability and digestibility

All digestion characteristics (W, %UIAA, %TDAA, %DUAA, and DUAA) were significantly affected by toasting. Differences between total AAN and N were significant, while %DUAA tended to a difference (P ≤ 0.15). The differences between total EAAN and NEAAN were only significant for DUAA (results not shown). Analysis (equation 7) showed that there was a significant interaction between AA and the temperature effect of toasting for all digestion characteristics, except for the k_d. Results were therefore presented for the individual amino acids.

Table 3 gives the washable fraction of AA. W was significantly (P ≤ 0.05) different between AA, but effects of toasting were not always significant. For most amino acids, there was a quadratic effect of duration of the treatment. In addition, there was an interaction between linear effects of temperature and duration on W. Table 4 gives the fractional degradation rate of AA. The k_d was significantly different between AA. Especially in untreated lupins, Met had a much higher k_d compared to the other AA. Toasting decreased the k_d of AA. Total AAN, EAAN and NEAAN tended to quadratic effects and showed significant linear effects of T and D, which indicates a curvi-linear response to the processing conditions. For EAAN, there was a tendency for interaction between T and D. For the individual amino acids, effects of the processing conditions were sometimes different.

Table 3 Effect of pressure toasting on the washable fraction of amino acid N in lupins (W, in %)[1].

Treatment	U[1]	100°C			118°C				136°C			SEM	T[2]		D		T × D
Treatment time		7	15	30	3	7	15	30	3	7	15		L	Q	L	Q	
Essential amino acids																	
Arg	50.1	46.2	52.8	48.7	46.0	54.6	46.9	31.2	35.6	40.0	35.0	2.33	NS	+	+	*	*
Cys	39.3	37.2	39.4	38.5	28.8	38.2	35.3	22.0	24.3	36.1	32.8	1.86	NS	-	*	*	NS
His	47.7	43.9	49.1	45.8	42.3	52.3	45.2	30.9	34.3	36.1	31.7	2.22	+	*	*	*	*
Ile	50.8	42.2	46.3	44.4	39.4	47.8	43.5	28.8	33.2	39.0	29.8	2.19	NS	*	*	*	*
Leu	49.8	42.6	45.9	44.2	38.4	45.4	41.2	25.5	29.6	35.5	29.1	2.40	NS	NS	*	*	*
Lys	57.5	43.0	46.7	46.4	40.7	47.8	43.4	28.9	39.5	44.2	33.8	2.26	NS	NS	+	*	*
Met	68.0	44.9	39.4	46.0	27.3	37.3	33.1	32.2	40.0	48.5	38.6	3.26	+	*	NS	-	NS
Phe	50.8	42.7	45.7	43.9	38.1	47.2	40.4	25.3	31.3	34.6	27.9	2.49	NS	+	NS	*	+
Thr	55.0	44.2	45.1	45.1	38.8	45.4	40.8	26.9	32.1	38.4	31.0	2.25	NS	-	NS	+	+
Tyr	55.2	47.8	51.2	48.2	44.0	54.6	45.5	30.6	36.8	39.3	32.6	2.54	NS	NS	+	+	+
Val	52.2	41.9	44.1	44.2	38.5	46.3	42.5	28.7	33.7	38.9	29.1	2.18	NS	NS	+	+	*
Non-essential amino acids																	
Ala	55.5	41.5	44.4	44.8	38.2	44.8	42.3	27.5	35.4	41.9	31.6	2.26	NS	-	+	+	*
Asp	43.3	38.2	45.2	44.3	42.1	47.0	42.9	27.5	32.4	38.0	32.3	1.89	+	*	*	*	*
Glu	52.3	47.3	52.6	48.6	45.4	53.2	46.2	30.0	33.4	38.7	35.3	2.48	NS	NS	+	+	+
Gly	53.0	44.8	47.9	46.5	41.3	48.4	43.7	28.6	34.3	39.8	33.7	2.22	NS	NS	*	*	*
Pro	48.2	40.0	45.0	43.4	34.1	48.5	36.6	23.6	18.3	26.4	24.3	3.24	NS	NS	NS	-	-
Ser	43.3	39.5	45.3	44.4	39.3	45.4	39.9	24.3	28.8	34.6	30.6	2.18	NS	NS	+	*	*
AAN	50.6	43.9	48.7	46.4	42.1	50.0	43.9	28.8	33.5	38.5	32.7	2.25	NS	+	+	*	*
EAAN	51.3	44.4	49.2	46.7	42.3	50.7	44.4	29.5	34.6	39.2	32.7	2.24	NS	+	*	*	*
NEAAN	49.5	43.0	48.1	46.1	41.8	49.1	43.2	27.8	31.8	37.5	32.7	2.27	NS	NS	+	*	*
N	46.6	42.9	41.6	43.1	32.7	36.7	30.7	28.1	31.3	31.5	29.9	1.96	*	*	NS	-	NS

1 For abbreviations see Table 1.
2 Interaction and linear (L) and quadratic (Q) effects of temperature (T) and duration (D) of the treatment. Significance levels: NS = P > 0.15; + = P < 0.15; * = P < 0.05; ** = P < 0.01; *** = P < 0.001. - = excluded from the model.

Table 4 Effect of pressure toasting on the fractional rate of degradation of amino acid N in lupins (k_d in % h^{-1}).

Treatment	U[1]	100°C			118°C				136°C			SE	T		D		T ×
		7	15	30	3	7	15	30	3	7	15		L	Q	L	Q	
Essential amino acids																	
Arg	14.4	9.1	4.9	5.7	6.9	4.2	3.6	4.3	4.6	5.5	3.7	0.9	*	+	*	*	+
Cys	12.9	8.5	4.7	4.5	7.1	4.7	3.2	3.3	3.9	4.9	3.8	0.8	*	NS	*	+	+
His	13.4	8.4	4.0	4.4	6.2	3.5	3.1	3.8	4.1	5.0	2.9	0.9	*	NS	*	+	+
Ile	14.3	8.8	4.7	5.0	7.0	4.3	3.2	3.4	3.8	4.6	2.9	1.0	*	+	*	*	+
Leu	13.7	8.6	4.4	4.6	6.4	4.1	3.0	3.4	3.8	4.7	2.8	0.9	*	+	*	*	+
Lys	21.3	11.9	7.8	6.9	10.1	6.9	5.7	5.8	6.3	9.6	7.8	1.3	NS	-	+	-	-
Met	(216.9)	15.4	11.8	7.3	13.9	10.7	7.5	8.7	9.8	11.4	9.4	0.8	*	+	*	+	*
Phe	14.7	9.0	4.9	4.8	6.8	4.2	3.6	3.7	4.0	5.2	3.0	1.0	+	NS	*	+	NS
Thr	17.0	9.5	5.5	5.3	7.5	5.1	4.0	4.2	4.7	6.0	3.8	1.1	*	NS	*	+	NS
Tyr	16.6	9.6	5.4	5.3	7.3	4.2	3.8	3.9	4.2	5.4	2.9	1.1	+	NS	*	+	NS
Val	13.7	8.7	4.6	4.7	7.0	4.5	3.3	3.6	4.0	4.7	3.1	0.9	*	NS	*	*	+
Non-essential amino acids																	
Asp	13.5	9.2	5.3	5.2	6.8	4.5	3.3	3.7	4.1	5.0	3.2	0.9	*	+	*	*	+
Ser	13.0	8.8	4.5	4.7	6.2	4.1	3.1	3.7	3.9	4.9	3.1	0.9	*	+	*	*	+
Glu	14.4	8.9	4.7	5.5	6.9	4.3	3.5	4.1	4.7	5.6	3.7	0.9	*	+	*	*	+
Gly	15.7	9.8	5.6	5.6	7.6	5.1	4.1	4.5	5.0	6.2	4.1	1.0	*	+	*	+	+
Ala	19.2	11.0	6.7	6.2	9.1	6.1	5.0	4.9	5.7	7.5	5.0	1.2	*	-	*	-	-
Pro	13.8	8.8	4.6	4.8	6.7	3.6	3.6	3.8	4.5	5.7	3.4	0.9	+	+	+	+	NS
AAN	14.7	9.2	5.1	5.4	7.1	4.5	3.6	4.1	4.5	5.6	3.6	0.9	*	+	*	+	NS
EAAN	14.9	9.2	5.0	5.4	7.2	4.5	3.7	4.1	4.5	5.5	3.6	1.0	*	+	*	+	+
NEAAN	14.4	9.2	5.1	5.4	7.0	4.5	3.6	4.1	4.6	5.6	3.6	0.9	*	+	*	+	NS
N	9.9	6.3	3.2	3.3	5.1	3.6	2.8	2.6	3.1	3.5	2.5	0.6	*	-	*	+	NS

[1] For abbreviations see Table 1.

[2] Interaction and linear (L) and quadratic (Q) effects of temperature (T) and duration (D) of the treatment. For significance levels see Table 3.

Table 5 gives the rumen undegraded fraction (UIAA) of AAN. Toasting increased UIAA for all amino acids. For all amino acids, as well as for N, the effects of T and D on UIAA were linear. Since there were no interactions between AA and T or D, differences between toasting conditions were similar among AA. The UIAA of untreated lupins ranged from 0.9% (Met) and 9.3% (Lys) to 17.9% (Ser) and 19.3% (Cys). For toasted lupins, UIAA ranged from 23.9% (Met) and 28.7% (Lys) to 48.3% (Phe) and 48.4% (Pro). The UIAA of AAN was smaller compared to N ($P \leq 0.0001$), due to a higher W and k_d of AAN. Met and Lys were more degradable ($P < 0.05$) than AAN and N. The relative increase in %UIAA after toasting was larger for total AAN compared to N.

Correction for microbial contamination resulted in significantly lower total AAN degradability (ranging from 3 to 9% units (results not shown). Relatively, differences in rumen undegraded AAN fraction ranged from 15 to 28%, but there was no systematic pattern with respect to the treatments. For individual amino acids %UIAA was very much affected by the correction for contamination (ranging from 0 to 19% units). For Lys, correction ranged from 7 to 21% units, while for Met it ranged from 14 to 33% units. For untreated lupins, correction for microbial contamination decreased %UIAA of Met from 17.7% to 0.9%. Correction for contamination also affected the ranking of treatments based on %UIAA, especially for Lys and Met.

Table 6 gives the intestinal digestibility of the rumen undegraded intake amino acids (%DUAA). After toasting at 100°C, %DUAA increased while for the other temperatures it decreased with increasing processing time. The %DUAA showed significant interaction between processing temperature and time, as well as a tendency to a quadratic effect of toasting temperature.

The total tract digestibility of AA was high. Toasting caused a slight, but significant decrease of the total tract digestibility (results not shown). The largest decrease of %TDAA was found for Val (from 98.4% to 96%). For the other AA the decrease was usually less than approximately 2% units.

Table 5 Effect of pressure toasting on the fraction of rumen undegraded intake amino acid N (UIAA, in %) in lupins.

Treatment	U[1]	100°C			118°C				136°C			SEM	T[2]		D		T×D
		7	15	30	3	7	15	30	3	7	15		L	Q	L	Q	
Essential amino acids																	
Arg	14.7	21.4	26.0	26.4	25.1	26.6	33.3	40.2	36.4	31.3	40.2	2.39	***	-	**	-	-
Cys	19.3	26.0	33.9	35.0	32.7	34.8	42.2	50.6	45.8	35.3	41.3	2.65	*	-	*	-	-
His	16.1	23.3	30.5	31.1	28.3	30.0	36.0	42.1	38.8	34.8	46.1	2.55	***	-	**	-	-
Ile	14.6	23.4	30.2	30.3	28.1	30.4	37.0	45.2	40.8	34.5	47.2	2.87	***	-	**	-	-
Leu	15.3	23.7	31.3	31.5	29.7	32.4	39.3	47.5	42.9	36.0	48.2	2.99	***	-	***	-	-
Lys	9.3	19.1	23.2	24.9	22.0	24.3	28.9	36.1	29.6	21.5	28.7	2.07	*	-	*	-	-
Met	(0.9)	15.5	20.4	24.4	21.9	22.5	29.7	27.7	22.8	17.7	23.9	2.92	+	+	+	-	NS
Phe	14.3	23.0	29.9	31.0	29.0	31.2	37.1	46.1	41.1	35.0	48.3	2.98	***	-	**	-	-
Thr	11.7	21.7	28.7	29.0	27.2	29.5	35.7	42.9	37.9	30.9	42.1	2.73	**	-	***	-	-
Tyr	11.9	20.1	25.7	27.4	25.2	26.7	33.4	41.9	37.3	31.9	45.6	2.92	***	-	**	-	-
Val	14.6	23.7	31.7	31.3	28.4	30.8	37.2	44.6	39.8	34.1	46.8	2.78	**	-	**	-	-
Non-essential amino acids																	
Ala	10.6	20.7	26.2	27.0	24.5	27.3	31.6	39.7	33.1	25.9	37.2	2.41	**	-	**	-	-
Asp	17.5	24.4	29.1	29.7	27.1	30.4	36.8	44.8	40.3	33.8	44.3	2.56	**	-	**	-	-
Glu	14.0	21.2	26.5	26.8	25.4	27.3	34.0	41.4	37.3	31.6	40.1	2.50	***	-	**	-	-
Gly	13.0	21.0	27.0	27.6	26.0	28.0	33.6	40.7	35.8	29.7	39.4	2.43	**	-	**	-	-
Pro	15.7	24.4	31.0	31.5	31.1	32.3	39.5	46.7	46.9	37.8	48.4	3.04	***	-	*	-	-
Ser	17.9	24.6	31.3	31.1	29.9	32.5	39.7	47.0	43.1	35.9	45.9	2.72	*	-	**	-	-
AAN	14.3	22.1	27.8	28.3	26.5	28.5	34.9	42.2	37.9	31.9	41.9	2.54	**	-	**	-	-
EAAN	14.0	21.9	27.6	28.2	26.3	28.2	34.5	41.7	37.4	31.6	41.9	2.53	***	-	**	-	-
NEAAN	14.8	22.4	28.1	28.4	26.8	29.1	35.4	43.0	38.8	32.3	42.0	2.55	**	-	**	-	-
N	20.2	27.9	37.9	36.8	36.3	39.6	47.3	50.1	45.4	43.5	49.5	2.78	***	-	**	-	-

[1] For abbreviations see Table 1.

[2] Interaction and linear (L) and quadratic (Q) effects of temperature (T) and duration (D) of the treatment. For significance levels see Table 3.

Table 6 Effect of pressure toasting on the intestinal digestibility of amino acid N in lupins (in % of rumen undegraded AAN).

Treatment	U[1]	100°C			118°C				136°C			SEM	T[2]		D		T x D
		7	15	30	3	7	15	30	3	7	15		L	Q	L	Q	
Essential amino acids																	
Arg	98.4	98.7	98.8	98.9	98.9	98.8	98.7	98.2	99.2	98.6	98.0	0.09	*	+	+	-	**
Cys	88.4	91.8	92.5	93.5	93.5	93.9	93.7	92.6	94.6	92.7	92.0	0.44	**	**	*	-	**
His	87.3	91.9	91.9	93.3	92.8	93.2	93.3	92.3	94.6	92.3	91.7	0.50	*	+	+	-	*
Ile	93.0	94.6	94.8	95.6	95.1	95.4	94.8	93.3	96.3	94.1	93.1	0.29	+	-	NS	-	**
Leu	94.2	95.5	95.9	96.3	96.0	96.4	95.7	94.4	97.0	95.1	94.1	0.26	*	+	+	-	**
Lys	84.3	93.0	94.3	94.7	94.2	94.4	94.8	94.3	95.4	93.8	92.4	0.83	*	*	*	-	*
Met	-	94.0	98.7	96.7	96.6	96.2	95.9	92.5	95.5	92.1	90.9	0.70	NS	-	NS	-	+
Phe	93.4	95.2	94.8	95.7	95.4	95.8	95.1	93.7	96.5	94.3	92.9	0.31	NS	-	NS	-	*
Thr	90.6	94.2	95.9	96.0	95.4	95.7	95.3	94.2	96.3	94.4	93.6	0.44	*	+	+	-	**
Tyr	95.0	96.6	96.2	96.8	96.6	96.7	96.4	95.2	97.6	96.0	94.7	0.24	+	-	NS	-	*
Val	89.1	92.7	93.6	94.1	93.4	93.8	93.4	91.8	94.8	92.3	91.5	0.42	+	-	NS	-	*
Non-essential amino acids																	
Ala	91.5	94.8	95.0	95.9	95.1	95.7	94.8	93.4	96.0	93.4	92.2	0.41	*	+	+	-	**
As	95.5	96.1	95.0	96.5	95.8	96.0	95.7	94.5	96.8	95.0	96.2	0.19	NS	-	NS	-	-
Glu	97.8	98.5	98.7	98.9	98.7	98.6	98.5	97.9	99.0	98.3	97.6	0.13	*	+	+	-	**
Gly	92.1	94.8	95.4	95.9	95.5	95.8	95.4	94.4	96.5	94.7	93.9	0.33	*	+	+	-	**
Pro	92.2	95.2	95.3	95.8	95.7	95.7	95.7	94.6	96.7	95.0	93.2	0.35	NS	-	NS	-	*
Ser	91.3	93.3	92.0	93.9	93.5	93.9	93.8	93.0	95.0	93.1	95.0	0.30	+	-	NS	-	-
AAN	94.2	96.0	96.1	96.7	96.4	96.5	96.3	95.4	97.2	95.8	95.3	0.22	+	+	+	-	**
EAAN	93.8	95.9	96.2	96.6	96.4	96.5	96.3	95.5	97.2	95.8	95.0	0.25	*	+	+	-	**
NEAAN	94.6	96.1	95.9	96.7	96.4	96.5	96.3	95.4	97.2	95.7	95.6	0.19	+	-	NS	-	*
N	92.3	94.5	94.9	95.3	95.1	95.2	95.3	94.0	95.9	94.4	93.9	0.26	*	-	NS	-	*

[1] For abbreviations see Table 1.

[2] Interaction and linear (L) and quadratic (Q) effects of temperature (T) and duration (D) of the treatment. For significance levels see Table 3.

Discussion

Amino acid profile and microbial contamination
The amino acid profile of untreated lupins (Table 1) was in good agreement with
results of previous studies (Cros et al., 1992; Benchaar et al., 1994; Gdala et al., 1996;
Moss et al., 1997), and given the range of the AA content observed in the different
lupins species (Hill, 1977). These results confirmed the low contents of Met and Cys in
lupins.
Moss et al. (1997) reported that 90 to 95% of total N consisted of AAN, which is
consistent with our results. However, table values (CVB, 1998) and results of a
previous study with *Lupinus angustifolius* (Goelema et al., 1995), indicated that AAN
comprised only 85% of total N, which suggests substantial variation in AAN content of
lupins.

The decreased Lys concentration after pressure toasting at 136°C was consistent with
the results of others for several feedstuffs (Mostaghi Nia and Ingalls, 1995; Hsu and
Satter, 1995; Schroeder et al., 1996; Dakowski et al., 1997). For legume seeds, Cros
et al. (1992), Benchaar et al. (1994) and Aldrich et al. (1995) reported no effects of
heat treatments on amino acid profile. The decrease in Lys may be due to the
irreversible formation of isopeptides or indigestible Maillard products which are not
hydrolyzed during acid hydrolysis during amino acid analysis. Since the decreased Lys
content was not observed at lower temperature, Maillard reactions were either not
occurring, or limited to a reversible stage.

Based on their DAPA content, it was concluded that in 3 toasted samples a small
microbial contamination occurred (Table 2). Crooker et al. (1986) suggested that the
occurrence of DAPA in feedstuffs was due to other components, co-eluting with DAPA
during analysis. Since the untreated and most treated lupins did not contain any
DAPA, it appears that for the contaminated samples, the drying procedure after
toasting (drying in a forced-air oven for 16 h at 35°C) was too slow to prevent
microbial fermentation. A too slow drying process might also explain the occurrence of
DAPA in some (processed) feeds as reported in literature, such as in soybean meal
(Crooker et al., 1986; Prestløkken et al., unpublished results), dried brewers grains and
canola meal (Boila and Ingalls, 1992). After washing, oven-drying at 60°C apparently

takes to much time to reduce the moisture content of the washed nylon bags to prevent microbial activity. This seems to be in agreement with the difference between the contamination between the oven dried and the freeze dried residue after 12 h rumen incubation (40.3 % versus 9.8%). These results suggest that a substantial part of the microbial AAN results from the fermentation during the drying of the bags. If this is true, correction for contamination based on DAPA results in an overestimation of the degradability and digestibility. It is therefore concluded that freeze-drying is the preferable method to dry nylon bags after *in situ* incubation.

Microbial contamination relatively increased with rumen incubation time, which is consistent with other studies (Varvikko and Lindberg, 1985; Varvikko, 1986; Beckers et al., 1995), and was significantly higher in residues of untoasted samples than of toasted samples, confirming the results of Crooker et al. (1986). The higher contamination for the untreated samples suggests a better accessibility of protein in the untreated samples. Contamination decreases, after an initial increment, when the substrate for fermentation diminishes (Varvikko and Lindberg, 1985), which was consistent with the results for the more intensive heat treatment (118 and 136°C). With soybean meal, Beckers et al. (1995) observed an increased contamination up to 24 h of incubation, which was consistent with our results for the mildly treated (100°C) or untreated samples. These findings contradicted previous results and our hypothesis, that contamination was to be expected at an earlier stage with the most degradable substrates. It also may suggest that the lower rumen degradability of the feeds was related to an impaired attachment of microbes to the substrate. The lower microbial contamination of the residue after mobile nylon bag incubation compared to the 12 h rumen incubation was consistent with the decreased availability of substrate.
In a number of studies, results from nylon bag incubations have also been corrected for microbial contamination. Several microbial markers were used, such as purines, DAPA (Erasmus et al., 1994) and ^{15}N, either to label the feed (Varvikko and Lindberg, 1985) or the rumen microbes (Beckers et al., 1995). Varvikko and Lindberg (1985) found differences in microbial contamination due to type of feed (rapeseed, barley, barley straw, rye grass), pore/sample size ($20\mu m/1$ g versus 40 $\mu m/5$ g) of the nylon bags, incubation time (5, 12, 24 h) and microbial marker (^{15}N and DAPA) used. The use of ^{15}N labelled rapeseed resulted in slightly smaller contaminations compared to DAPA. With DAPA as microbial markers and oven-drying after washing the incubated

nylon bags, Varvikko and Lindberg (1985) found results similar to ours for lupins. Results of Crooker et al. (1986) for bacterial contamination of incubated soybean meal were lower than our results. With freeze dried residues and using ^{15}N labelled microbes as markers, Beckers et al. (1995) observed lower microbial contaminations compared to our results and the DAPA results of Varvikko and Lindberg (1985), but it was not always consistent with the results of Crooker et al. (1986). Thus, further research is necessary to quantify microbial contamination in feeds after incubation, and in its origin.

Labelling of feed with ^{15}N will overcome the problem of fermentation possibly occurring during the drying of the bags. On the other hand, rumen degradation might be underestimated, since N incorporated in the microbes which originates from the soluble and degraded labelled feed AAN are considered as undegraded.

The present study shows that contamination of incubation residues was substantial, which was consistent with results of studies in which other microbial markers were used. The drying procedure for the incubated nylon bags after washing may have great impact on the degree of contamination. It is concluded that differences due to contamination may be acceptable for evaluation of effects on total AAN, but are unacceptable when studying degradability and intestinal digestibility of individual amino acids. Results of corrections with a fixed DAPA/microbial AA ratio (Storm and Ørskov, 1983), as was used in the present study, may therefore have limited quantitative applicability, although it can be useful for qualitative comparisons.

Amino acid degradability and digestibility

The N degradability of the untreated lupins can vary greatly between studies, as was discussed by Goelema et al. (1998[a,b]). Between AA, also significant differences were found for W, k_d and, consequently, for %UIAA. Differences in solubility and degradability between the AA may be related to the AA profile of the different protein fractions (Derbyshire et al., 1976; Hill, 1977). AA profiles of feed were shown to be different in residues after washing, after rumen incubation and in the rumen undegraded AAN fraction. This was consistent with results of Susmel et al. (1989), Cros et al. (1992) and Benchaar et al. (1994), but in contrast to results of Ganev et al. (1979) and Weakley et al. (1983), who found no systematic differences in AA profile after rumen incubation. However, these authors did not correct for microbial

contamination. Taking into account contamination, differences in degradability between AA were also observed by Varvikko and Lindberg (1985), Varvikko (1986), and Beckers et al. (1995).

The Met content in lupins is very low (Hill, 1997). After 8 h of rumen incubation, no detectable Met was found, resulting in an k_d of 217%/h, and in a rumen undegraded fraction of 0.9%. Because of the very different k_d and UIAA compared to the other AA, results for Met were omitted from the calculation of the standard error. Met and Lys were the most degradable AA in our study, while Cys, Ser and Asp were most resistant to microbial fermentation. Without corrections for contamination Arg, Tyr and Glu had the highest degradability, while Cys, Ser and Asp were most resistant to fermentation.

In studies with *Lupinus albus*, Cros et al. (1992) observed the highest degradability for Arg and Glu, and the lowest for Met and Lys, whereas Benchaar et al. (1994) found highest degradabilities for Arg and Glu, while Val and Ala had the lowest degradability. No studies were reported on AA degradability of lupins where microbial contamination was taken into account. However, available data for lupin AA degradability suggests, that differences in AA between studies are also affected by the correction for microbial contamination.

Correction for microbial contamination affected the degradability of total AAN and of most individual AA. Especially for Lys and Met, the ranking of %UIAA changed after correction, which is related to the relatively low concentration of Met, the high degradability of Lys and Met compared to the other amino acids, and the decrease of Lys concentration in the feed after toasting at 136°C. The difference in ranking with correction could lead to misinterpretations regarding the effectivity of the heat treatment. The significantly higher rumen undegraded fraction of uncorrected N compared to total AAN showed that calculating UIAA based on the N degradability and the feed AA profile generally will result in an overestimation of the amount of intestinal available total AAN. However, for individual amino acids it may result in an underestimation of DUAA.

The 30 min treatment at 118°C resulted for most AA in the highest increase of digestible rumen undegraded AAN (results not shown), except for DUAA of His, Ile, Leu and Val, which were highest after the 136°C/15 min treatment. So, for most amino acids, toasting for 30 min at 118°C was the optimum treatment to increase DUAA.

Toasting significantly increased the fraction of rumen undegraded amino acids (Table 5). Since there were no interactions between AA and T or D, effects of toasting conditions among AA were similar. This suggests, that toasting has lead to an alteration of the overall protein structure, rather than modifying protein degradability of specific AA, such as Lys due to Maillard reactions (Cleale IV et al., 1987). This is consistent with the rather small effect on ADIN after toasting at 132°C (Goelema et al., 1998[a]), but inconsistent with the decreased Lys concentration after toasting at 118 and 136°C. Thus, it was concluded that effects on protein degradability were predominantly caused by denaturation, while at higher temperatures, Maillard reactions may have occurred as well. This was confirmed by the observed increased brown coloring of the samples and the decreased Lys content after toasting at 136°C

Masoero et al. (1994) reported higher intestinal digestibility for AAN compared to N. This was consistent with our results before correction for contamination. However, corrected AAN intestinal digestibility was significantly larger than uncorrected N digestibility. Total tract AA digestibility of canola meal decreased after autoclaving for 45 min, but was not reduced after treatment for 15 min (Mostaghi Nia and Ingalls, 1995). This is consistent with our results after prolonged toasting (> 15 min) at 118 and 136°C (Table 6).

Total tract digestibility was similar for uncorrected AAN and N (results not shown), but was significantly higher for corrected AAN compared to uncorrected N. Therefore, uncorrected N digestibility will underestimate the digestibility. On the other hand, the mobile nylon bag digestibility will overestimate the small intestinal digestibility, since it also includes digestion in the large intestine (Mostaghi Nia and Ingalls, 1995). Since autoclaving for 45 min at 127°C increased the AA digestion in the large intestine, this might also be an explanation for the increased total intestinal digestibility after toasting.

The increments of DUAA are much larger in our study than those found by Mostaghi Nia and Ingalls (1995) for canola meal. Toasting at 118° for 15 min was more effective in increasing DUAA than autoclaving for 15 min at 127°C canola meal. This may be related to the higher degradability of lupins compared to the canola meal (which has undergone an atmospheric toasting treatment after oil-extraction), as well as to the

difference in sensitivity to heat treatments between lupins and canola. This is consistent with the results we obtained after toasting of lupins and fullfat rapeseed in a previous study (Goelema et al., 1995).

Conclusions

Toasting significantly decreased rumen degradability of AAN by decreasing its W and k_d. The most intensive treatment slightly decreased the total tract digestibility of AA. As a result, the amount of intestinal digestible AA substantially increased. For most AA, toasting for 30 min at 118°C resulted in the highest increase of DUAA, or a result which was similar to treatment at 136°C.

Since processing conditions affected degradability of individual AA in a similar way, it was concluded that toasting predominantly resulted in denaturation of lupin protein, rather than altering protein degradability of some specific AA preferentially, for instance via Maillard reactions.

Using DAPA as a microbial marker revealed contamination of some dried toasted lupins, as well as contamination of in situ incubation residues. Based on the increased contamination in oven dried residues after washing and rumen incubation, and the lower contamination after freeze-drying of rumen incubation residues it was concluded that freeze-drying could overcome a substantial part of the contamination.

Correction for microbial contamination resulted in a higher AAN degradability compared to uncorrected N degradability, as well as in a other ranking of treatments with respect to degradability of individual amino acids. Neglecting the contamination therefore may result in misinterpretation of the effectivity of treatments.

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CHAPTER 6

Effect of various conditions during pressure toasting on rumen degradability and intestinal digestibility of faba beans, peas, lupins and soybeans

4. IN SITU DEGRADABILITY AND DIGESTIBILITY OF STARCH IN FABA BEANS AND PEAS

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Effect of Various Conditions during Pressure Toasting on Rumen Degradability and Intestinal Digestibility of Faba Beans, Peas, Lupins and Soybeans

4. In situ degradability and digestibility of starch in faba beans and peas

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Abstract

The effects of various combinations of processing time and temperature during (pressure) toasting on the digestive behaviour of starch of faba beans and peas were studied. Whole seeds were treated at 100, 118 or 136°C for 3, 7, 15 and 30 min. Untreated samples were used as controls. Ground samples were incubated in the rumen and intestines of dairy cows to measure in situ rumen undegraded intake starch (%UIS) and intestinal digestible rumen undegraded starch (DUS).

The treatments significantly increased %UIS, by decreasing the washable fraction (W) and the rate of degradation (k_d). Effects of processing conditions were not significant for total tract starch digestibility (%TDS) and intestinal digestibility of starch (%DUS), but values were higher after toasting. Values for %TDS and %DUS, as measured by mobile nylon bag incubations, were low compared to in vivo results from literature. The largest increase of %UIS and DUS was observed after toasting for 15 min at 136°C. Using standard procedure for calculating starch degradability, %UIS increased by 80 and 57% for faba beans and peas, respectively, while for DUS increases were 87 and 49%. The effects on %UIS were inconsistent with the increased degree of gelatinization after toasting, measured either in vitro or with differential scanning calorimetry (DSC). The latter results showed that toasting, especially at 136°C, resulted in a considerable gelatinization of starch.

Toasting changed the distribution of starch over different particle size classes, as was determined by wet and dry sieve analysis. The fraction of starch in the particles < 71 μm decreased after toasting, which was consistent with the large decrease of W. The filter solubility (FS) of starch was much smaller than W, and was only slightly decreased after toasting. The use of FS instead of W (the standard procedure) in the calculation of %UIS resulted in a drastic decrease of the effect of toasting on %UIS. Based on the strong correlation between the rumen undegraded protein (%UIP) and %UIS and the results of sieve analysis it was concluded that denaturation of the protein matrix as well as the change in the distribution of starch of the particles were responsible for the increase of %UIS and DUS.

Key words: Pressure toasting, Peas, Faba beans, Starch degradability, Digestibility, Gelatinization

Introduction

Several studies in literature have shown that heat treatments can be used successfully to increase the amount of rumen undegraded protein of various protein sources. Studies of effects of processing on starch degradability were mainly set up to increase rumen degradability of corn and sorghum starch (Theurer, 1986; Owens et al., 1986). Previously, we showed (Goelema et al., 1998[a]), that pressure toasting for 3 min at 132°C was a very effective method to reduce the in situ degradability of legume seed protein and starch in ruminants. For protein, these results were in agreement with those of other studies on legume seeds (Aguilera et al., 1992; Sommer et al., 1994; Singh et al., 1995). Most studies also showed that heat treatments, such as for instance extrusion, increased the in situ starch degradability (Walhain et al., 1992; Focant et al., 1990). Sommer et al. (1994), on the other hand, reported a decreased in vitro starch degradability of field peas after dry heat treatment at 90, 110 and 130°C and also after moist heat treatment at 90°C. However, moist heat treatment at 110 and 130°C increased starch degradability. Since a detailed description of the treatments and of the in vitro method was not given, it is difficult to relate these effects to other results from literature.

Goelema et al. (1998[a]) hypothesized that the in situ starch degradability decrease was related to the decrease of the protein degradability. Moreover, the specific conditions during pressure toasting (the low moisture level and the absence of mechanical damage during processing) may impose physical changes on the starch structure that result in a decrease of the starch degradability. Furthermore, it was unknown to what extent the processing temperature or time used in the previous experiment was important in explaining these effects.

In this study, the effects of temperature and residence time during pressure toasting of faba beans and peas on the in situ rumen degradability and intestinal digestibility of starch are evaluated. In previous papers, effects on the protein degradability and digestibility (Goelema et al., 1998[b]) and on related laboratory measurements for protein (Goelema et al., 1998[c]) are described.

Materials and methods

Samples and treatments

Faba beans (*Vicia faba* cv. Alfred) and peas (*Pisum sativum*) were heat processed in a laboratory scale pressurized toaster (Van der Poel et al., 1990). The seeds were toasted at 100°C for 7, 15 or 30 min; at 118°C for 3, 7,15 or 30 min, or at 136°C for 3, 7 or 15 min. Treatments were repeated on two consecutive days. After toasting, the samples were oven-dried at 35°C for 16 h. Untreated samples of the feedstuffs were used as controls.

Table 1 Chemical composition of untreated faba beans and peas.

	Faba beans	Peas
Dry matter (g/kg)	887.3	883.6
In dry matter (g/kg^{-1})		
Organic matter	959.8	966.5
Crude protein	312.1	249.8
Crude fat	11.4	11.8
Crude fibre	82.1	53.6
Starch	417.2	491.2
Neutral detergent fibre	119.9	97.8
Acid detergent fibre	116.7	71.9
Acid detergent lignin	13.9	7.6

In situ incubations

Rumen incubations were carried out with nylon bags (40 μm pore size) for 0 (blank), 2, 4, 8, 12, 24 and 48 h, according to Dutch standard methods (CVB, 1996). Before rumen incubation, samples were ground (3 mm screen, Retsch centrifugal mill). Four rumen-cannulated, lactating Holstein cross Friesian cows were used for rumen incubation. Two other lactating Holstein cross Friesian cows, fitted with a cannula in the proximal duodenum, were used to measure intestinal protein digestibility with the mobile nylon bag technique. More details on the procedures for rumen incubations and intestinal incubations have been described by Goelema et al. (1998[b]).

Physico-chemical analyses

Total starch and starch degree of gelatinization were determined enzymatically, as described by Goelema et al. (1998[a]). All other chemical analyses and solubility measurements were carried out as described by Goelema et al. (1998[b]).

Denaturation enthalpy (δH) of the samples was determined by DSC in duplicate, using a Mettler-Toledo DSC12-E, as described by Thomas et al. (1998). Onset (T_o), peak (T_p) and endset temperature (T_e) of the denaturation peaks were determined as described by Goelema et al. (1998[b]). The δH was calculated by integration of the peak surface (from T_o to T_e), using the Mettler system TA89E software package. The δH is a measure for the amount of native starch present in the sample. Thus, a difference in δH between toasted and untreated samples represents the degree of gelatinization to the heat treatment.

Particle size distribution of the 3 mm ground samples, also used for in situ incubations, was determined by wet and dry sieve analysis (Retsch AS 200), as described by Goelema et al. (1999). Only the untreated seeds and the seeds toasted for 15 min at 136°C were analyzed.
Sieve fractions were analyzed for DM and starch and were expressed as a fraction of the starch content of the sample. If sieve fractions were insufficient to analyse, samples were pooled with other fractions. The fraction < 71 μm after wet sieve analysis was calculated by subtraction. For wet sieve analysis, the filter solubility procedure (Weisbjerg et al., 1990) was used to divide the fraction < 71 μm into the ('soluble') fraction < 8 μm and the fraction of particles between 8 and 71 μm. The distribution of starch over the sieve fractions was used to calculate an apparent mean starch particle size ($X_{50,starch}$), using the logarithmic normal distribution as described by Waldo et al. (1971).

Calculation of degradability and digestibility

Starch was classified into two fractions: a readily available fraction (W), measured as the fraction disappearing after washing (0 h incubation) and a potentially degradable fraction (D = 100 - W). 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). Rumen undegraded intake starch (%UIS) was calculated for the Dutch standard outflow rate (k_p) of 6%/h (equations 1 and 2), according to Tamminga et

al. (1994). It was assumed that 10% of W of starch escapes rumen fermentation (Tamminga et al., 1994).

$$\%UIS \qquad = 0.1 * W + D * (k_p / (k_p + k_d) \qquad\qquad (1)$$

$$UIS \ (g/kg \ DM) \quad = \%UIS / 100 * starch \qquad\qquad (2)$$

The starch residue remaining after intestinal incubations (IS, in g/kg DM), was used to calculate total starch digestibility (%TDS) as a fraction of starch (in g/kg DM) and as a fraction of the undegraded intake starch (%DUS), using equations 3 trough 5 (Tamminga et al., 1994).

$$\%TDS \qquad = 100 * (starch - IS) / starch \qquad\qquad (3)$$

$$\%DUS \qquad = 100 * (UIS - IS) / UIS \qquad\qquad (4)$$

$$DUS \ (g/kg \ DM) \quad = UIS * \%DUS \qquad\qquad (5)$$

Statistical analysis

Analysis of variance was conducted using the General Linear Models (GLM) procedure of SAS (SAS, 1989) with equation 6. Treatment means were compared pairwise using Tukey's HSD-test ($P \leq 0.05$).

$$Y_{ij} \qquad = \mu + Treatment_i + D_j + \epsilon_{ij} \qquad\qquad (6)$$

where, Y_{ij} is the dependent variable under examination (W, k_d, %UIS, %TDS, %DUS, DUS), μ the overall mean, treatment$_i$ the treatment effect (i = 1 - 11), D_j the treatment day effect (j = 1, 2) and ϵ_{ij} the residual error term.

Linear correlations were analyzed with proc CORR (SAS, 1989). Regression analysis was carried out with the GLM procedure, using equation 7 (SAS, 1989), after omitting the untreated controls from the dataset. For regression, processing temperature was linearly transformed into increments from 100°C (*i.e.* 0, 18 and 36). If possible, models were reduced in complexity by excluding non-significant quadratic effects (P > 0.10).

$$Y_{ij} \qquad = a + D_i + ß_1 X_1 + ß_2 X_2 + ß_3 (X_1 \times X_2) + ß_4 X_1^{\ 1} + ß_5 X_2^{\ 2} + \epsilon_{ij}, \qquad (7)$$

where, Y_{ij} is the dependent variable under examination (W, k_d, %UIS, %TDS, %DUS, DUS), a the intercept, D_i the treatment day effect (i= 1, 2), X_1 the temperature increment (in °C), X_2 the residence time (in min), $ß_1$ the linear effect of temperature, $ß_2$ the linear effect of time, $ß_3$ the interaction of time and treatment, $ß_4$ the quadratic effect of temperature, $ß_5$ the quadratic effect of time and ϵ_{ij} the residual error term (j = 1-11).

Table 2 List of abbreviations used in the paper.

Abbreviation	parameter
CP	crude protein content (N x 6.25, g/kg DM)
D	potentially degradable fraction (%)
DM	dry matter content (g/kg)
DUS	intestinal digestible UIS, as % of UIS or in g/kg DM
DVE	intestinal absorbable protein (g/kg DM)
δH	DSC gelatinization enthalpy (J/kg starch)
FS	filter soluble fraction (%)
IS	indigestible starch (g/kg DM)
k_d	fractional degradation rate (%/h)
k_p	fractional outflow rate (%/h)
SEM	standard error of the mean
SGD	starch gelatinization degree (in %)
TDS	total digestibility of starch (in %)
T_e	DSC endset temperature (°C) of gelatinization
T_o	DSC onset temperature (°C) of gelatinization
T_p	DSC peak temperature (°C) of gelatinization
UIS	rumen undegraded intake starch, as % of starch content
W	washable fraction (%)
$X_{50,\ starch}$	apparent mean starch particle size (μm)

Results

Starch degradability, digestion and gelatinization
Table 2 gives an overview of the abbreviations used in the paper. Toasting decreased the W of starch of faba beans (Table 3) and peas (Table 4), especially at higher toasting temperatures. This effect was largely responsible for the increase of %UIS, although for

faba beans, the lower k_d was also associated with the increased %UIS.

For faba beans and peas, the processing temperature linearly decreased W, while time was quadratically related to W. For the k_d of starch in faba beans, the effect of temperature and time during processing was linear, while for starch in peas a quadratic relation with temperature was found.

The %TDS and %DUS were not significantly affected by different conditions during toasting, but %TDS and %DUS were higher after toasting.

DUS significantly increased after processing. For faba beans, there was a significant interaction effect of processing time and temperature. For peas, temperature linearly affected DUS.The degree of starch gelatinization was mainly affected by toasting at 136°C. This explained the significant interaction between temperature and time during processing (Tables 3,4), as well as a quadratic effect of temperature. SGD slightly decreased after toasting at 100°C and 118°C (not significant), but significantly increased after toasting at 136°C. At this temperature, an increased processing time resulted in an increase of SGD.

Table 3 Effects of pressure toasting on starch degradation and digestion characteristics and starch gelatinization degree (SGD) of faba beans[1].

	W (%)	k_d (%/h)	UIS (%)	TDS (%)	DUS (%)	DUS g/kg DM	SGD (%)
Treatments[2]:							
untreated	58.6^a	4.68	29.2^e	86.3	52.9^b	64.3^f	8.1^{cd}
100/7	54.2^{ab}	4.51	31.6^{de}	91.3	72.6^{ab}	95.5^{def}	6.2^d
100/15	51.4^{abc}	5.18	31.2^{de}	88.3	62.6^{ab}	81.7^{ef}	5.6^d
100/30	51.1^{abc}	4.66	32.7^{de}	91.7	74.5^{ab}	104.2^{cdef}	5.5^d
118/3	45.3^{bcd}	4.84	34.8^{de}	88.8	67.7^{ab}	98.7^{cdef}	6.7^d
118/7	40.4^{cde}	4.73	37.4^{cd}	90.4	74.4^{ab}	115.8^{bcde}	6.8^d
118/15	38.5^{de}	3.60	42.3^{bc}	86.8	69.0^{ab}	122.2^{bcde}	6.7^d
118/30	34.1^{def}	3.87	43.5^{bc}	90.2	77.6^{ab}	142.1^{abc}	7.7^d
136/3	31.5^{efg}	4.08	43.9^b	87.8	72.2^{ab}	131.8^{bcd}	10.7^{bc}
136/7	25.9^{fg}	3.87	47.7^{ab}	87.6	74.1^{ab}	150.5^{ab}	12.4^b
136/15	20.7^g	3.44	52.5^a	91.8	84.5^a	184.0^a	15.3^a
SEM	2.58	0.14	1.61	0.59	1.95	7.23	0.66
Treatment day[3]	NS	NS	NS	NS	NS	NS	NS
Temperature	***	***	***	NS	NS	NS	*
Time	*	*	**	NS	NS	NS	+
Time x temp.	NS	-	+	NS	NS	**	***
Temp x temp	-	-	-	-	-	*	***
Time x time	*	-	*	-	-	-	-

[1] For abbreviations see table 2. Within columns, different superscripts indicate significant differences (P < 0.05, Tukey's HSD-test).

[2] Codes denote the processing temperature (in °C) and the processing time (in min) *e.g.* the 100 7 treated seeds have been toasted for 7 min at 100°C.

[3] Probabilities: NS = P > 0.1; + = P ≤ 0.1; * = P ≤ 0.05; ** = P ≤ 0.01; *** = P ≤ 0.001, -: excluded from the model.

Table 4 Effects of pressure toasting on starch degradation and digestion characteristics and in vitro starch gelatinization degree (SGD) of peas[1].

Treatments[2]:	W (%)	k_d (%/h)	UIS (%)	TDS (%)	DUS (%)	DUS g/kg DM	SGD (%)
untreated	52.2^a	4.61	32.3^e	84.5	51.8^b	81.0^e	11.5^c
100/7	45.2^{ab}	5.17	34.0^{de}	87.3	62.5^{ab}	102.0^{de}	10.2^c
100/15	-	-	-	-	-	-	10.0^c
100/30	43.4^{ab}	4.55	36.6^{cde}	86.4	62.7^{ab}	109.4^{cde}	8.9^c
118/3	40.1^{abc}	3.87	40.5^{bcde}	85.6	64.6^{ab}	125.3^{bcde}	11.8^c
118/7	34.1^{bcd}	4.17	42.4^{abcd}	87.0	69.3^{ab}	142.4^{abcd}	12.6^c
118/15	27.2^{cde}	4.63	43.9^{abc}	87.0	70.0^{ab}	146.7^{abcd}	11.8^c
118/30	23.7^{def}	4.24	47.2^{ab}	86.4	70.9^{ab}	162.5^{abc}	12.9^c
136/3	21.6^{def}	4.42	47.3^{ab}	89.9	78.7^a	179.4^{ab}	25.4^b
136/7	15.8^{ef}	4.75	48.6^{ab}	87.1	73.6^a	174.1^{ab}	28.5^b
136/15	11.1^f	4.79	50.6^a	89.4	79.0^a	190.3^a	39.0^a
SEM	2.95	0.11	1.42	0.43	1.87	8.00	2.06
Treatment day[3]	NS	NS	NS	NS	NS	NS	NS
Temperature	***	*	***	NS	*	***	**
Time	*	NS	NS	NS	NS	NS	+
Time x temp.	NS	NS	NS	NS	NS	NS	**
Temp. x temp.	-	*	-	-	-	-	***
Time x time	+	-	-	-	-	-	-

[1] For abbreviations see table 2. Within columns, different superscripts indicate significant differences (P < 0.05, Tukey's HSD-test).

[2] For treatment codes see table 3.

[3] For probability levels see table 3.

Toasting significantly affected the DSC gelatinization characteristics (Table 5). Compared to the untreated faba beans and peas, the residual enthalpy (δH) decreased, especially after toasting at 136°C. Concomitantly, the T_o, T_p and T_e increased.

Table 5 Effects of pressure toasting on starch gelatinization of faba beans and pea[1], as measured by differential scanning calorimetry (DSC).

	Faba beans				Peas			
	T_o	T_p	T_e	δH	T_o	T_p	T_e	δH
	°C	°C	°C	J/g starch	°C	°C	°C	J/g starch
Treatments[2]								
untreated	62.7^{cd}	71.6^{e}	81.1^{d}	5.0^{a}	60.8^{ef}	68.6^{h}	77.7^{e}	4.9^{a}
100/7	62.2^{cd}	72.9^{de}	80.7^{d}	4.9^{a}	58.0^{g}	68.9^{h}	77.8^{e}	4.2^{ab}
100/15	62.4^{d}	73.7^{de}	81.4^{d}	4.4^{abc}	59.4^{fg}	70.6^{g}	78.5^{de}	3.9^{abc}
100/30	62.5^{cd}	74.0^{de}	82.2^{cd}	4.6^{ab}	60.4^{fg}	71.9^{f}	80.3^{cde}	4.3^{ab}
118/3	62.6^{cd}	74.5^{de}	81.4^{d}	4.1^{abc}	59.4^{fg}	72.1^{f}	80.4^{cde}	3.9^{abc}
118/7	63.7^{bc}	75.6^{bc}	82.6^{bcd}	4.1^{abc}	61.3^{f}	74.9^{e}	84.4^{bcd}	4.3^{ab}
118/15	64.5^{b}	77.1^{ab}	84.6^{ab}	3.9^{abcd}	63.4^{cde}	76.2^{d}	85.3^{bc}	4.0^{abc}
118/30	66.4^{a}	79.0^{a}	86.4^{a}	4.2^{abc}	65.3^{bc}	77.2^{c}	86.2^{abc}	3.6^{bcd}
136/3	66.0^{a}	77.4^{ab}	84.3^{abc}	3.1^{bcd}	63.7^{cd}	75.4^{de}	85.9^{abc}	2.8^{cde}
136/7	66.1^{a}	79.1^{a}	85.3^{a}	2.4^{d}	67.1^{ab}	78.5^{b}	89.1^{ab}	2.5^{de}
136/15	71.0^{a}	79.1^{a}	89.1^{a}	2.8^{cd}	69.7^{a}	81.8^{a}	91.7^{a}	1.6^{e}
SEM	0.56	0.57	0.56	0.19	0.76	0.87	1.01	0.20
Treatment day[3]	NS	NS	NS	NS	NS	NS	NS	NS
Temperature	NS	***	NS	***	NS	***	***	**
Time	NS	*	*	*	**	***	*	NS
Temp. x time	***	*	**	+	*	*	NS	**
Temp. x temp.	***	-	*	-	*	-	-	***
Time x time	-	-	+	*	**	***	*	-

[1] For abbreviations see table 2. Within columns, different superscripts indicate significant differences (P < 0.05, Tukey's HSD-test). ND: not detected.

[2] For treatment codes see table 3.

[3] For probability levels see table 3.

Particle size

Results of sieve analysis (Table 6) showed, that for toasted faba beans and peas a higher proportion of starch was in the coarser feed particles (Table 6). The larger contribution of the fraction of particles < 71μm after wet sieve analysis resulted in considerable differences between the results of dry and wet sieve analysis. However, the effect of the treatment on the distribution of starch over the dry and wet sieve fractions was similar. Toasting resulted in a significant decrease of the contribution of the starch fraction of particles < 71 μm. Wet sieve analysis showed, that toasting resulted in a shift from particles of 8 to 71 μm to particles of 71 to 160 μm.

For untreated faba beans and peas, the fraction < 71 μm contained 62.6% and 60.1% of the starch, respectively. After toasting, this decreased to 34.8% and 27.7% of the starch. The shift in the starch distribution over the different size classes after wet sieve analysis resulted in a significant increase of the $X_{50,starch}$. For both types of sieve analysis, faba beans and peas had a similar $X_{50,starch}$ before, as well as after toasting.

Table 6 Effects of pressure toasting of faba beans and peas on the starch distribution over different particle size classes[1].

Type of sieve analysis	Faba beans				Peas			
	Dry		Wet		Dry		Wet	
	U[2] (%)	T[2] (%)	U (%)	T (%)	U (%)	T (%)	U (%)	T (%)
< 8 μm (filter solubility)	-	-	8.1^a	10.6^b	-	-	7.6	14.5
8 - 71 μm	-	-	54.6^a	24.1^b	-	-	52.6^a	13.2^b
< 71 μm	32.4^a	28.0^b	62.6^a	34.8^b	33.5^a	20.7^b	60.1^a	27.7^b
71 - < 160 μm	4.7	9.7	0.6^a	19.9^b	3.6^a	16.3^b	0.5^a	26.8^b
160 - < 315 μm	5.7	5.5	0.9^a	2.8^b	5.4	6.5	1.0	6.1
315 - < 630 μm	11.8	11.9	3.9^a	6.8^b	12.3	12.8	4.0^a	6.4^b
630 - < 1250 μm	31.5	32.3	14.2	17.2	31.5	31.6	14.4	15.7
1250 - < 2500 μm	14.0	12.6	17.5	18.5	13.5	12.1	19.6	17.3
≥ 2500	0.0	0.0	0.3	0.1	0.0	0.0	0.2	0.1
SEM[2]	7.67	7.95	5.48	7.49	7.64	8.44	5.84	7.49
X$_{50.starch}$ (μm)	283	286	15^a	167^b	275	308	21^a	192^b

[1] Different superscripts a and b indicate significant (P ≤ 0.05) differences between untoasted (U) and toasted (T, pressure toasted for 15 min at 136°C) samples, within type of sieve analysis.

[2] Standard error of the mean, pooled over the fractions from < 71 to ≥ 2500 μm.

Solubility

The filter solubility (FS) of starch increased after toasting (Table 7), but this was only significant for faba beans. On the other hand, the W decreased after toasting. The difference between W and FS represents the fraction of undegraded particles which are lost from the bags during washing. Taking FS as the fraction immediately available for fermentation, and assuming that the washable, non-soluble particles have the same k_d as the particles remaining in the bags resulted in a much higher %UIS for the untreated seeds. For the toasted seeds, however, the correction was much smaller. Consequently, the effect of toasting on %UIS diminished after this correction (Table 7).

Table 7 Effects of pressure toasting on solubility and corrected starch degradability of faba beans and peas[1].

	Faba beans			Peas		
	U	T		U	T	
	(%)	(%)	SEM	(%)	(%)	SEM
Parameter						
W (%)	58.6[a]	20.7[b]	11.0	52.2[a]	11.1[b]	11.9
FS (%)	8.1[a]	10.6[b]	0.73	7.6	14.5	2.10
k_d	4.68	3.44	0.54	4.61	4.79	0.15
%UIS	29.3[a]	52.5[b]	6.77	32.3[a]	50.6[b]	5.32
%UIS$_c$	52.9	57.9	2.50	53.1	49.0	1.31

[1] %UIS$_c$ = undegraded intake starch calculated using FS instead of standard procedures. Different superscripts indicate significant ($P \leq 0.05$) differences between untoasted (U) and treated (T, toasted for 15 min at 136°C) samples.

Discussion

Chemical composition, starch degradability and digestion

The chemical composition of the untreated faba beans and peas (Table 1) was in agreement with tabular values (CVB, 1998) and those reported in other studies (Dixon and Hosking, 1992; Goelema et al., 1998[a]). Toasting affected neither protein nor starch content of the seeds (results not shown).

Starch degradability varies between feedstuffs (Tamminga et al., 1990) due to differences in granular size, granular form, amylose to amylopectin ratio and the related degree of crystallinity, as well as due to differences in surface structure and composition of the layers surrounding the granules (Colonna, 1992; Gallant et al., 1992). Processing affects the digestive behaviour of starch by changing its granular structure and by changing particle size.

In our study, rumen degradability and intestinal digestibility of untreated faba beans and peas was very similar, which is in agreement with the resemblance in chemical composition, in the composition of their isolated starch granules (an amylose content of 33% and 0.1% lipids for both seeds and 0.9 0.7% protein g for faba beans and peas, respectively (Colonna et al., 1992), as well as in the gelatinization properties measured by DSC (Wolters and Cone, 1992)).

Pressure toasting increased %UIS for both legume seeds, mainly by decreasing W. The decrease of W and the increase of %UIS were consistent with results from a previous study (Goelema et al., 1998[a]).

A large part of the effect of toasting on W and, consequently, on %UIS was related to the change in starch distribution over the different particle size classes after toasting. Drying and storage of the toasted samples may have led to retrogradation of the gelatinized starch (Kayisu and Hood, 1979; Dreher et al., 1984). Since retrograded starch is difficult to solubilize, this may decrease W as well. Toasting especially affected the contribution of the particles < 71 μm to the starch distribution. Assuming that this fraction is comparable to the fraction disappearing from the nylon bags after incubation and washing, this immediately stresses the importance of the availability of this fraction for fermentation. Goelema et al. (1998[a]) assumed that 90% of the W fraction was immediately available for fermentation. Results of Nocek and Tamminga (1991) previously showed that this assumption resulted in a reasonable correlation between in situ and in vivo results for starch degradability, which was later confirmed by Sauvant et al. (1994). The results of the present study show, that the corrected washable fraction (0.9 x W) largely exceeds the FS of starch (Table 7).

Goelema et al. (1998[a]) attributed the decreased W to a decreased swelling power and solubility of starch. The results of the present study, however, show that compared to W, FS of starch only slightly decreased after toasting for 15 min at 136°C (Table 7).

When the FS instead of W was assumed to be the readily available fraction, %UIS was only slightly affected by toasting. The differences between FS and W agreed with results of Lund and Tothi (unpublished results). These authors observed a large difference between the FS and the so-called a fraction (the intercept from the degradation curve) for barley and maize. In their study, FS decreased after expander treatment from 6 to 3% for barley and maize, and from 17 to 7%, respectively, while a increased from 49 to 67% and from 22 to 32%

For starch-rich feedstuffs, such as faba beans and peas, the filtration method may lead to an underestimation of the solubility, due to gelling of starch during solubilization. The layer of solubilized starch surrounding the starch containing particles is not removed during the measurement, since no stirring action was used. Washing in a washing machine probably removes this layer and thus facilitates further solubilization. Insoluble particles < 40 μm may also leave the bags during washing.

Based on the conflicting results for W and FS and the large impact of it on %UIS, it was questioned whether the in situ method was suitable for measuring starch degradability and for studying the effects of processing on starch degradability. Apart from the statistical inferences with respect to degradability originating from the difference between W and FS (Dhanoa et al., 1996), the most important question is, whether the starch lost from the bags is very rapidly available, as was assumed for N lost from the bags (Tamminga et al., 1994). Others, however, assumed that the small particles have a degradation rate similar to that of the particles remaining in the bags (Weisbjerg et al., 1990; Madsen et al., 1995). The latter assumption leads to a lower rumen degradability. Since native starch is insoluble in cold water, the washable fraction consists mainly of soluble sugars, oligosaccharides and granules which are damaged by grinding. It was assumed that this fraction can be hydrolyzed at a much faster speed compared to the starch which is still embedded in the protein matrix.

The degradation rate of the washable, insoluble particles is unknown. Therefore it is not known whether %UIS can be calculated based on FS, or by using the standard formula from equation 1. Further research in this field is necessary to elucidate the discrepancy between the different approaches to calculate %UIS using nylon bag studies.

Since pressure toasting reduced rumen protein degradability (Goelema et al., 1998[a,b]), the effects of a reduced accessibility of the protein matrix embedding the starch in the

endosperm may have influenced the starch degradability as well. This was confirmed by the strong correlation between protein and starch degradability (r= 0.97, P=0.0001). This was not only true for the degradability, but also for the effects observed for solubility and particle size. The effects on starch and protein were confounded, and therefore the effects on degradability of starch and protein are not independent.

Total tract starch digestibility and intestinal digestibility of untreated faba beans and peas were low (Tables 3, 4). In a study by Mupeta, Weisbjerg and Hvelplund (unpublished results) with tropical and temperate grains, the total tract starch digestibilities after subsequent 16 h rumen incubation, in vitro (pepsin-HCL) and intestinal incubation with mobile nylon bags, were also low compared to in vivo results. In their study, total tract starch digestibility ranged from 50 to 75%, which is even lower than the 86.3 and 84.5% in our study for faba beans and peas, respectively. Thus, mobile nylon bag starch digestibility may give an underestimation of in vivo starch digestibility.

After toasting, the total tract digestibility and the intestinal digestibility of starch numerically increased (not significant). A higher %TDS is normally found after processing treatments, and usually coincides with an increased rumen degradability (Owens et al., 1986; Theurer, 1986). The higher digestibility may be related to the inactivation of heat-labile proteinaceous α-amylase inhibitors, which are present in many legume seeds (Dreher et al., 1984), although they are not present in peas (Liener, 1980). The higher %DUS for toasted faba beans and peas may be the result of protecting the easily accessible starch from rumen degradation, which was in agreement with Owens et al. (1986). This can be due to changes during passage of the forestomaches and/or due to a longer residence time. Consequently, the starch surface or its embedding matrix is altered in such a way, that it becomes more digestible in the intestines.

A high intestinal digestibility after extrusion of peas was sometimes attributed to the increased degree of gelatinization (Focant et al., 1990; Walhain et al., 1992). The significant correlation of δH with %DUS for faba beans and peas in our study (correlation coefficient r = -0.47 and -0.78 respectively, P $\leq$ 0.03) suggests that gelatinization has contributed to the higher intestinal digestibility of rumen undegraded starch. In turn, the higher intestinal digestibility may have affected values for %TDS as well, which was confirmed by the positive correlation (r = 0.78, P $\leq$ 0.0001). Gelatinized starch may

retrograde upon cooling, drying and storage (Dreher et al., 1984; Keetels, 1995). According to Englyst et al. (1996), retrograded starch contributes to the fraction which is indigestible in the small intestine, but this was not in agreement with the results for %DUS.

Starch gelatinization parameters

Heat treatments may lead to gelatinization at temperatures ranging from 50 to 80°C when sufficient water is available. A minimal water-to-starch ratio of 0.3:1 is necessary to start gelatinization, but for complete gelatinization a water to starch ratio of 1.5:1 is required (Lund, 1984). Untreated seeds contain approximately 8-12% of moisture. Moisture levels for faba beans and peas were 30.2% and 23.8% after the 100°C/30 min treatment, 34.9% and 24.0% after 118°/30 min, and 31.9% and 24.3% after the 136°C/15 min treatment. This means that for all toasting conditions, moisture levels were sufficient for partial gelatinization only, which is in coherence with the results of SGD and DSC (Tables 4, 5, 6). Although moisture levels after 30 min toasting at 100°C and 118°C were similar to after 15 min toasting at 136°C, the former treatments only slightly affected SGD and δH. A low moisture level during processing limits gelatinization at lower temperatures (<100 °C), due to an increase of the gelatinization temperature (Lund, 1984; Keetels, 1995, Eliasson and Gudmundsson, 1996). Tovar and Melito (1996) showed that atmospheric steaming (98°C, 90 min) and autoclaving (121°C, 15 min) reduced in vitro starch digestibility by Thermamyl and amyloglucosidase, which is consistent with the decrease of SGD under mild toasting treatments in the present experiment. According to Colonna et al. (1992), the structural changes in starch after heating under low moisture conditions and high temperatures are different from those occurring at the lower temperatures and high moisture levels. Under low moisture conditions, the structural changes may be limited to a melting of the crystallites, without a necessary disappearance of the granular shape.

Starch gelatinization enthalpy of untreated faba beans and peas in our study was considerable lower than in studies where isolated starch granules were analyzed (Biliadaris et al., 1980; Vasanthan et al., 1995). Murray et al. (1995) showed, that after purification and isolation of protein δH increased, which was due to a changed contribution of for instance the hydrophobic interactions (Murray et al. 1995). Likewise, starch gelatinization enthalpy may be affected by the surrounding seed matrix. Wolters and Cone (1992), however, showed that isolated starch fractions and whole meals from cereal and

legume grains had only slightly higher values for δH.

The peak temperature of untreated faba beans was similar to results of Wright and Boulter (1980). For peas, T_p was similar to results of Wright and Boulter (1980) and Vasanthan et al. (1995), but higher than results from a previous study (Goelema et al., unpublished). Since sample preparation and heating rate during analysis in the present study were similar to those in the previous study (Goelema et al., unpublished). Since other studies in our laboratory showed a large difference between peas of different origin, with δH of 8.0 and 11.2 J/g starch, variation between varieties of peas probably played an important role in this discrepancy (Eliasson and Gudmundsson, 1996).

The increase of the SGD after toasting was consistent with the decrease of the DSC gelatinization enthalpy (r = -0.77 and -0.92, P $\leq$ 0.0001 for faba beans and peas, respectively). This was in agreement with results of Wolters and Cone (1992) who found a strong correlation between δH and digestion by α-amylase. SGD changes after toasting were smaller than for δH, which may be due to interactions between starch and the non-starch components in the samples during in vitro digestion.

The shift of onset, endset and peak temperatures of gelatinization to higher temperatures after the treatments was consistent with results reported by Kulp and Lorenz (1981), Cooke and Gidley (1992) and Eliasson and Gudmunddson (1996). Since individual granules show slight differences in gelatinization temperature. For individual granules, gelatinization takes place over a temperature range of 1 to 2°C, while for the total starch it might be 10 to 15°C (Eliasson and Gudmunddson, 1996). During mild heat treatments and limited amounts of water, the less perfect crystallites gelatinize first. Later on, the more perfect crystallites may melt but, due to higher degree of order in the granules, this occurs at a higher temperature. When part of the starch is pre-gelatinized during toasting, only the more perfect crystallites are present in the native form, resulting in the observed shift of the gelatinization temperatures during DSC.

Conclusions

Toasting decreased the washable starch fraction of peas and faba beans, and the fractional degradation rate of starch in faba beans. Using standard procedures for calculating in situ starch degradability, toasting was effective in increasing the fraction of

rumen undegraded starch. This was coherent with the increased $X_{50,starch}$, in contrast with the increased degree of gelatinization, measured either with DSC, or in vitro. It was therefore concluded that the effects of particle size dominated over the effects of gelatinization. The discrepancy between the results based on filter solubility compared to standard procedures, stresses the need for further research on the fermentation of the washable fraction. Literature, on the other hand, indicates that results from standard procedures used in the present study significantly correlate with in situ results.

It is concluded that pressure toasting increased the amount of rumen undegraded intake starch and intestinal digestible rumen undegraded intake starch, by reducing the W and, for faba beans, also the k_d.

Acknowledgements

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GENERAL DISCUSSION

J.O. Goelema

Introduction

The importance of legumes seeds for the Dutch feed industry

In this thesis are described the effects of toasting, expander treatment and pelleting on in situ rumen degradability and intestinal digestibility of legume seeds.

In the early nineties, there was a growing interest in legume seeds as animal feed ingredients in the Netherlands, related to an EU subsidy for using legume seeds in compound feeds. The use of legume seeds as concentrate ingredients showed a gradual increase from 0.2 million tons in 1983 (1.3% of the total concentrate feedstuffs), to a maximum of 1 million tons in 1988/89 (5.2%). Since then, the contribution has slightly decreased, levelling to about 4% of the current use of 17.1 million tons concentrate feedstuffs used in 1995/1996 (Anonymous, 1998). The data from 1982 to 1992 also show, that legumes are predominantly used for pig feeds (about 65%), while the remainder is almost equally divided over feeds for poultry and ruminants.

Byproducts from all kinds of food production processes are the most important concentrate ingredients, ranging from 51% in 1993/1994 to 55.1% in 1995/1996. Within the byproducts, those from the edible oil industry contributed about 54%. Most of these byproducts are included in concentrates for dairy cows, which contributed 25% of the total concentrate production in 1993/1994, and 23% of the total concentrate production of 16.4 x million tons in 1996/1997. It was estimated that more than 95% of the concentrates for dairy cows are fed in the (expander) pelleted form although there is an increasing interest in using single (unpelleted) feedstuffs as 'top-dressing' over roughages or in mixed rations.

When used in concentrates for ruminants, legume seeds provide the animals with protein of high rumen degradability, whereas the starch, present in, for example, peas and faba beans has an intermediate rumen degradability.

For high-producing dairy cows, even an optimal rumen fermentation results in insufficient microbial protein synthesis to maintain milk productions of over approximately 25 kg/cow/day. To meet the protein requirements of these animals, feeding rumen undegraded protein is necessary. Two strategies can be followed, either using protein sources with a low rumen degradability, such as (processed) animal proteins, or decreasing the rumen degradability of plant proteins by processing. However, in large

parts of Europe, the use of animal proteins in ruminant concentrates is not allowed, due to the risk of Bovine Spongiform Encephalopathy (BSE).

Several chemical (Varvikko et al., 1983; Cleale IV et al., 1987; Stern et al., 1994) and physical treatments (Aguilera et al., 1992; Arieli et al., 1989; 1995; Focant et al., 1990; Schroeder et al., 1996) have been used to decrease rumen protein degradability. In this study, physical treatments were used to decrease rumen degradability of protein in legume seeds.

Pressure toasting has been previously used in our laboratory to improve the nutritional value of phaseolus beans for monogastric animals (Van der Poel et al., 1990). A preliminary study with toasted phaseolus beans (R. Zom, unpublished results) showed, that in situ rumen protein degradability decreased, especially when toasting temperature was increased from 102 to 136°C. That was the reason for studying this treatment in more detail.

Effects of processing on in situ digestive behaviour of legume seeds

In this project, four different legume seeds were studied. There was a large variation in chemical composition between the legume seeds (Chapters 2, 4). Peas and faba beans were chosen, based on their similarity in protein (250 and 312 g/kg DM) and starch content (420 and 490 g/kg DM). Lupins have a similar protein content (324 g/kg DM), but contain hardly any starch, more fat (72 g/kg DM) and much more crude fibre (144 g/kg DM). Although the currently available varieties cannot be grown in most EU countries, the commercially useful soybeans were chosen because they combine a high protein content (394 g/kg DM) and a high fat content (198 g/kg DM).

Apart from differences in protein content, the seeds of different legume species have a different protein structure and a different amino acid profile. These differences were held responsible for the differences in sensitivity to (pressure) toasting between legume seeds (Chapters 2, 4), which was related to the differences in solubility and degradability of the protein fractions. Based on their protein and their starch content and composition, peas and faba beans were expected to respond in a similar way to the heat treatment, which was confirmed by most results (Chapter 2, 4, 5, 6). Lupins and soybeans showed large similarities with respect to the effects of toasting on denaturation characteristics protein (Chapter 4), which was attributed to the similarity in their protein composition.

The in situ method was used to incubate samples of untreated and processed seeds in the rumen, in order to quantify the degradability and digestibility of the samples. The readily available fraction of protein and starch was measured as the fraction disappearing from the nylon bags after washing in a washing machine. From literature, various methods have been described to quantify the readily available fraction, such as measuring solubility in various media or by using the intercept of the degradation curve.

The preferred way to measure the washable fraction (W), the potentially degradable (D) and the truly undegradable fraction (U), as well as the rate of degradation (k_d) of the potentially degradable fraction is by using the one sample, one particle size and one pore size. Following this procedure, the degradation characteristics are dependent, which implies that one cannot take the separate characteristics from different studies and use them to calculate a reliable estimate of the rumen degradability.

Studies which are based on combinations of in situ and laboratory methods to determine the degradation characteristics mentioned above, used either differently ground samples, or a different pore size, or both, which results in fractions which are not complementary to each other.

When nylon bags are washed in a washing machine, the pore size of the bags undoubtedly has an effect on how many small, insoluble particles leave the bags. This will be affected by the intensity of the washing process and, if applied, the spinning procedure. As long as it is unclear at what rate these lost small particles are degraded or to what extent they are escaping degradation through the rumen outflow, discussions as to whether the washable fraction is representative for the immediately available fraction will continue.

The availability of the washable fraction for rumen microbes probably depends on its chemical composition. The soluble N fractions consist among others of free amino acids, small peptides, and non-protein N. It has been shown, that the in vitro fermentation of soluble N fractions largely varies between feedstuffs (Mahadevan et al., 1978), which means that the availability should be determined as well as only quantifying the solubility. Although the cold-water soluble fraction of native starch is usually small, grinding may result in the breaking of glycosidic bonds and the crystalline structure of the starch granules, thus increasing the water-soluble and the washable fractions. In our experiments (Chapters 4, 6) the sieve fraction < 71 μm of peas and faba beans after dry and wet sieving were relatively rich in N and starch. In other studies the smaller particles were

sometimes relatively low in N, whereas in other feedstuffs, N content hardly changed with increasing particle size (Michalet-Doreau and Cerneau, 1991). Wolters and Cone (1992) observed, that in some feedstuffs, grinding resulted in the separation of individual starch granules, whereas for others it resulted in particles containing larger groups of associated granules. This probably also affects the distribution of starch over the different particle size classes.

A newly discovered factor (Chapter 4, 6) is that processing affects particle size distribution after grinding. The distribution of N and starch over the particle size classes changed after toasting (Chapter 4). In our study, the apparent mean particle size for N and starch of the toasted seeds was larger than before toasting.

Since particle size distribution of the incubated samples is usually not reported, it is unknown whether the changes observed in our study are representative of the effect on particle size after (heat) treatments. For a proper comparison of the effects of heat treatments, untreated and treated samples should be ground in the same way, (in contrast to what is sometimes done). Likewise, studies on the effects of treatments such as pelleting and expander processing -which result in forms which are normally fed to the animals- should not be carried out with ground samples from the pellets or the expandate, since grinding may interfere with the measured in situ results, which do not occur in vivo. Moreover, when in a study the process itself or its conditions are of special interest, the replications for the statistical evaluation should originate from multiple batches or runs of treatments, rather than from different cows used for the rumen incubation.

Pressure toasting
In agreement with the initial hypothesis, toasting decreased the rumen protein degradability of legume seeds, by decreasing the readily available protein fraction, as well as the rate of degradation of the potentially degradable fraction.

For starch, it was assumed that toasting would result in gelatinization and, consequently, in an increased rumen starch degradability. This study confirmed that toasting resulted in gelatinization of starch, especially at processing temperatures exceeding 130°C. This was observed both by enzymatic (in vitro) analysis, and by differential scanning calorimetric (DSC) measurement. However, contrary to expectations, in situ rumen starch degradability was reduced, rather than increased. The effect on starch degradability was mainly caused

by decreasing its washable fraction. This was inconsistent with our hypothesis, and with most reported studies of effect of processing on starch degradability. The following conditions during toasting are assumed to have played an essential role in our results:

1) low moisture levels,
2) relatively low temperatures,
3) processing of whole seeds instead of ground mashes,
4) no shearing action,

In situation of an excess of water (500 - 700 g moisture/kg), heat treatment leads to swelling of the starch granules and dissolution of the granules (Colonna et al., 1992). Although gelatinization in an excess of water leads to a complete disappearance of the crystalline structure, low moisture conditions may result in melting of the crystallites only, without affecting the granular shape (Colonna et al., 1992). Therefore, processing may result in a melting of the less perfect crystallites, while the most perfect ones remain in their stable form.

Limiting moisture levels increase gelatinization temperatures (Lund, 1984; Eliasson and Gudmundsson, 1996) to values exceeding the lower toasting temperatures of 100 and 118°C used in this study. When moisture levels and/or temperatures are insufficient for complete gelatinization, partial gelatinization may occur locally (Chapter 6).

Whole seeds are more slowly hydrated and heated than ground mashes. When whole seeds are toasted, the presence of the intact seed hulls probably prevents the popping effect due to the 'flash off', observed after expander processing of ground mashes. Therefore, the physical structure of toasted whole seeds will be very different from that of expanded mashes. This will change the effects of the treatment on starch degradability, even when the same processing time and temperatures are applied.

When gelatinized starch is cooled and/or stored at low temperature, it may recrystallize, which means that gelatinized starch will become more crystalline, which is associated with a lower solubility. This change is called retrogradation, and this may contribute to the formation of slowly degradable or even indigestible starch fractions, the so-called 'resistant' starch fraction (Englyst et al., 1996).

This retrograded surface may enclose the other starch and thus indirectly also limit its solubilization (Chapter 7). This effect was seen after pelleting an expander treated concentrate. Compared to the expandate, pelleting decreased the washable starch fraction, although the degradation rate was substantially enhanced (Goelema et al., 1996).

Thomas (1998) used the in vitro gas production technique (Theodorou et al., 1993, 1994) with sheep rumen fluid as inoculum, to measure the fermentation of processed tapioca starch. His results indicated that, after long-term storage, the moment at which the maximum in vitro gas production rate was reached was earlier for pre-gelatinized compared to native tapioca starch, whereas total cumulative gas production and volatile fatty acid (VFA) production were reduced. Based on these observations, Thomas (1998) hypothesized, that during storage, a part of the pre-gelatinized starch had retrograded, whereas another part was still present in its gelatinized state. A higher degree of gelatinization increased the rate of fermentation, whereas recrystallization of the gelatinized starch resulted in a slowly or even undegradable starch fraction, explaining the decreased total gas production and VFA production. Our results for peas, toasted at 136°C are in agreement with this. When the degree of gelatinization increased (ranging from 25 to 39%), k_d numerically increased (not significant), which was in line with the lower DSC gelatinization enthalpy. For faba beans, toasting at 136°C resulted in a lower degree of starch gelatinization (ranging from 11 to 15%), and k_d decreased when gelatinization increased. This suggests that a minimal degree of starch gelatinization is necessary to observe an increased k_d for starch after toasting.

On the other hand, toasting resulted in a consistent decrease of the washable fraction, even after toasting at the highest temperature of 136°C. This can be related to an increased amount of retrograded starch. The hypothesis proposed by Thomas (1998) also applies to the explanation for the decreased starch degradability after toasting. When a starchy feedstuff is heated with steam, condensation of steam increases its moisture content. If the temperature of the seeds during processing exceeds the gelatinization temperature at the actual moisture content, gelatinization starts. Depending on the moisture content, processing temperature and time, this gelatinization varies from a local and partial one to complete gelatinization, which increases starch degradability. Upon cooling and drying, however, recrystallization can take place. Since retrograded starch is difficult to solubilize, it directly affects the size of the washable fraction.

In conclusion, above a certain level of (in vitro) gelatinization, which is probably higher than 15% SGD, 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 W of starch. Since gelatinization and retrogradation counter-act with respect to rumen starch degradability, different processing conditions lead to different outcomes.

Shear forces during or after heat processing, such as during steam-flaking, expander treatment, extrusion or pelleting, may further disrupt the granular structure of starch and interact with gelatinization and retrogradation, but also affect the particle size. Since the chemical composition of feedstuffs and the structure, gelatinization and retrogradation properties of starches in different feedstuffs vary greatly, notably when shear is involved, differences between feedstuffs upon processing can be expected.

Apart from the specific changes of the protein and starch fraction in faba beans and peas, an interaction between the two components is also likely. This interaction may have originated from the strong association between protein and starch, but also from the effect of heat treatment on the protein fraction. Thorne et al. (1983) and Franco et al. (1995) suggested, that complexes between protein and starch are formed upon heat treatment. It is assumed that when the protein matrix becomes denatured, it reduces the accessibility for amylolytic enzymes as well (Holm et al., 1985). However, it is unclear whether this effect of protein on starch degradability originates from the small protein fraction in the granular starch itself, or from the other protein in the seed.

To what extent the observed decreases of the k_d and the W of starch are due to the retrogradation of starch, or to the denaturation of protein, is unknown. More systematic research in this field, focussing on the conditions which influence the structural properties of starch, and their effects on digestion characteristics of heat treated and subsequently dried or cooled feedstuffs is required. The use of genetically modified feedstuffs, which vary in amylose and amylopectin content of starch and in starch content, might be very attractive to study the effects of gelatinization, retrogradation and the impact of the protein content.

Effects of toasting on the rumen degradability and intestinal digestibility of the individual amino acids were evaluated in lupins (Chapter 7). The amino acids showed differences

in washable fraction, rate of degradation, and, consequently in rumen degradability. The results further showed, that all amino acids responded in a similar way to the processing conditions. However, due to the initial differences in degradability, some amino acids showed a larger decrease in protein degradability than others. It was concluded that toasting resulted in denaturation of protein as a whole, rather than altering the degradability of specific amino acid preferentially, as might have been expected for lysine due to Maillard reactions. The results were consistent with observation of Marsman et al. (1998), who concluded that toasting under mild conditions involves the formation of non-covalent, instead of covalent bonds. This was also supported by the observation that the amount of acid detergent insoluble N, an indicator for excessive heat damage of proteins (Van Soest, 1994; Hsu and Satter, 1995), remained negligible after 3 min toasting at 132°C (Chapter 1). However, some brown coloring appeared after treatment at 136°C, which indicates that at that temperature Maillard products were probably formed. This was confirmed by the lysine loss after the most intensive treatment, although the intestinal digestibility of lysine in toasted lupins was always higher than in the untreated lupins. Toasting generally increased the in situ intestinal digestibility of the rumen undegraded fraction, except for toasting at 136°C, which reduced the digestibility. The higher digestibility after toasting was previously also observed in vitro and in vivo in pigs (Van der Poel et al., 1990). It was associated with the inactivation of antinutritional factors, as well as with the structural changes in the storage protein after toasting.

Incubation residues were corrected for the contamination with protein of rumen microbial origin, using DAPA as microbial marker. Although it is generally assumed that for protein-rich feedstuffs the effects on practical implications of contamination are negligible, our results indicate otherwise. Contamination was generally increased with rumen incubation time. Consistent with the decrease of available substrate, the microbial contamination decreased for toasted lupin samples after 24 h of rumen incubation. Corrected total amino acid N had a higher degradability and a higher intestinal digestibility than total N, which was not corrected for microbial contamination. Moreover, correction for microbial contamination affected the ranking of treatment with respect to the degradability of the individual amino acids in lupins, especially for methionine and lysine. Using the total protein degradability and the feed amino acid profile for calculating the amount of rumen undegradable amino acids may therefore lead to erroneous results, and

may also lead to misinterpretations of the effectivity of (heat) treatments in altering rumen amino acid degradability.

Surprisingly, the study also indicated, that a substantial part of the contamination may originate from (aerobic) fermentation due to too slow oven-drying of heat treated feedstuffs and incubation residues. The results showed that freeze-drying was preferable compared to 24 h oven-drying at 60°C.

With respect to lupins, one might question whether protein should be protected from rumen degradation, since the amino acid profile of lupins is deficient in both methionine and lysine, limiting amino acids for dairy cows. Relative to microbial protein (Storm and Ørskov, 1983), the amino acid profile of rumen undegraded lupin protein remains deficient in these two amino acids. The use of protected synthetic amino acids could overcome part of the problems in balancing the amino acid profile of rumen undegraded protein with animal requirements.

Expander treatment

The other treatment which was used to decrease the rumen degradability of a mixture of legume seeds was expander treatment (Chapter 2). Several studies have been published in which extrusion was shown to be effective in reducing protein degradability of legume seeds, though the processing costs of this treatment are relatively high (Melcion and Van der Poel, 1993). Another relatively new, and cheaper treatment than extrusion is expander processing. In our study, expander treatment at 115°C did not reduce protein degradability, despite other successful attempts (Nielsen, 1994; Prestløkken, 1994; Arieli et al., 1995; Lund and Tothi, unpublished). Thus, our results were in disagreement with the prior hypothesis that expander treatment decreases protein degradability. In the published studies on expander treatment cereal grains were mainly used, which have a lower protein content, and smaller seeds than legumes. Some of these experiment were carried out with finely ground mashes, whereas in our experiment, a coarsely broken mash was used. The larger particle size of the legume seeds used in our study most likely hampered the transfer of heat and moisture, and -in turn- limited the denaturation of protein. This was supported by the small decrease in protein dispersibility and nitrogen solubility after expander treatment, relative to what was observed after toasting (Chapter 2).

In the other published studies, processing temperatures often exceeded the temperature of 115°C used in our study, although this could not be judged very well in all cases, due to the sometimes unclear presentation of the processing conditions. Another important factor is the residence time in the expander, which was only 8 s in our study. It was hypothesized that longer processing times, smaller particle size and a higher processing temperature would have resulted in a more effective treatment. The results of Nielsen (1994) for peas suggest, that the minimal processing temperature during expander treatment to reduce protein degradability is between 122 and 127°C. Although this is also dependent on the processing time, the author did not show the residence times in the conditioner and the expander.

For starch, expander treatment increased rumen degradability, which is in agreement with most in vitro and in vivo results after extrusion of peas (Focant et al., 1990; Walhain et al., 1992) and expander treatment of cereals (Harstad et al., 1996). Arieli et al. (1995), however, reported a decreased in situ rumen starch degradability of several cereals. The increased starch degradability after expander treatment in our experiment was due to a higher washable fraction and a rate of degradation, which was negatively associated with particle size and starch gelatinization.

These results demonstrate the effect of expander treatment on the particle size and its impact on degradability. The particle size of the broken mixture of legume seeds reduced after expander processing, which was positively associated with the washable protein fraction and, consequently, with the rumen protein degradability. Although the results were not consistent with our hypothesis that expander treatment decreases protein degradability, they are very interesting, since the conditions applied have characteristics similar to those used in the (expander) processes frequently carried out in the feed industry to improve the physical quality of pelleted feeds. For that purpose, steam is added to obtain processing temperatures ranging from 80 to 100°C. Under those conditions, protein denaturation is very limited, especially in concentrates for dairy cows (Goelema et al., 1996), which mainly consist of byproducts from the food industry However, in vitro gelatinization of starch increased (Goelema et al., 1996). Shear forces during expander processing were held responsible for the decrease in particle size, which was positively associated with starch gelatinization, and rumen degradability of protein and

starch (Chapter 2). Apart from the particle size reducing effect of the shear forces, they may have altered the surface to a more porous, brittle structure. This facilitates the hydration and, subsequently, rumen fermentation.

Thus, expander treatment involves an important shearing effect, resulting in a decreased particle size, an improved pellet durability and hardness, but also in an increased rumen degradability. However, at higher temperatures (> 115°C), the heat treatment during expander processing may have resulted in protein denaturation, and thus lead to a decreased rumen protein degradability (Prestløkken, 1994). On the other hand, results of Arieli et al. (1995) suggest, that there are conditions which reduce in situ starch degradability after expander treatment.

It is concluded that further research should aim to quantify and optimize the effects of processing conditions (temperature, moisture level, residence time, shearing action) on rumen degradability of protein and starch.

Pelleting

It was estimated that for the manufacture of pelleted animal feed between 420 and 630 MJ ton[-1] animal feed is used (Thomas, 1998), of which 60% is used for conditioning, pelleting and cooling. When heat-treated feedstuffs are used as concentrate ingredients, the energy consumption by the pelleting process may be even higher. Thus, pelleting is an important factor in determining the costs of pelleted animal feeds, which sharply contrasts with the small number of studies on digestive behaviour of pelleted concentrates for animals. The reported studies refer mainly to monogastric animals, and the results are therefore not generally applicable to ruminants.

Although the effects of pelleting depend on the applied conditions during the conditioning phase, the results obtained in our study with the coarse mash are generally consistent with results of other studies carried out previously in our laboratory (see General Introduction and Goelema et al., 1996), using various feed ingredients, and several levels of protein and starch.

All these experiments consistently showed that rumen degradability of protein and starch increased with pelleting. This was consistent with our previous hypothesis. Various factors may be responsible for the increase. The positive correlation between particle size and degradability after pelleting a mixture of legume seeds (Chapter 2) confirmed the idea that

particle size reduction played an important role in explaining the increased rumen degradability. Apart from affecting the particle size, the structure of the pelleted product may have become more porous and more accessible, resulting in an increased rate of degradation.

The overall effect of pelleting on the amount of intestinal absorbable protein in concentrates is unknown. If energy is not limiting, the increased rumen protein degradability may result in an increased microbial protein synthesis, which is favored by the increased starch degradability. On the other hand, effects of pelleting on the efficiency of microbial protein synthesis are unknown. The effects of particle size reduction on in situ protein degradability may be partially compensated for by an increased outflow of soluble protein. This can be the case for the small, insoluble particles as well. Their specific functional weight may even be high enough to escape from fermentation by passage from the rumen, which prevents them from being captured by rumen microbes.

However, based on studies presently available, it cannot be decided whether, nor to what extent, the effects on in situ protein degradability are influenced by in vivo changes in microbial protein synthesis, nor the extent to which the increased degradability is compensated for by an increased rumen outflow.

Combinations of toasting, expander treatment and pelleting
The combination of toasting, expander treatment and pelleting (Chapter 2) showed that, under the conditions tested, toasting had the largest impact on rumen degradability of a broken mixture of faba beans, peas and lupins. Subsequent expander treatment and pelleting resulted in relatively small changes. Consequently, the increased UIP and UIS after toasting were maintained after expander treatment and pelleting. However, with less severe toasting treatments (lower temperature, shorter processing time), expander treatment and pelleting may reduce the beneficial effect of toasting on UIP, as was shown for a concentrate mixture including lupins which were toasted for 1.5 min at 130°C (Goelema et al., 1995).

Effects of processing on laboratory parameters of legume seeds
Effects of heat treatment can also be evaluated by several laboratory measurements. Protein dispersibility (PDI) or solubility (NSI) are very common methods to indicate the level of denaturation of, for example, commercial soybean products. Our results indicate

that, except for lupins, there are strong correlations between PDI, NSI (Chapter 1, 2) and the in situ protein degradation characteristics, such as the washable fraction, the rate of degradation and the rumen undegraded protein fraction and the amount of intestinal digestible rumen undegradable protein (DUP). The exception for lupins is probably due to the relatively low PDI for untreated lupins (Chapters 1, 4).

Results for PDI and NSI in water are very similar for rumen protein degradation characteristics, although NSI gives slightly lower values (Chapter 1). Despite the good correlations between PDI, NSI and rumen protein degradability (Chapter 1) at levels below approximately 20% PDI, the differences in protein degradation are poorly discriminated. Results for the predicted DUP from PDI (Chapter 4) further confirmed this effect, although DUP of toasted legume seeds could be estimated from PDI with an accuracy varying from 4.9 (for soybeans) to 13.5% (for lupins) for the mean observed PDI (Chapter 4).

DSC was also used to measure the extent of protein denaturation after toasting. Both DSC and PDI results correlated very well with in situ results for protein (Chapter 4). Based on the DSC results it was possible to discriminate between differences in denaturation and protein degradability of samples from intensive treatments, where PDI failed. For the milder treatments, however, PDI was more discriminating for DUP. Prediction of DUP based on the DSC denaturation enthalpy resulted in a similar accuracy as based on PDI, ranging from 8.9 (faba beans) to 11.6% (soybeans).

Thus, the application of DSC results to explain the altered protein degradability showed great promise (Chapter 4). Comparison of our results with those in the literature was hampered by the variation in sample preparation and in scanning conditions. The results of Murray et al. (1985) demonstrated, that purification of seed protein or starch fractions may result in completely different enthalpy levels. This may interfere with the effects of processing as well. It can therefore be concluded that to study the effects on protein degradability, a standardized DSC analysis should be carried out preferably with the (ground) whole seeds, rather than with purified protein or starch fractions.

DSC was also used to quantify the extent of starch gelatinization after toasting. These results showed good correlation with the in vitro degree of gelatinization (SGD), as measured by enzymatic digestion with amyloglucosidase. However, SGD probably also reflects the particle size of the samples, which, within certain limits, is shown to be of less importance in DSC measurements (M.R. Roth, unpublished results). Both DSC and SGD

results are influenced by other processes than gelatinization as well. For instance, retrogradation will affect the gelatinization enthalpy and presumably also SGD, although the magnitude of these effects were not studied in our experiments. This was confirmed by Kayisu and Hood (1979), who observed a decreased pancreatic alpha-amylase digestibility of starches after drying and storage.

Further research should elucidate whether DSC can also be used to predict the effects of heat treatment on rumen degradability of feedstuffs.

Effect of toasting on in vivo digestive behaviour of protein and starch.
The results of toasting on degradation and digestibility of legume seeds, as described in this thesis, are all based on in situ measurements. A proper in vivo evaluation of the results, especially those which were in disagreement with prior hypotheses seems necessary, since the number of studies in this area is limited.

The disagreement between the (decreased) in situ starch degradability and the increased degree of gelatinization (measured in vitro or by DSC) is fascinating and gives rise to numerous discussions with other scientist and representatives from the feed industry. These discussions concentrated on:

1) the possible role of denaturation of the protein matrix surrounding the starch granules,
2) the availability of the washable starch fraction (W),
3) the general applicability of the observed effect for legumes

If denaturation of protein (1) plays an important role, one would expect a smaller effect of toasting on the starch degradability of seeds which have a higher starch/protein ratio compared to faba beans and peas, such as cereal grains.

In the case that the washable starch fraction is readily available and completely degraded in the rumen (2), which is not unlikely (Chapter 1), the difference in in situ starch degradability will also be observed in vivo. However, when the fraction of particles between 40 and 71 μm, 'the so-called particle loss', has a slower degradation rate than the fraction < 40 μm, the amount of UIS will be underestimated, which is especially the case for the untreated faba beans and peas, due to their higher fraction of lost particles. Consequently, the effect of toasting on UIS will be overestimated (Chapter 6).

In close cooperation with the department of Animal Science of the Agricultural University in Äs (Norway), two experiments were carried out, focussing on reduction of rumen degradability of cereal starches by toasting. In the first experiment, an in situ screening was carried out with 3 cereals (barley, oats, corn), 3 processing temperatures (100, 118 and 136°C) and 5 processing times (1.5, 3, 7, 15, 30 min). The samples were ground over a 1.5 mm screen. The results showed, that the results for barley and oats were similar to those of faba beans and peas, whereas corn starch responded differently. For barley, the amount of rumen undegraded intake starch increased from 13% to a maximum of 33% UIS, whereas for oats, it increased from 9 to 25% UIS. For corn, UIS decreased after toasting, from approximately 54% UIS to a minimum of 46%. For barley and oats, toasting decreased the washable fraction (W) of starch and at the lower toasting temperatures, also its rate of degradation (k_d). For corn, k_d remained unchanged, but W increased after toasting at 100 and 118°C, whereas at 136°C, the k_d increased and W decreased with increasing processing time (Goelema, Harstad and Gotvassli, unpublished results). .

The results for barley and oats are consistent with our results for faba beans and peas, despite the large difference in initial starch degradability and starch and protein content in these seeds. Results for corn were inconsistent with the results for faba beans, peas, barley and oats, which suggests that the starch to protein ratio indeed may play a critical role in obtaining an effect of toasting on UIS. This seems to be contradict the strong association between protein and starch in corn and sorghum which is (at least partially) held responsible for the slow degradability of the starch in these feedstuffs (Holm et al., 1985).

The results obtained demonstrate, that the results of toasting on in situ starch degradability are not exclusively due to specific characteristics of legume seeds, and that the underlying mechanisms are the same for other starchy feedstuffs.

The second experiment was an in vivo evaluation with lactating dairy cows (4-5 months in lactation), in a 3 x 3 Latin square design. Ground barley, either untreated (B0) or toasted (B1 and B2) were used. The samples were ground over a 3 mm screen. The in situ UIS of the samples was 10, 20 and 22%, respectively, whereas the apparent in vivo UIS was 15, 19 and 20% (Harstad, Gotvassli, Goelema and Tamminga, unpublished results).

These results suggest that the effects of toasting on in situ starch degradability based on

in situ measurement were overestimated, but that the order of the degradability remained unchanged. The in situ results for the toasted samples are in line with the in vivo results and support the hypothesis stated above, that only a part of the washable fraction is immediately available and incorporated in the microbial mass, and that, especially when the washable fraction is large, a substantial part of the washable starch fraction escapes fermentation via rumen outflow.

The in vivo evaluation also showed, that duodenal protein flow was significantly increased by toasting. This was consistent with the observed increase on in situ UIP (from 19% to about 28 and 31%, respectively). On the other hand, it can not be excluded that the higher duodenal protein flow originated from an improved microbial protein synthesis, due to synchronization of available N and energy for the rumen microbes.

Feeding toasted barley increased average daily milk yield by 1.5 kg/cow. This might be related to the increased duodenal flow of starch, which has probably resulted in an increased intestinal glucose supply. Since duodenal protein supply was also increased, this enabled milk protein concentration to increase slightly. The concomitantly observed decrease of milk fat concentration can be explained by a dilution effect, which is related to the limited amount of available lipogenic energy for milk fat synthesis. Consequently, milk protein production significantly ($P < 0.05$) increased, whereas milk fat production decreased. The results of this study show, that the observed effects of toasting on in vivo starch degradability were in agreement with the in situ results for starch. The observed production response with toasted barley is probably related to optimization of the fermentation processes in the rumen, as well as to the increased supply of intestinal digestible starch and protein. Regarding the small differences in in situ degradability of starch and protein, the observed production response is surprisingly large. Further evaluation of the microbial protein synthesis will give more insight in the mechanisms responsible for the observed production responses.

Practical implications and conclusions

In market systems where milk fat production is limited by quota systems, an increased milk protein production is the most economical way to increase profits. In this respect, toasting seems to be an interesting method to improve the nutritive value of legume seeds and cereals, since the profits can be achieved even with a relatively short treatment at

atmospheric conditions (B1), which can be carried out with equipment presently available on the market. Toasting numerically increased the N balance (though not significant), which is very interesting from an ecological point of view. Although toasting requires fossil energy, which can be considered a disadvantage, one might speculate that it enables the use of home-grown concentrate ingredients. In turn, this might reduce the imports of (protein) supplements from all over the world, which saves on the energy costs for transport. Whether this ever happens depends probably predominantly on political and macro-economic situations, rather than on the sole question of whether it is ecologically beneficial. It is therefore very difficult to decide which impact the results for legumes (and cereals) presented in this thesis have on the use of these feedstuffs in the feed industry as a whole, the EC and in particular in The Netherlands.

Nevertheless, the following conclusions can be drawn:
-       Toasting decreases in situ rumen degradability of protein and starch.
-       Pelleting increases in situ rumen degradability of protein and starch.
-       Expander processing under mild conditions increases in situ rumen degradability of protein and starch.
-       Effects of toasting, expander treatment and pelleting on in situ degradability are associated with changes in protein denaturation and starch gelatinization, but also in particle size.
-       Toasting at 132°C for 3 min has more impact on in situ rumen degradability of starch and protein than the tested conditions of expander treatment and pelleting.
-       Effects of toasting on the amount of intestinal digestible protein in legume seeds can be accurately predicted from the changes in protein dispersibility index or in denaturation enthalpy, as measured by differential scanning calorimetry.
-       Toasting may affect production responses in dairy cows, resulting in an improved N balance and an increase in the farmers' profits.

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SUMMARY

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Summary

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Introduction

In this thesis effects of toasting, expander treatment and pelleting on in situ rumen degradability and intestinal digestibility of legume seeds are described.

In the feeding of ruminants, particularly in dairy cattle, there is a great interest in digestive behaviour of feed in forestomach and intestines, because of at least two reasons:

1) Degradation of feed protein and microbial protein synthesis are rarely balanced. This imbalance results in unnecessary N losses from the rumen, because ammonia, resulting from deamination of amino acids and degradation of non-protein N, accumulates shortly after the feed is ingested, and may have left the rumen before it can be incorporated into microbial protein.

2) The ratio in which the different VFA (acetic, propionic, and butyric acid) and lactic acid are formed in the rumen is largely dependent on the rate at which the substrate (structural carbohydrates, non-structural carbohydrates, and protein) is degraded. The imbalance between microbial degradation and synthesis as well as the ratio in which VFA are formed are important determinants of the type of nutrients that come available for the animal. These in turn are important factors determining the level and composition of ruminant production.

It is easy to understand that it would be advantageous to maximize the degradation of structural carbohydrates in the rumen. Degradation of protein and non-structural carbohydrates can be restricted to what is necessary for maintaining an efficient microbial activity and growth, provided that the undegraded fraction is digested in the small intestine. Digestion of feed protein and non-structural carbohydrates in the small intestine has also the advantage that less nitrogen and energy is lost.

For high producing dairy cows, even an optimal rumen fermentation results in insufficient microbial protein synthesis for maintaining milk productions of over 25 kg/cow/day. To meet the protein requirements of those animals, feeding rumen undegraded protein is necessary. Two strategies can be followed, either using protein sources with a naturally

low rumen degradability, like (processed) animal proteins, or decreasing the rumen degradability of plant proteins by processing. In the present study, physical treatments were used to decrease rumen degradability of protein in legume seeds. When legume seeds are used in concentrates for ruminants, they provide the animals with highly rumen degradable protein, whereas the starch, present in for instance peas and faba beans has an intermediate rumen degradability.

Therefore, the feeding of untreated legume seeds to dairy cows in situations where energy availability is the limiting factor will increase the N-excess, which results in a less efficient utilization of N.

This thesis describes the effects of processing of legume seeds on digestive behaviour of protein and starch in the rumen and intestines of dairy cows. This information enables feed manufacturers and nutritionists to formulate concentrates and rations which:

1. Optimize rumen fermentation processes
2. Influence the site of digestion, by shifting it from the rumen to the intestines, which quantitatively alters the supply of nutrients
3. Contribute to the reduction of unnecessary losses from the rumen.

In the following chapters, the results of three experiments are described. In these experiments effects of 3 methods of processing are studied:

A. Pressure toasting
B. Conditioning/expander treatment
C. Conditioning/pelleting

In the study outlined in chapter 1, the effects of pressure toasting of whole and broken peas, lupins and faba beans on in situ degradability of protein and starch and intestinal digestibility of protein were studied. To test for associative effects on rumen degradability and intestinal digestibility after toasting, a mixture of peas, lupins and faba beans was examined and results were compared with weighted averages of separately processed feedstuffs.

Pressure toasting for 3 min at 132°C decreased in situ protein degradability of peas, lupins and faba beans and in situ starch degradability of peas and faba beans, especially when broken vs. whole seeds were processed. Undegraded intake protein (%UIP)

increased after toasting whole or broken seeds from 25% to 44% and 52% for peas, from 22% to 47% and 51% for lupins and from 20% to 48% and 57% for faba beans, respectively. Undegraded intake starch (UIS) increased from 39% to 50% and 53% after toasting whole and broken peas and from 33% to 53% and 60% for toasted whole and broken faba beans, respectively. Total tract protein digestibility, measured after 12 h rumen and subsequent intestinal incubation, remained unchanged for peas and faba beans, but decreased for toasted broken lupins. After pressure toasting, washable fractions (W) of all legume seeds decreased for both constituents, the fractional rate of degradation (k_d) of protein decreased, while the k_d of starch increased. It was concluded that protein degradability decreased after pressure toasting, without seriously affecting its total tract protein digestibility.

Toasting a mixture of peas, faba beans and lupins resulted in higher starch degradabilities than expected, based on the separately treated feedstuffs. The k_d's of the mixtures were higher than expected: 5.49 vs. 4.29%/h for whole seeds and 5.01 vs. 4.18%/h for broken seeds, respectively. Consequently, UIS was lower than expected (47% vs. 51% for whole seeds and 50% vs. 57% for broken seeds).

In Chapter 2, the effects of pressure toasting, expander treatment and pelleting on the rumen degradability and intestinal digestibility was studied. In this experiment, a mixture of coarsely broken peas, lupins and faba beans was used. The broken feedstuffs were from the same batches as those used in the experiment described in Chapter 1. The treatments included pressure toasting (3 min, 132°C), expander treatment (8 s, 115°C) and pelleting (10 s, 80°C). In contrast to Chapter 1, samples were incubated without grinding which means that the physical form (broken seeds, expanded broken seeds or pelleted broken seeds, or combinations of these treatment) was maintained during the incubations.

Toasting was more effective in altering rumen degradability of protein than expander treatment or pelleting. Toasting decreased rumen protein degradability significantly, mainly by reducing its k_d. Expander treatment and pelleting increased the W of protein, whereas pelleting additionally increased k_d, resulting in a decreased UIP. The observed in situ effects of both expander treatment and pelleting could be explained by particle size reduction during processing. Combinations of toasting, expander treatment and pelleting resulted sometimes in interactions, but the order of treatments hardly affected the effects on protein and starch degradability.

Comparisons with literature showed, that the effects on starch and protein degradability are very much dependent on the conditions applied during processing. A concept was proposed, describing the effects of heat, moisture level and shear and pressure during steam treatment on in situ starch degradability.

In the Chapters 3 to 6, the results of an experiment are described in which whole faba beans, peas, lupins and soybeans were toasted at 100, 118 or 136°C for 3, 7, 15 or 30 min. This study was set up to determine the optimal treatment and to study the differences in sensitivity with respect to toasting between different legume seeds.

Ground samples were incubated in the rumen to measure in situ rumen degradability and in the intestines of dairy cows to measure the intestinal digestibility of protein and starch. In Chapter 3, the effects of toasting on the rumen degradability and intestinal digestibility were described, as well as the effect on the intestinal absorbable protein (DVE) content of faba beans, peas, lupins and soybeans.

Toasting significantly increased UIP by decreasing both the W and the k_d of crude protein (CP). The UIP of the untreated faba beans, peas and lupins was 21, 21, and 21. Toasting at 136°C for 3 min resulted in an increase of UIP. For faba beans, peas and lupins UIP was 44, 47 and 49%, respectively, which was consistent with the results described in Chapter 1.

Total tract protein digestibility of faba beans, peas and soybeans was not affected by the treatment, but for lupins it was slightly decreased.

Faba beans and peas show great resemblance in chemical composition, and were also similar with respect to the rumen degradability and the sensitivity to the different processing conditions. Likewise, lupins and soybeans showed a similar behaviour after toasting.

For faba beans and peas, W consistently decreased when temperature or time of processing were increased. For lupins and soybeans, W decreased after toasting at 100°C, but slightly increased after toasting at higher temperatures. The k_d was strongly decreased after toasting at 136°C. Generally, higher temperatures during processing resulted in a higher UIP and DVE. The largest increases of UIP and DVE were found after toasting for 15 min at 136°C. The fraction of UIP increased by 152%, 156%, 142% and 56%, while for DVE, the increases were 91%, 80%, 76% and 71% for faba beans, peas, lupins and soybeans, respectively.

Correcting for the loss of small particles from the nylon bags increased the levels of UIP,

as well as the effect of toasting on UIP and DVE. Based on the results of this study, it was concluded that toasting is an economical method to increase the protein value of faba beans, peas, lupins and soybeans.

In Chapter 4, the results of toasting on in situ protein degradability and digestibility characteristics, as described in Chapter 3, were compared with the results of laboratory measurements. Protein dispersibility index (PDI) and denaturation enthalpy (δH) according to differential scanning calorimetry (DSC) were used to determine effects of toasting on protein denaturation. Dry and wet sieve analyses were used to characterize particle size distribution.

For all legume seeds, the PDI significantly decreased after toasting. Differences in PDI due to processing conditions, however, differed between legume seeds. The untreated legume seeds had a similar denaturation enthalpy (δH_{tot}). After toasting, the residual denaturation enthalpy was decreased. For lupins and soybeans, two distinct endothermic transition peaks were observed, which were attributable to the 7S and 11S protein fractions in these seeds. For faba beans and peas, only one peak was observed. The first peak in lupins and soybeans was more sensitive to toasting than the second one. The first peak disappeared after intermediate toasting conditions, whereas the second peak was only completely denatured after 15 min toasting at 136°C.

The lowest PDI and denaturation δH_{tot} values coincided with the highest values for the intestinally digestible undegraded protein (DUP) concentration. Based on linear regression, DUP could be predicted very well from PDI, except for lupins, where it failed to discriminate the differences in DUP due to processing conditions. The denaturation δH_{tot} was more discriminating than PDI at higher DUP values. For lupins and soybeans, differences between observed and predicted values were larger for untreated and mild toasting treatments, even though mean prediction errors for DUP were only 10%.

There were only small differences in in vitro protein digestibility (IVPD) due to toasting. Only severe toasting decreased IVPD for peas and lupins. Correlations of IVPD and in situ intestinal digestibility and total tract protein digestibility (%TDP) were poor. A significant positive correlation of IVPD was only found with %TDP (r = 0.80, P ≤ 0.0001) for lupins. Results of wet sieve analysis indicated that ground toasted legume seeds had a larger apparent mean N particle size than untoasted seeds, which coincided with a decreased rumen protein degradability.

It was concluded that PDI and denaturation δH_{tot} are useful indicators for protein

denaturation, which can be used to evaluate effects on DUP after toasting.

The IVPD, however, failed to discriminate between in situ digestibility of untreated and toasted legume seeds in general, but could be used to evaluate effects on %TDP for lupins.

In Chapter 5, the effects of toasting on the rumen degradability and intestinal digestibility of amino acids in lupins were described. Diaminopimelic acid (DAPA) was used to correct for microbial contamination of feedstuffs and incubation residues.

Toasting significantly decreased rumen degradability of amino acid N (AAN) by decreasing its washable fraction and its rate of degradation. Toasting for 15 min at 136°C slightly decreased the total tract digestibility of AA, but intestinal digestibility of rumen undegraded intake AA (UIAA) was increased after toasting. As a result, the amount of intestinal digestible rumen undegraded intake AA (DUAA) substantially increased. Effects of processing time and temperature showed a curvi-linear response for rumen degradability. For most amino acids, toasting for 30 min at 118°C resulted in a maximal or similar response to toasting at 136°C. Toasting for 30 min at 118°C increased the fraction UIAA of total AAN from 14.3 to 42.2%. For lysine and methionine, UIAA increased from 9.3 to 36.1% and from 0.9 to 27.7%, respectively. Since processing conditions affected degradability of individual AA in a similar way, it was concluded that toasting predominantly resulted in denaturation of protein, rather than decreasing protein degradability of some specific AA preferentially, for instance through Maillard reactions. The use of DAPA as a microbial marker revealed contamination of some toasted and dried lupins and in situ residues. The microbial contamination showed a positive relation with rumen incubation time. Based on the increased contamination in oven dried residues after washing, and in oven dried rumen incubation residues, compared to freeze dried residues, it was concluded that freeze-drying could overcome a substantial part of the contamination. Correction for microbial contamination resulted in a higher AAN degradability compared to uncorrected N degradability. Moreover, correction affected the ranking of treatments with respect to degradability of individual amino acids.

In Chapter 6, the effects of toasting on the digestive behaviour of starch in faba beans and peas were described. Toasting significantly increased the fraction of rumen undegraded starch (UIS), by decreasing the W and the k_d. Toasting faba beans and peas for 3 min toasting at 136°C resulted in an UIS of 44 and 47%, respectively. This was consistent with

the results after toasting in Chapter 1. Effects of processing conditions were not significant for total tract starch digestibility (%TDS) and intestinal digestibility of starch (%DUS) but values were numerically higher after toasting. Values for %TDS and %DUS, as measured by mobile nylon bag incubations, were low compared to in vivo results from literature. The largest increase of UIS and DUS was observed after toasting for 15 min at 136°C. Using standard procedures for calculating starch degradability, UIS increased by 80 and 57% for faba beans and peas, respectively, while for DUS increases were 87 and 49%. The effects on UIS were inconsistent with the increased degree of gelatinization after toasting, measured either in vitro or with differential scanning calorimetry (DSC). The latter results showed that toasting, especially at 136°C, resulted in a considerable gelatinization of starch. Toasting changed the distribution of starch over different particle size classes, as was determined by wet and dry sieve analysis. The fraction of starch in the particles < 71 μm decreased after toasting, which was in line with the large decrease of W. The filter solubility (FS) of starch was much smaller than W, and was only slightly decreased after toasting. The use of FS instead of W (the standard procedure) in the calculation of %UIS resulted in a drastic decrease of the effect of toasting on UIS. Based on the strong correlation between the UIP (Chapter 3) and UIS and the results of sieve analysis it was concluded that denaturation of the protein matrix as well as the change in the distribution of starch of the particles were responsible for the increase of UIS and DUS.

Effect of toasting on in vivo digestive behaviour of protein and starch.
The results of toasting on degradation and digestibility of legume seeds, as described in this thesis, are all based on in situ measurements. A proper in vivo evaluation of the results, especially those which were in disagreement with prior hypothesis seems necessary, since the number of studies in this area is limited.
In cooperation with the department of Animal Science of the Agricultural University in Ås (Norway), two experiments were carried out, focussing on reduction of rumen degradability of cereal starches by toasting. In the first experiment, an in situ screening was carried out with 3 cereals (barley, oats, corn), 3 processing temperatures (100, 118 and 136°C) and 5 processing times (1.5, 3, 7, 15, 30 min) (J.O. Goelema, O.M. Harstad and T. Gotvassli, unpublished results). The results for barley and oats were coherent with the results for faba beans and peas. Despite the large difference in initial starch degradability and starch and protein content in these seeds, toasting resulted in an increase of UIS for barley and oats. For corn, UIS decreased after toasting, which was in contrast with the results for

faba beans, peas, barley and oats. This suggests that the starch to protein ratio indeed may play a critical role in obtaining an effect of toasting on UIS. The results obtained demonstrate, that the results of toasting on in situ starch degradability are not exclusively due to specific characteristics of legume seeds, and that the underlying mechanisms are the same for other starchy feedstuffs.

The second experiment was an in vivo evaluation with lactating dairy cows (T. Gotvassli, O.M. Harstad, J.O. Goelema, and S. Tamminga, unpublished results). Untreated barley was compared with two batches of toasted barley (2 different treatments). The results suggested, that the starch degradability of untreated barley based on in situ measurements was overestimated, but that the in situ results for the toasted samples were in good agreement with the in vivo results. Moreover, the order of the degradability of the untoasted and two treated barleys remained unchanged. These results support the hypothesis stated above, that only a part of the washable fraction is immediately available and incorporated in the microbial mass, and that, especially when the washable fraction is large, a substantial part of the washable starch fraction escapes fermentation via rumen outflow. Feeding toasted barley also affected milk production: daily milk yield increased by 1.5 kg/cow, milk protein concentration increased, and milk fat concentration decreased, while the N-balance increased.

Summarized, the following conclusions can be drawn:
- Toasting decreases in situ rumen degradability of protein and starch.
- Pelleting increases in situ rumen degradability of protein and starch.
- Expander processing under mild conditions increased in situ rumen degradability of protein and starch.
- Effects of toasting, expander treatment and pelleting on in situ degradability are associated with changes in protein denaturation, starch gelatinization but also in particle size.
- Toasting at 132°C for 3 min has more impact on in situ rumen degradability of starch and protein than the tested conditions of expander treatment and pelleting.
- Effects of toasting on the amount of intestinal digestible protein in legume seeds can be accurately predicted from the changes in protein dispersibility index or in denaturation enthalpy, as measured by differential scanning calorimetry.
- Toasting may affect production responses in dairy cows, resulting in an improved N balance and an increase of the farmers profits.

SAMENVATTING

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Samenvatting

J.O. Goelema

Inleiding

Het verteringsgedrag van voer in de voormagen en darmen van melkkoeien staat sterk in de belangstelling. Hiervoor zijn tenminste twee redenen, te weten:

1) De afbraak van voereiwit en de synthese van microbieel eiwit zijn zelden met elkaar in evenwicht. Hierdoor ontstaan onnodige stikstof (N) verliezen uit de pens.

2) De verhouding waarin de verschillende vluchtige vetzuren (VFA: azijnzuur, propionzuur en boterzuur) en melkzuur gevormd worden, is grotendeels afhankelijk van de snelheid waarmee het substraat (structurele en niet-structurele koolhydraten en eiwit) wordt afgebroken. De mate waarin de afbraak van voereiwit en de synthese van microbieel eiwit met elkaar in evenwicht zijn en de verhouding waarin de VFA worden gevormd, zijn van belang voor de soort nutriënten die voor het dier beschikbaar komen, waardoor de productie en samenstelling van de melk beïnvloed wordt.

Het is duidelijk dat het voordelig is om de afbraak van structurele koolhydraten in de pens te maximaliseren. De afbraak van eiwit en niet-structurele koolhydraten kan worden beperkt tot het niveau dat nodig is voor de handhaving van een efficiënte microbiële activiteit en groei, onder de aanname dat de fractie die onafgebroken de pens verlaat, wordt verteerd in de dunne darm. Vertering van voereiwit en niet-structurele koolhydraten in de dunne darm heeft tevens als voordeel dat er minder N en energie in de pens verloren gaat. Voor hoogproductief melkvee is zelfs bij een optimale pensfermentatie de microbiële eiwitsynthese onvoldoende voor het handhaven van producties van meer dan 25 kg melk per koe per dag. Om te voorzien in de behoefte van deze dieren moet tevens pensbestendig voereiwit gevoerd worden. Twee strategieën kunnen worden gevolgd: óf men maakt gebruik van voedermiddelen met van nature een hoge bestendigheid, zoals bijvoorbeeld (behandeld) dierlijk eiwit, óf men maakt gebruik van plantaardige eiwitten waarvan de eiwitbestendigheid met behulp van technologische behandelingen is verhoogd.

In het hier beschreven onderzoek zijn fysische behandelingen onderzocht, met als doel

de verhoging van de pensbestendigheid van eiwit in de zaden van vlinderbloemigen. Wanneer onbehandelde zaden van vlinderbloemigen als eiwitbron in mengvoer voor rundvee gebruikt wordt, betekent dit dat de dieren een snel afbreekbare eiwitbron aangeboden krijgen. Het zetmeel in zaden van vlinderbloemigen zoals bijvoorbeeld erwten en faba bonen, heeft echter een meer geleidelijke afbraak.

In voedingssituaties voor melkkoeien waarin pensenergie een beperkende factor is, zal het voeren van onbehandelde zaden van vlinderbloemigen tot een verhoging van het N-overschot leidden, wat resulteert in een slechtere efficiëntie van N-benutting.

In dit proefschrift worden de effecten beschreven van technologische behandelingen van zaden van vlinderbloemigen op het verteringsgedrag van eiwit en zetmeel in de pens en darmen van melkkoeien. Deze informatie stelt mengvoederfabrikanten en nutritionisten in staat om krachtvoeders en rantsoenen samen te stellen die:

1. Leiden tot een optimale pensfermentatie.
2. De plaats van vertering gericht veranderen, wat de beschikbaarheid van nutriënten beïnvloedt.
3. Bijdragen tot een vermindering van de onnodige verliezen die in de pens ontstaan.

In de hoofdstukken worden de resultaten van drie experimenten beschreven. In deze experimenten zijn drie technologische behandelingen bestudeerd:

A. Druktoasten
B. Conditioneren/expanderen
C. Conditioneren/pelleteren

In het experiment dat in hoofdstuk 1 beschreven staat, worden de effecten van druktoasten beschreven. Erwten, lupinen en faba bonen werden geheel, of in grof gebroken vorm, getoast. Van de onbehandelde en de behandelde zaden zijn met de nylon zakjes methode de in situ pensafbraak en darmverteerbaarheid van eiwit en zetmeel bepaald. Tevens is onderzocht of er interactie optreedt indien een mengsel van de zaden getoast wordt. Hiervoor zijn de resultaten van een getoast mengsel van erwten, bonen of lupinen vergeleken met het gewogen gemiddelde van de afzonderlijk getoaste zaden. Druktoasten gedurende 3 min bij 132°C verlaagde de in situ eiwitafbraak van erwten, lupinen en faba bonen en de in situ zetmeelafbraak van erwten en faba bonen, vooral

indien gebroken in plaats van hele zaden werden behandeld.

Door het toasten steeg de eiwitbestendigheid (UIP) van hele en gebroken zaden van 25% naar respectievelijk 44 en 52% voor erwten, van 22% naar 47 en 51% voor lupinen en van 20% naar 48 en 52% voor faba bonen. De zetmeelbestendigheid (UIS) nam toe van 39% naar 50% en 53% voor respectievelijk hele en gebroken erwten en van 33% naar 53% en 60% voor hele en gebroken faba bonen. De totale eiwitverteerbaarheid van erwten en faba bonen, gemeten na 12 uur pensincubatie en vervolgens darmincubatie, werd niet beïnvloed door toasten, maar voor gebroken lupinen werd na toasten een lichte daling waargenomen. Na toasten daalde de uitwasbare fractie (W) van eiwit en zetmeel. Na toasten daalde de fractionele afbraaksnelheid (k_d) van eiwit, terwijl de k_d van zetmeel steeg. Er werd geconcludeerd dat de eiwitbestendigheid steeg, zonder dat de totale eiwitverteerbaarheid sterk beïnvloed werd.

Het toasten van een mengsel van erwten, faba bonen en lupinen resulteerde in een lagere zetmeelbestendigheid dan werd verwacht op basis van de afzonderlijk getoaste zaden. De k_d's van het mengsel waren hoger dan verwacht (5.49 versus 4.29%/uur en 5.01 en 4.18%/uur voor respectievelijk de hele en de gebroken zaden), waardoor de UIS lager was dan werd verwacht (47 versus 51% voor hele zaden en 50 versus 57% voor gebroken zaden).

In hoofdstuk 3 zijn de effecten van druktoasten, expanderen en pelleteren op de pensafbraak en darmverteerbaarheid beschreven. In het experiment werd een mengsel van grof gebroken mengsel van erwten, faba bonen en lupinen gebruikt. De gebroken zaden waren afkomstig van dezelfde partijen als in hoofdstuk 1. In het experiment zijn drie behandelingen bestudeerd: druktoasten (3 min, 132°C), expanderen (8 s, 115°C) en pelleteren (10 s, 80°C). In tegenstelling tot in hoofdstuk 1 werden de monster voor incubatie in de pens niet gemalen. Dit betekent dat de fysische vorm van de zaden (grof gebroken, geëxpandeerde gebroken of gepelleteerde gebroken zaden, of de diverse onderzochte combinaties van deze behandelingen) gehandhaafd bleef.

Toasten had meer effect op de pensafbraak van eiwit dan expanderen of pelleteren. Door toasten steeg de eiwitbestendigheid significant, vooral door een daling van de k_d.

Expanderen en pelleteren verhoogden de W, terwijl pelleteren tevens de k_d verhoogde. Dit resulteerde in een daling van de UIP. De waargenomen veranderingen in in situ afbraak konden worden gerelateerd aan de veranderingen van de deeltjesgrootte tijdens de behandelingen. Combinaties van toasten, expanderen en pelleteren resulteerden soms

in interacties, maar de volgorde van de behandelingen had nauwelijks invloed op het uiteindelijke effect op de pensafbraak en darmverteerbaarheid eiwit en zetmeel. Vergelijking van deze resultaten met resultaten die in de literatuur beschreven zijn gaf aan, dat de effecten van technologische behandelingen in sterke mate afhankelijk zijn van de gebruikte instellingen tijdens de behandelingen. Er is een concept voorgesteld dat de effecten van hitte, vochtgehalte, afschuifkrachten ('shear') en druk tijdens stoombehandelingen op de in situ zetmeelafbraak beschrijft.

In de hoofdstukken 3 tot en met 6 zijn de resultaten van een experiment met faba bonen, erwten, lupinen en sojabonen beschreven. In dat experiment zijn hele zaden getoast bij 100, 118 of 136°C gedurende 3, 7, 15 of 30 min. De studie was opgezet om de optimale behandelingscombinatie tijdens toasten te bepalen en om de verschillen in gevoeligheid tussen de zaden van vlinderbloemigen met betrekking tot de hittebehandeling te bestuderen. De monsters werden in gemalen vorm geïncubeerd om de pensafbraak en darmverteerbaarheid te bepalen. Tevens zijn in het laboratorium diverse andere metingen verricht om de waargenomen verschillen mogelijk te kunnen verklaren.

In hoofdstuk 3 zijn de effecten van toasten op de pensafbraak en darmverteerbaarheid van eiwit de eiwitwaarde (DVE) beschreven. Toasten leidde tot een significante verhoging van de UIP, doordat zowel de W als de k_d afnam. De UIP van de onbehandelde faba bonen, erwten, lupinen en sojabonen bedroeg respectievelijk 21, 21, 21 en 28%. Het gedurende 3 min toasten bij 136°C leidde tot een verhoging van de UIP. Voor faba bonen, erwten, lupinen en sojabonen steeg de UIP tot respectievelijk 44, 47, 49 en 41%. Deze resultaten kwamen overeen met de resultaten die in hoofdstuk 1 beschreven zijn.

De totale eiwitverteerbaarheid van faba bonen, erwten en sojabonen werd niet beïnvloed door toasten, terwijl voor lupinen een lichte daling gevonden optrad.

Faba bonen en erwten vertoonden grote overeenkomst in chemische samenstelling en tevens in de mate waarin de pensafbraak veranderde onder invloed van de toastbehandeling. Lupinen en sojabonen vertoonden eveneens een vergelijkbare gevoeligheid voor de toastbehandelingen. Een verhoging van de toasttemperatuur of de procestijd leidde voor faba bonen en erwten tot een consistente daling van de W. Voor lupinen en sojabonen daalde de W indien bij 100°C de procestijd verlengd werd, maar de W steeg indien bij een hogere temperatuur getoast werd. Door te toasten bij 136°C daalde de k_d sterk. In het algemeen leidden hogere toasttemperaturen en langere procestijden tot een hogere UIP en DVE. De grootste toename werd gevonden na 15 min

toasten bij 136°C. De UIP van faba bonen, erwten, lupinen en sojabonen nam respectievelijk met 152%, 156%, 142% en 56% toe, terwijl de DVE met 91%, 80%, 76% en 71% toenam.

De correctie voor het verlies aan kleine, onoplosbare deeltjes uit de nylon zakjes leidde tot een verhoging van de UIP en de DVE, en tot een de groter effect van de toastbehandeling op de UIP en de DVE. Op basis van deze studie werd geconcludeerd dat toasten een economische methode is voor de verhoging van de DVE van faba bonen, erwten, lupinen en sojabonen.

In hoofdstuk 4 zijn de effecten van toasten op de pensafbraak en darmverteerbaarheid van eiwit vergeleken met de resultaten van laboratorium bepalingen. De eiwitdispergeerbaarheid (PDI) en de denaturatie enthalpie (δH), gemeten met differential scanning calorimetry (DSC), zijn gebruikt om de effecten van toasten op de denaturatie van eiwit te meten. Droge en natte zeefanalyses zijn uitgevoerd om de verdeling van de deeltjesgrootte vast te stellen. Voor alle zaden van vlinderbloemigen leidde toasten tot een significante daling van de PDI. De mate waarin de PDI daalde als gevolg van de toastcondities, verschilde echter tussen de zaden. De verschillend onbehandelde zaden van hadden een vergelijkbare denaturatie enthalpie (δH$_{tot}$). Na toasten daalde de residuele denaturatie enthalpie. Voor lupinen en sojabonen werden twee verschillende overgangspieken waargenomen, die toegeschreven werden aan de twee belangrijkste eiwitfracties in deze zaden, de 7S en de 11S fractie. Voor faba bonen en erwten werd slechts 1 eiwitpiek waargenomen.

De eerste piek voor lupinen en sojabonen was gevoeliger voor toasten dan de tweede piek. De eerste piek verdween na een matig intensieve toastbehandeling, terwijl de tweede piek pas volledig verdwenen was na 15 min toasten bij 136°C.

De laagste waarden voor de PDI en δH$_{tot}$ kwamen overeen met de hoogste waarden voor de hoeveelheid darmverteerbaar bestendig eiwit (DUP). De DUP kon op basis van de PDI met lineaire regressie goed voorspeld worden, behalve voor lupinen. Voor lupinen kon op basis van de PDI geen onderscheid tussen de verschillende procesomstandigheden worden gemaakt. Op basis van de denaturatie δH$_{tot}$ kon, vergeleken met de PDI, beter onderscheid gemaakt worden tussen de hogere waarden van de DUP. Voor lupinen en sojabonen waren de verschillen tussen de waargenomen en de op basis van lineaire regressie geschatte waarden groter voor onbehandelde en relatief mild behandelde zaden, alhoewel de gemiddelde schattingsfout voor de DUP slechts 10% bedroeg.

De verschillen in in vitro eiwit verteerbaarheid (IVPD) tussen de verschillende toastcondities waren relatief klein. Alleen voor erwten en lupinen leidde een intensieve toastbehandeling tot een daling van de IVPD. Er bestond daarom slechts een zwakke correlaties tussen de IVPD en de in situ darmverteerbaarheid en totale verteerbaarheid (%TDP) van eiwit. Er bestond alleen een significante positieve correlatie tussen de IVPD en de %TDP (r = 0.80, P ≤ 0.0001) voor lupinen. De resultaten van natte zeefanalyse gaven aan dat de schijnbare gemiddelde deeltjesgrootte voor N voor getoaste zaden van vlinderbloemigen groter was dan voor ongetoaste zaden. Deze stijging kwam overeen met een stijging van de eiwitbestendigheid (UIP).

Er werd geconcludeerd dat de PDI en de denaturatie δH_{tot} bruikbare parameters voor eiwitdenaturatie zijn, die gebruikt kunnen worden voor de voorspelling van de effecten van toasten op de DUP. In het algemeen bleek de IVPD ongeschikt om onderscheid te maken tussen de in situ verteerbaarheid van onbehandelde en getoaste zaden van vlinderbloemigen, alhoewel deze parameter wel gebruikt kan worden voor de evaluatie van de effecten van toasten op de %TDP van lupinen.

In hoofdstuk 5 zijn de effecten van toasten op de pensafbraak en darmverteerbaarheid van individuele aminozuren in lupinen beschreven. Diaminopimeline zuur (DAPA) werd gebruikt om te corrigeren voor de microbiële contaminatie van voedermiddelen en incubatieresiduen.

Toasten leidde tot een significante daling van de pensafbraak van aminozuur N (AAN), door een verlaging van de W en de k_d. Gedurende 15 min bij 136°C toasten leidde tot een lichte daling van de totale verteerbaarheid, maar de darmverteerbaarheid van de pensbestendige aminozuren (UIAA) nam toe na toasten. Hierdoor leidde toasten tot een aanzienlijke stijging van de hoeveelheid darmverteerbare bestendige aminozuren (DUAA) De pensbestendigheid vertoonde een curvelineair verband met de procestijd en -temperatuur. Voor de meeste aminozuren leidde 30 min toasten bij 118° tot een maximale of vergelijkbare respons als toasten bij 136°C. Gedurende 30 minuten toasten bij 118°C leidde tot een verhoging van de UIAA van totaal AAN van 14.3 naar 42.2%. Voor lysine en methionine nam de UIAA toe van respectievelijk 9.3 en 0.9% naar 36.1 en 27.7%.

Aangezien de procescondities de bestendigheid van de individuele aminozuren op dezelfde manier beïnvloedden, werd geconcludeerd dat toasten met name resulteert in de denaturatie van eiwit. Hierdoor worden alle aminozuren vergelijkbaar beïnvloed, in

plaats van een specifieke reactie van enkele aminozuren, bijvoorbeeld via Maillard reacties.

Het gebruik van DAPA als microbiële merkstof toonde aan dat enkele getoaste en vervolgens gedroogde lupinen, en de gedroogde in situ incubatieresiduen microbiële contaminatie vertoonden. Er bleek positieve relatie te bestaan tussen de incubatietijd in de pens, en de mate van contaminatie. Aangezien de contaminatie in de gevriesdroogde incubatie residuen veel geringer was dan in de gestoofdroogde residuen na pensincubatie en/of uitwassen, werd geconcludeerd dat vriesdrogen een aanzienlijk deel van de contaminatie kan voorkomen. De correctie voor microbiële contaminatie op basis van DAPA resulteerde in een lagere AAN bestendigheid vergeleken met de ongecorrigeerde eiwitbestendigheid. Tevens beïnvloedde de correctie de volgorde van de behandelingen met betrekking tot de bestendigheid van de individuele aminozuren.

In hoofdstuk 6 zijn de effecten beschreven van toasten op de pensafbraak en darmverteerbaarheid van zetmeel in faba bonen en erwten. Toasten leidde tot een significante verhoging van de zetmeelbestendigheid (UIS), door een daling van de W en de k_d. Drie minuten toasten bij 136°C leidde tot een UIS voor faba bonen en erwten van 44 en 47%. Dit was consistent met de resultaten na toasten, zoals beschreven in hoofdstuk 1. De effecten van de proces condities waren niet significant voor de totale verteerbaarheid van zetmeel (%TDS), noch voor de darmverteerbaarheid van bestendig zetmeel (%DUS), alhoewel de waarden van de getoaste zaden numeriek gezien hoger waren. De waarden voor de %TDS en de %DUS, gemeten met de mobiele nylon zakjes methode waren lager dan de literatuur aangaf voor de in vivo resultaten.

De grootste stijging van de UIS en de DUS werd waargenomen na 15 min toasten bij 136°C. Op basis van de standaard berekeningsmethode voor bestendig zetmeel steeg de UIS met respectievelijk 80 en 57%, voor faba bonen en erwten. De DUS nam toe met 87 en 49%. De toename van de UIS was in tegenspraak met de stijging van de in vitro ontsluitingsgraad van zetmeel en de daling van de zetmeel onsluitingsenthalpie, gemeten met DSC. Beide methoden gaven aan dat toasten, vooral bij 136°C, resulteert in een aanzienlijke ontsluiting van zetmeel. Toasten veranderde daarnaast eveneens de verdeling van zetmeel over de deeltjesgrootte klassen, zoals bepaald werd met natte en droge zeefanalyse. De fractie zetmeel in de deeltjes < 71 μm daalde na toasten, wat overeenkwam met de aanzienlijke daling van de W na toasten. De filter oplosbaarheid (FS) van zetmeel was veel kleiner dan de W, en vertoonde na toasten slechts een geringe

daling. Het gebruik van de FS in plaats van de W (de standaard methode) voor de berekening van de UIS resulteerde dan ook in aanzienlijk kleiner effect van toasten op de UIS. Op basis van de sterke correlatie tussen de UIP (hoofdstuk 3) en de UIS en de resultaten van de zeefanalyses, werd geconcludeerd dat zowel de denaturatie van de eiwit matrix als de verandering in de verdeling van zetmeel over de deeltjesgrootteklassen geleid hebben tot de stijging van de UIS en de DUS na toasten.

De effecten van toasten op het in vivo verteringsgedrag van eiwit en zetmeel
De in dit proefschrift beschreven resultaten van toasten op de pensafbraak en darmverteerbaarheid van zaden van vlinderbloemigen zijn allemaal gebaseerd op in situ metingen. Een in vivo evaluatie van de resultaten, met name van die resultaten die niet overeenkwamen met de oorspronkelijke hypothesen lijkt zinvol, aangezien het aantal studies dat op dit gebied is uitgevoerd, beperkt is.
In samenwerking met de vakgroep Animal Science van de Agricultural University in Ås (Noorwegen), zij twee experimenten uitgevoerd, die gericht waren op het verhogen van de zetmeelbestendigheid van granen. Het eerste experiment bestond uit een in situ screening waarin 3 granen (gerst, haver en mais) getoaste werden bij 3 proces temperaturen (100, 118 en 136°C) en 5 proces tijden (1.5, 3, 7, 15, 30 min) (J.O. Goelema, O.M. Harstad en T. Gotvassli, ongepubliceerde resultaten). De resultaten voor gerst en haver kwamen overeen met de resultaten voor faba bonen en erwten. Ondanks het grote verschil in zetmeelbestendigheid en eiwitgehalte resulteerde toasten ook voor gerst en haver in een toename van de zetmeelbestendigheid. Voor mais werd echter een daling van de bestendigheid gevonden. Dit suggereert, dat de verhouding zetmeel/eiwit een belangrijke rol kan spelen bij de daling van de UIS na toasten. Deze resultaten tonen tevens aan, dat de effecten van toasten op de zetmeelbestendigheid niet alleen voor zaden van vlinderbloemigen gelden, maar dat de daaraan ten grondslag liggende mechanismen ook voor ander zetmeelbronnen gelden.
Het tweede experiment bestond uit een in vivo evaluatie met lacterende melkkoeien (T. Gotvassli, O.M. Harstad, J.O. Goelema en S. Tamminga, ongepubliceerde resultaten). Onbehandelde gerst werd vergeleken met getoaste gerst (2 verschillende behandelingen). De resultaten toonden aan, dat de zetmeelbestendigheid van de onbehandelde gerst met in situ metingen werd overschat, maar dat de in situ resultaten voor de getoaste monsters goed overeenstemden met de in vivo resultaten. Tevens bleek de volgorde van de bestendigheid van de ongetoaste en getoaste gerst ongewijzigd. Deze resultaten

bevestigden de eerdere hypothese, dat slechts een gedeelte van de uitwasbare zetmeelfractie direct beschikbaar is, en benut wordt voor microbiële groei. Tevens gaf het aan, dat een aanzienlijk deel van de uitwasbare fractie de pensfermentatie kan ontsnappen via de passage. Het voeren van de getoaste gerst had ook effect op de melkproductie: de gemiddelde melkproductie steeg met 1.5 kg/koe, het melkeiwitgehalte steeg, het melkvetgehalte daalde. Hierdoor steeg tevens de N-balans.

Op basis van de in dit proefschrift beschreven resultaten zijn de volgende conclusies getrokken:
- Toasten verhoogt de in situ bestendigheid van eiwit en zetmeel.
- Expanderen onder milde condities verlaagt de in situ bestendigheid van eiwit en zetmeel.
- Pelleteren verlaagt de in situ bestendigheid van eiwit en zetmeel.
- De effecten van toasten, expanderen en pelleteren op de in situ bestendigheid zijn gerelateerd aan de veranderingen in eiwitdenaturatie, zetmeelontsluiting en deeltjesgrootte
- Gedurende 3 min toasten bij 132°C heeft meer effect op de in situ pensafbraak van eiwit en zetmeel dan de onderzocht condities tijdens expanderen en pelleteren.
- De effecten van toasten op de hoeveelheid darmverteerbaar bestendig eiwit in zaden van vlinderbloemigen kan nauwkeurig voorspeld worden op basis van de verandering in eiwitdispergeerbaarheid (PDI) of de denaturatie enthalpie, gemeten met differential scanning calorimetry.
- Het voeren van getoaste grondstoffen kan de melkproductie van melkkoeien beïnvloeden, wat kan resulteren in een verhoging van de N-balans en het inkomen van melkveehouders.

Curriculum Vitae

Jacob Otto Goelema werd op 23 juni 1965 geboren te Zuidlaren. In juni 1984 behaalde hij het Atheneum diploma aan het Augustinuscollege te Groningen. In september van datzelfde jaar begon hij met een studie Nederlandse Landbouw, oriëntatie preventieve gezondheidszorg aan de Rijks Hogere Landbouwschool te Groningen. Na afronding van deze studie kwam hij in oktober 1988 als statistisch/epidemiologisch analist in dienst van de Landbouwuniversiteit te Wageningen, bij de sectie Gezondheids- en Ziekteleer van de vakgroep Veehouderij. In september 1991 startte hij met het doorstroomprogramma Zoötechniek aan de Landbouwuniversiteit. In juni 1993 studeerde hij af met als oriëntatie Veevoeding en als afstudeervakken Veevoeding en Agrarische Bedrijfseconomie. In september 1993 werd hij aangesteld als Assistent In Opleiding (AIO) bij de vakgroep Veevoeding. De resultaten van de in het kader van dit AIO-project uitgevoerde onderzoek zijn grotendeels beschreven in dit proefschrift. Sinds 15 oktober 1998 is hij als nutritionist werkzaam bij Pre-Mervo U.A. te Utrecht.