Röbäcksdalen
meddelar

The effect of different processing methods on rumen degradation of protein and starch in threshed peas

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1 Abstract

Legumes have increased in interest as a locally produced low input protein feed for ruminants in northern Europe. Peas are of special interest in Sweden since they are possible to cultivate on most farms. Protein feed is also a limited resource in organic farming, especially for high producing dairy milk cattle. Farmers often have to complement feed rations with conventional produced protein concentrate. The limitation with all legume grains, including peas, is that their protein is very soluble in the rumen. As a result, the pea protein is almost immediately degraded to a great deal and much of the protein, which is transformed to ammonia, is lost through the rumen wall, transported with the blood and excecreted with the urine. The consequence is that much nitrogen is lost to the environment instead of being synthesised as microbe protein. Different methods of feed processing, including preservation of feed and milling, rolling and heat treatment could improve protein utilisation. Of great importance affecting rumen degradation rate is particle size in processed peas. The extent to which peas are processed may also affect the passage rate through the rumen. A combination of different processing: preservation methods (i.e. wet or dry), grinding, flaking and cracking might give an additive effect in reducing protein degradation in the rumen.

2 Abbreviations list

AA	amino acid
CP	crude protein
DM	dry matter
ECM	energy corrected milk
FCM	fat corrected milk
FSG	functional specific gravity
RDP	rumen degradable protein
SBM	soy bean meal
SG	specific gravity
VFA	volatile fatty acids

3 Introduction

Legume seeds have attracted attention as components of feedstuffs for dairy cows as protein supplements in recent years mainly because: (1) legume seeds usually have a particularly high protein and/or starch content; and (2) a diversity of legume species, with high seed yields, are well suited to the various ecological and climatic conditions in many countries. However, the use of legume seeds in feeding dairy cows is limited and the utilisation of N is inefficient under certain conditions. An important reason is that the content of soluble or rapidly degradable protein of many legume seeds is high. These cause an imbalance between feed protein breakdown and microbial protein synthesis, resulting in unnecessary N loss from the rumen. Often there is an overall surplus of rumen-degraded protein. This is because microbial growth rate and protein synthesis cannot be increased at the same time as NH_3 concentration is maximised, and subsequently after early rapid degradation, a shortage of feed N in the rumen will occur (Yu *et al.,* 2002).

Microorganisms and their proteolytic enzymes must gain access to their substrate in order for protein breakdown to occur. Soluble proteins are more susceptible to degradation than are insoluble proteins, but exceptions to this rule do exist. Cross-linking, particularly through disulphide bonds has a major influence on protein degradability. A reduction in the rate or extent of degradation of protein of legume seeds in the rumen will only be beneficial to the animal when the protein escaping degradation in the rumen is digested and amino acids (AA) and peptides are absorbed from the small intestine (Yu *et al.,* 2002). The principal limitations to the more efficient use of established grain legume crops for increased animal performance appear to be the high proportion of the grain legume protein present as rumen degradable protein, and the low sulphur-containing amino acid content of the protein. Effective low cost means to increase the escape of grain legume protein from the rumen and hence the undegraded dietary protein content is needed (Dixon & Hosking, 1992).

From sowing to harvest, peas need 110-120 days, earliness depends on cultivars (Johansson, 1999). Peas, as all pulses, are usually harvested as grains when the water content is below 20 per cent (Johansson, 1999). The breeding of pea varieties have improved pea cultivating properties, earliness, stem strength and suitability for combine harvesting, unfortunately in many cases resulting in declined protein value and decreased quality (Saastamoinen, 1990). Modern varieties with stiffer straws are easier to thresh than older varieties (Johansson, 1999; Bingefors *et al*., 1979).

4 Nutritional characteristics of peas

4.1 Gross composition in peas

The main nutrients of interest in peas are starch and protein, which in average constitute about 440 and 225 g kg^{-1} DM respectively from the crop. Neutral detergent fibre (NDF) constitute on average 63 g kg^{-1} DM in whole peas. Total free sugars in whole peas constitutes about 125 g kg^{-1} DM. The main sugars are raffinose, stachyose and verbascose, at 12, 32, 19 g kg^{-1} DM each. Fat content, which is low in peas compared to other feedstuffs (Khorasani & Kennelly, 1997; Dandanell-Daveby, 1997), ranges between 10 – 24 g kg^{-1} DM. Dominating fatty acids in peas is oleic and linoleic acid. Ash content is normally about 33 g kg^{-1} DM. Legumes are good sources of minerals and water-soluble vitamins, especially thiamine, riboflavin and niacin (Dandanell, 1997). The energy content of peas is similar to corn and wheat (Corbett 1997). Thomke (1979) implies that there is no difference in amino acid pattern between white flowered or variegated peas.

4.2 Protein

Even though starch is the major constituent, peas are considered a protein crop. Protein content in peas ranges between 180 – 300 g kg^{-1} DM. Pea proteins are fractionated into albumins (210 g kg^{-1}), globulins (e.g. legumin and vicilin; 660 g kg^{-1}) and glutelins (120 g kg^{-1}), divided after their solubility (Van Soest, 1994; Dandanell-Daveby, 1997). Albumins are water-soluble, globulins are salt soluble and glutelins are dilute alkali soluble (Van Soest, 1994; Yu *et al.,* 2002). Legumes tend to contain more soluble globulins and albumins, but these are sensitive to heat denaturation that renders them water insoluble (Van Soest, 1994). Pea protein is from the nutritional standpoint of a good quality, with high content of lysine and threonine. However the total content of sulphur containing AA is low, despite the rather high sulphur content of albumins. This shortage of sulphur AA decreases the nutritional values of peas (Dandanell-Daveby, 1997), however this decrease is less important in ruminant nutrition due to the extensive digestion of protein by ruminal microbes (Dixon & Hosking, 1992). Both methionine and cystine are first limiting AA in peas (Dixon & Hosking, 1992; Dandanell-Daveby, 1997). Since lysine is the first limiting AA in cereals, combining peas and cereals means improved protein quality in the mixture.

Proteins are macromolecular polypeptides, consisting of covalently bound α-amino acid residues forming the primary structure of the protein. The polypeptide secondary structure comprises helical coils, hold together by non-covalent bonds such as hydrogen bonds. This structure has several forms, the α-helix, a β-pleated sheet and the triple helix. Side chains in the α-helix are projected outward. Even in water-soluble protein, 40% of these chains are hydrophobic. The α-helices are positioned to minimize exposure of hydrophobic groups in aqueous solutions. The tertiary structure is folded and twisted positioning of the secondary structure, also stabilized by hydrogen bonds. Stable disulfide bonds occur when two polypeptide-SH group-containing chains are in close vicinity. Finally, the quarternary structure describes the way two or more polypeptides are associated, often involving for instance nonpolypeptide groups (Goelema, 1999).

Depending on its function in the plant, a varying proportion of the protein is present in the soluble fraction. 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 cellulase. 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 a quantitatively important fraction of total protein. Because part of the embryo is in fact a small plant, the characteristics of these proteins often is similar to that of growing plants. Storage proteins are often contained in protein bodies and are to be utilised by the embryo in its initial growth phase. Depending on their structure, susceptibility to proteolysis varies. Probably, the number of disulfide bonds is the primary structural characteristic that decreases susceptibility of proteins to proteolysis. They are known to be of particular importance in animal protein (Goelema, 1999).

The native protein configuration as it relates to starch granules can influence starch degradation. Starch granules can be embedded in a protein matrix of the endosperm of cereal grains, especially in corn and sorghum. Processing can disrupt this matrix and allow starch to be more accessible to enzymatic digestion. However, certain types of processing (those causing gelatinisation) may lead to formation of indigestible starch-protein complexes (Nocek & Tamminga, 1991).

4.3 Starch

Starch is the major storage carbohydrate as well as the dominating individual component in peas. In many plants starch is present in granules in leaves, stems, tubers and roots (potato, tapioca), fruits and seeds (cereals, legumes) (Van Soest, 1994), and varies in shape and size (Goelema, 1999). Pea starch makes up a large proportion of the seed, 410-540 g kg^{-1} DM, and is the major storage form of polysaccharides (Corbett, 1997; Khorasani & Kennelly, 1997). As illustrated in Figure 1, the glucose molecules in starch are polymerised into two polysaccharides: amylose and amylopectin. These polysaccharides are packed into a semi crystalline structure – the starch granule (Eliasson, 1988). A proteinaceous layer including some fat surrounds the starch granules. The surface sometimes has indentations, grooves, fissures and pores, which may serve as starting points for microbial attack (Goelema, 1999). Cousin (1997) suggests that smooth seeded varieties of peas have round granules, whereas most of those varieties with wrinkled-seed have composite granules. In most plants, a single starch granule is formed inside the amyloplast. In some plants (e.g. oats) several small granules (4-10 μm) are formed, which aggregate to a much larger complex (20-150 μm). Starch is sometimes classified as a soluble carbohydrate because of its gelatinisation and partial solubility in hot water. More properly it can be regarded as a reserve carbohydrate (Van Soest, 1994). The nutritive availability of starch depends on the animal's ability to cleave the glycosidic bonds, and animal digestive enzymes can hydrolyse the α- (1, 6) linkages in starch (Van Soest, 1994). The glucose units in amylose are linked by α-(1,4)-bonds, whereas α-(1,6)-linkages are present at the branching points of amylopectin. Unlike the β-(1,6)-glucose linkages in cellulose, the α-(1,4)-linkages in starch can be digested by enzymes produced by the small intestine. Consequently, starch which escapes digestion in the rumen can be digested in the intestine (McAllister & Cheng, 1996). The proportions of the different components affect the functional properties of the starch (Van Soest, 1994). Typical for legume starches is a high amylose content, and the starch granules contain only traces of lipids, are elliptical or oval in shape with a diameter of 19-28μm (Dandanell-Daveby, 1997). The non-soluble, rumen-degradable fraction has a slow rate of degradation in the rumen. In high concentrate diets, the ruminal degradation rate of pea starch is similar to corn and much slower than wheat, oats or barley (Corbett, 1997).

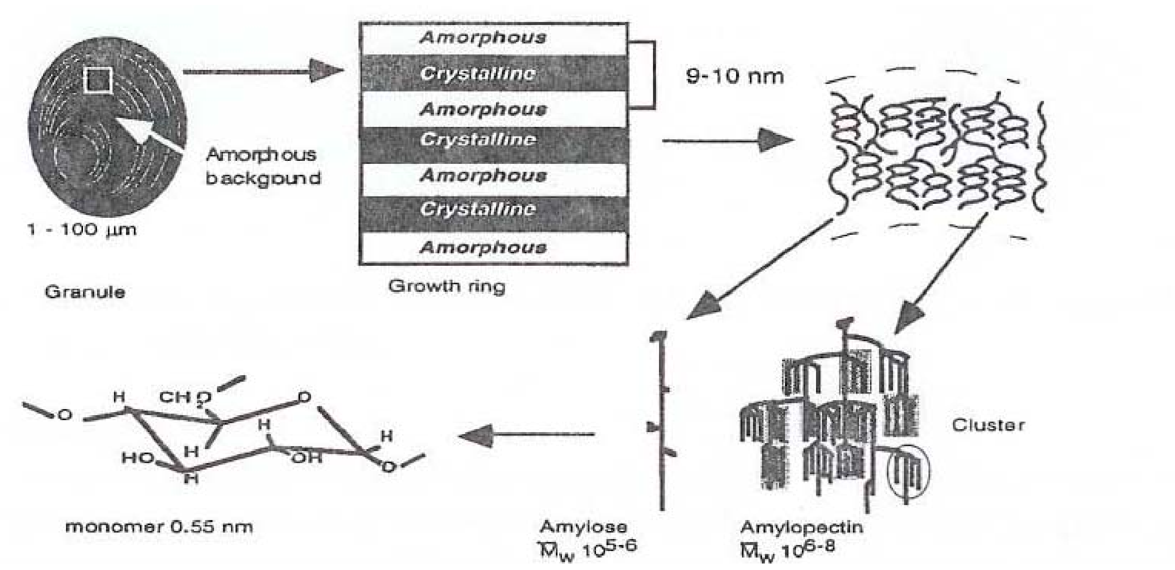

Figure 1. Schematic representation of the different structural levels of starch in the granule (Buleon *et al.*, 1998).

To increase milk production, it can be beneficial to decrease starch escape in the rumen (Nocek & Tamminga, 1991). Starch escaping rumen degradation decreases fermentation losses in the rumen and will, if digested in the intestines, also supply more glucose, which as an important precursor of lactose may result in sparing of AA (Yu *et al.*, 2002).

Noteworthy is that both Goelema (1999) and Yu *et al.*, (2002) claims that most starches in legume seeds are degraded very rapidly, in contrast to others who conclude that most legume starches degrade slowly (Corbett, 1995; 1997).

4.4 Antinutritional factors in grain legumes

In general, peas have a relatively low content of antinutritional substances. The main concern is the tannin content, since tannins can complex with feed proteins and microbial enzyme systems, reducing rumen microbial activity and digestion of dietary substrates (Dixon & Hosking, 1992). Tannins are generally considered heat stable, and in peas they are concentrated in the hull, and the content is related to flower colour rather than seed colour (Dandanell-Daveby, 1997). Both broad bean and variegated peas have 2-3 times higher tannin content than white flowered peas. However, tannin content does not appear to interfere with nutritional value for ruminants. Thomke (1979) observed no difference in digestibility and energy value between white flowered or variegated peas when fed to sheep. Other antinutritional factors to be considered in peas are lectins, protease inhibitors, and phytates. Lectins are heat labile proteins that can bind glycoproteins and carbohydrates, interfering with the absorption of digestion end products in the small intestine (Dixon & Hosking, 1992). In dehulled peas, lectin content increases during development (Dandanell-Daveby, 1997), but most lectin activity is inactivated by rumen fermentation (Dixon & Hosking, 1992). Protease inhibitor activity is particularly low in most pea cultivars, and like lectins most protease inhibitors are heat labile and are of little concern in processed legumes. In most legumes, phytate phosphorous account for about 80 % of the total phosphorous as a storage deposit. Phytate may interfere with essential minerals and reduce solubility and activity of proteins, but is effectively reduced by fermentation (Dandanell-Daveby, 1997).

Information on the ability of rumen fermentation to modify other antinutritional factors such as protease inhibitors, phytic acid and the raffinose family of oligosaccharides appears to be lacking. It is possible that effective protection against rumen degradation of grain legumes also protects inactivation of antinutritional factors and increases the passage of antinutritional factors to the small intestine (Dixon & Hosking 1992).

4.5 Time of harvest

In peas, protein is deposited during early development of the seeds, earlier than starch, resulting in large variations in protein and starch content (Håkansson *et al.*, 1986; Åman & Graham, 1987). In mature seeds, the protein content is highly variable, influenced by both genetic and environmental factors (Åman & Graham, 1987). The starch content of mature peas also varies greatly, and differences in starch structure and properties can occur among cultivars of the same species even under identical environmental conditions (Ratnayake *et al.,* 2001). The amylose component of starch increases during maturity, whilst there are no changes in the fine structure of amylopectin molecules (Biliaderis, 1982). It has been shown that most of the variation in protein content is explained by differences in starch content (Reichert & MacKenzie, 1982). Carbohydrate content is according to Black *et al.* (1998) highly and negatively correlated with protein content. Variability in protein content of field pea (cv. Trapper) was not related to the degree of maturation (Reichert & MacKenzie, 1982). Their results suggested that only peas within a narrow range of protein content can be used if uniform products are to be achieved (Reichert & MacKenzie, 1982). In field pea cv. Simo the gross chemical composition of pea seeds changes during maturation (Åman & Graham, 1987). When threshing Simo 86 days after sowing, the pea seeds had a higher content of fat, ash, sugars, protein and non-starch polysaccharides than pea seeds threshed 98 days after sowing. Glucose and sucrose content decreased almost by half, whilst the non-starch polysaccharide residues rhamnose, arabinose, xylose and galactose actually increased slightly at the later harvest occasion. The total AA content of pea seed increased between the two harvest occasions (Åman & Graham, 1987). Black *et al.* (1998) studied how physico-chemical properties varied in field peas; they concluded that year-to-year effects are of lesser concern in predicting quality parameters than genotypic effects. Another study performed of Kosson *et al.* (1994) showed that effectiveness of dehulling, and associated changes in gross composition (proteins, lipids and ash) is affected by pea type (smooth or wrinkled), kernel size, and kernel hardiness. Dandanell-Daveby (1997) summarizes results from three pea varieties; considerable chemical changes occurred during growth and maturation in both dehulled peas and hulls (Figure 2). Sucrose concentration was very high in the young dehulled pea, but decreased rapidly until starch concentration reached its maximum during development, where after starch content decreased somewhat. Crude protein showed an opposite pattern to starch. The concentration of dietary fibre and the raffinose series of oligosaccharides generally increased during growth and maturation. The hull content of NDF increased dramatically during development.

5 The digestive process

5.1 Particle size

Particle size and outflow from the rumen have a major influence on the digestive process. Breakdown of long feed particles has been suggested as the rate-limiting step in ruminant digestion. Major modes of particle breakdown occur through mastication, rumination, microbial fermentation and detrition by ruminal musculature activity. The force required to shear lignified fibres is increased. In milling, lignin becomes selectively distributed among the larger particles (Van Soest, 1994). Particle size reduction effectively increases surface area and makes particles more fragile and accessible for digestion. Decreasing particle size also increases particle density and specific gravity. These processes cause stratification of particles within the rumen: small particles sink (higher density) and are passed out of the rumen, and larger particles (lower density) rise within the ruminal strata and thus become further subjected to microbial breakdown and possibly rumination. It has also been suggested that chemical as well as physical composition will affect extent of digestion and particle breakdown. Particle breakdown is a dynamic process, and breakdown of large particles does not necessarily mean progression into the next smaller particle size pool. Particle size reduction is a function of initial particle size, rate of DM digestion, and quantity of DM that represents a given pool size (Nocek & Tamminga, 1991).

Rates of particulate and fluid passage will strongly influence whether or not starch will be digested in the rumen or escape to the lower gut (Nocek & Tamminga, 1991). Initially, feed particles are less dense than water (specific gravity <1.0). As hydration takes place, density of feed particles increases and hydration potential decreases. In addition, physical compaction associated with chewing, digestion etc causes particle density to increase. Chemical composition influences hydration and therefore density. Cellulosic materials resist hydration, whereas carboxyl groups in hemicellulose and phenolic groups of lignin contribute to ion-exchange capacity and hydration (Nocek & Tamminga, 1991). Heavy particles have been shown to shift through rumen stratification to a greater degree and to become more accessible for passage than lighter particles (Nocek & Tamminga, 1991). No critical specific gravity has been characterized for passage of particles through the rumen.

Critical particle size has been used as a concept in the development of models of digesta flow. The large size of the reticulo-omasal orifice in cattle is such that whole grains that are not crushed during eating are likely to pass through to the lower gut. This is more common in adult cattle, and when whole grain is fed as a supplement to roughage based diets (Orskov, 1980). However, this concept is contradicted when discussing critical particle size for passage out of the rumen, which has been suggested to be approximately 1.2 mm (Poppi *et al.,* 1980; Poppi *et al*., 1981). Poppi *et al.* (1981) concluded that particles greater than 1180 µm strongly resist escape

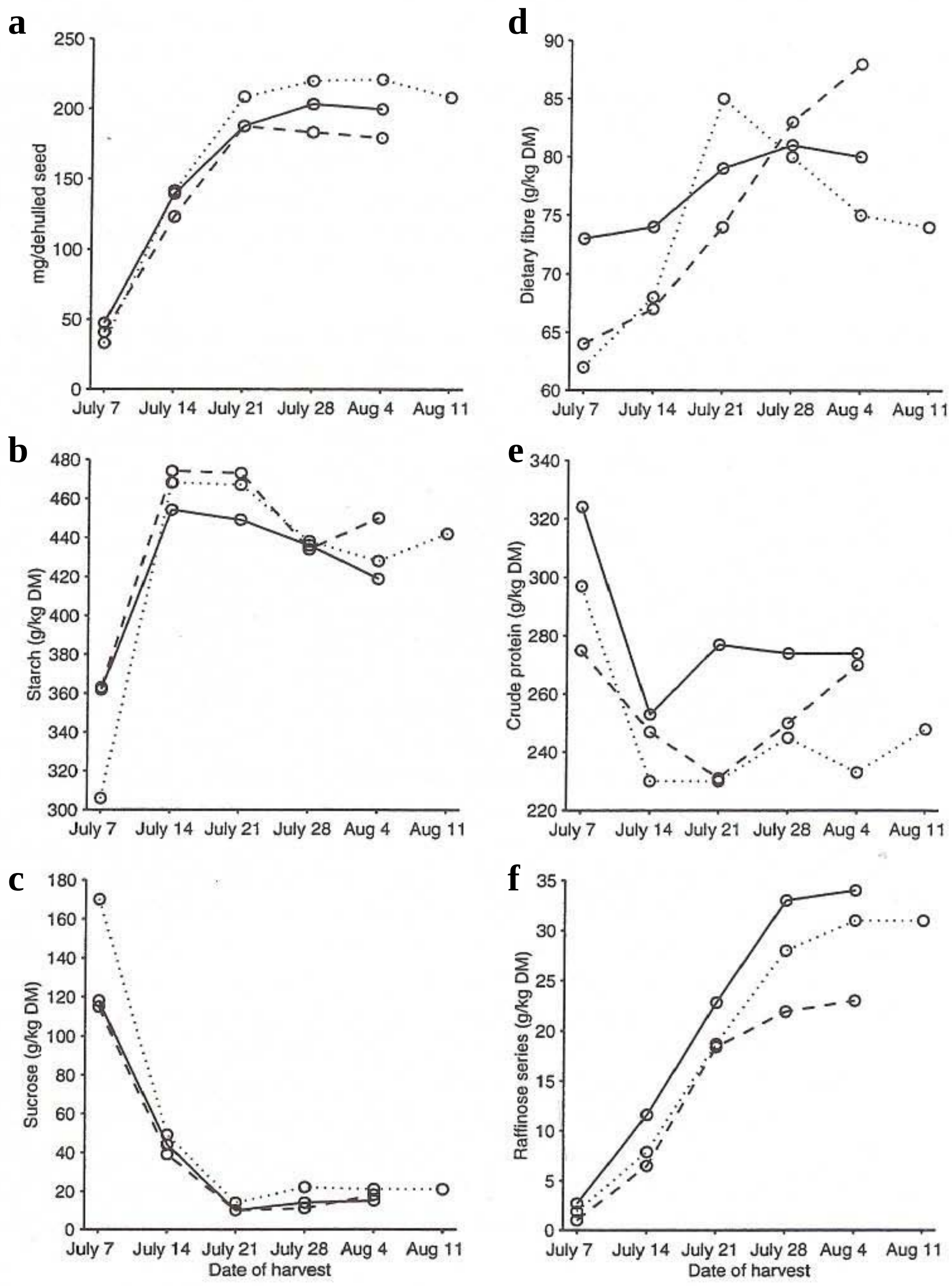

Figure 2. Developmental changes in dehulled peas: Capella dotted line; Vreta dashed line; Timo full line. a) Weight of dehulled peas, b) starch, c) sucrose, d) dietary fibre, e) crude protein, f) raffinose, series of oligosaccharides (Dandanell-Daveby, 1997).

from the rumen and seldom escape[1] (Figure 3). Since most of the grain legume seeds are too large to pass readily from the rumen of either sheep or cattle, it seems likely that much grain legume material of particle size equivalent to the more coarsely ground treatments will be retained in the rumen for long periods and be fermented (Dixon & Hosking, 1992). The concept of critical particle size divides rumen particles into two pools: a large particle pool, which cannot pass out of the rumen, and a small particle pool, which can leave the rumen (Poppi *et al.*, 1980).

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[1] Outflow of small particles, those passing through a screen with 1.18-mm pores often is considered the rate limiting step in passage of ruminal solids. Factors that enhance movement of small particles towards the reticulo-omasal orifice are thought to be important determinants of digesta passage. Particle specific gravity (SG) may be one such factor. Their size and SG affect Ruminal retention time of inert particles. A SG of about 1.2 maximizes ruminal outflow of inert particles. Functional SG (FSG) is considered a more physiological measurement of feed particle density, because it represents the density of individual feed particles along with associated gas-filled spaces and bound water (Siciliano-Jones & Murphy, 1991).

Poppi *et al.* (1981) suggest that the particle size of digesta does not change significantly after it leaves the rumen-reticulum and that faecal particle size is a good estimate of particle sizes leaving the rumen. Little is known about factors that regulate rate of propulsion of ingesta through the rumen and abomasum. It is reasonably clear that size of ruminal particles alone does not regulate rate of passage. Density of particles appears also to play a role, but that role is not well understood. Cell wall content of the forage appears to affect particle passage, but the mechanism is not understood. Consistency of the ingesta may also play a role in passage (Martz & Belyea, 1986).

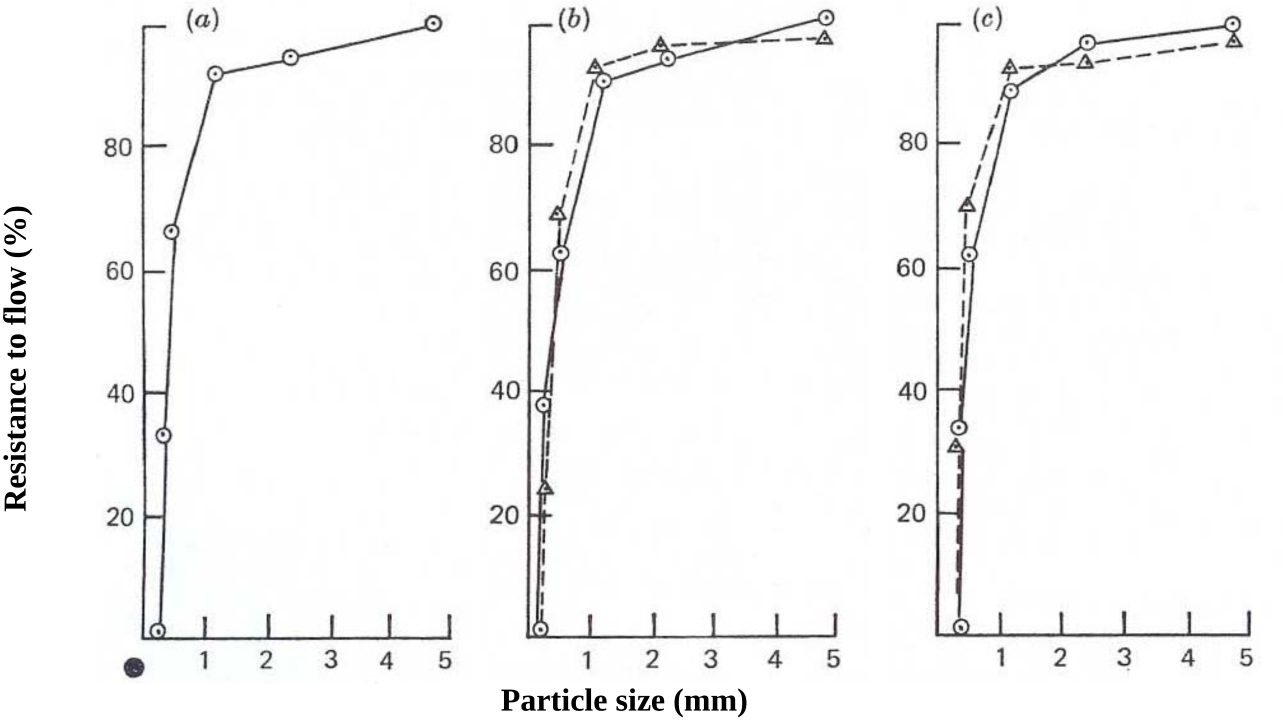

Figure 3. Relative resistance to flow from the rumen of different size particles in a) all ten feeds; b) five grasses (⊙) and five legumes (△); and c) young (⊙) and mature (△) grasses and legumes (Poppi *et al.*, 1980).

Pea organic matter (OM), protein and starch degradation generally increases as particle size decreases[2] (Bayourthe *et al.*, 2000). A similar difference in OM disappearance was observed[3]. For all particle sizes, CP were more degraded than starch during the first 8 h of rumen incubation which supports previous reports that the protein matrix first must be disrupted for release of starch (Bayourthe *et al.*, 2000). Therefore, particle size of pea strongly influences protein and starch degradation. Michalet-Doreau and Ould-Bah (1992) concludes that feed degradation rate generally increases with grinding fineness, and that differences between feeds are important. Protein degradability of pea and lupin decreases by 10 and 24 points, respectively, when grinding changes from 0.8 mm to 6 or 4 mm, whereas soya bean meal degradability was unchanged within the same variation range (Michalet-Doreau & Ould-Bah, 1992). Dixon & Hosking (1992) suggest that for rumen fractional outflow rates of the order of 0.02 h^{-1}, at least 0.80 of DM of lupin, pea, cowpea, navy bean and lablab grain is fermented in the rumen. The N of these grain legumes may be even more fermented. At higher fractional outflow rates associated with higher intakes, particularly in cattle, substantial proportions of ingested grain

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[2] Before grounding, using a laboratory grooved-roller mill, the cotyledons and hulls were separated from the pea seeds in order to obtain seven degrees of grinding. One hundred grams of each sample were dry sieved in triplicate for 15 min. Bags with samples (3 g) were then incubated in the rumen for 2, 4, 8, 16, 24 and 48 h.

[3] Particles of 112 to 267 µm diameter had the highest disappearance values; particles of 2025 to 1042 the least and particles of 502 to 753 µm had intermediate values until 2 h after rumen incubation. By 16 h, particles of different sizes were degraded to similar extent.

legumes are likely to escape fermentation and pass to the small intestine (Dixon & Hosking, 1992).

Nitrogen content in peas ground with a hammer mill increases with particle size, but if a 6 mm screen is used, particles larger than 2.5 mm have the same N content as whole peas (Michalet-Doureau & Cernau, 1991). According to Michalet-Doureau & Cernau (1991), a large part of the N becomes soluble during wet sieving, resulting in N losses especially when using a 0.8 or 3 mm screen. When studying degradability *in sacco*, N degradability of peas ground with a hammer mill increases when smaller screens are used i.e. smaller particles (< 2.5 mm) have a larger rapidly degradable fraction than larger particles (> 2.5 mm) (Michalet-Doureau & Cerneau, 1991). With whole, intact seeds, legume seeds were more susceptible than both husked and naked cereal grains (Nordin & Campling, 1976)[4]. Breaking of all seeds brought about marked increases in their losses of DM, and field peas had intermediate losses (Nordin & Campling, 1976). Maaroufi *et al.* (2000) investigated the physical and chemical characteristics of pea flour fractions. They noted great differences in chemical composition of the granulometric fractions: the smaller the size, the higher the contents of both CP and starch, and the lower the cell wall contents. Hence they concluded grinding of pea seeds might result in physical separation of the botanical constituents of the pea seed: with coarse fractions in which hulls are accumulated together with most parietal constituents and finer fractions enriched with kernels and cellular constituents and the smallest fraction mainly composed of starch granules. In another trial Maaroufi *et al.,* (1998) investigated the influence of particle size from peas on the extent of gas production in rumen fluid *in vitro*. The degradation rate increased with the decrease of particle size and the chemical composition and the specific surface area had great influence for the degradation rate of pea flour (Figure 4).

Goelema *et al.* (1998) obtained maximum dehulling and minimal particle size reduction on grain legumes ground with a roller mill (Roskamp TP 900-36) with three roller pairs. In the actual trial the gap widths of the roller pairs 1, 2 and 3 were 4.15, 3.20 and 3.90mm for peas; DM content in the seeds was 0.94-0.95. The reduction in modulus of fineness was consistent with the increase of fractional degradation rate (Goelema *et al.*, 1999) that is in agreement with both Michalet-Doureau & Cernau (1991) and Michalet-Doreau & Ould-Bah (1992).

5.2 Particle size determination

Particle size may be measured in two ways: by dry sieving or by slurring wet suspensions through calibrated screens arranged by decreasing pore opening from top to bottom. Since the rumen is a wet system, wet methods (i.e. wet sieving) of sizing particles in feeds and rumen contents have come to be favoured. Soaking the feed in water will dissolve much of the finely divided non-cell wall matter; however, in ruminant feeds it often is more relevant to measure the insoluble fibrous matter. Legumes shatter into short particles, while grass particles are more needle-like. The dry method has problems with the electrostatic charge on particles, which leads to aggregates, particularly in smaller particles. Sample size must be small, and the ration of sample size to screen area controlled. The expression of particle size as a single number offers special problems. The distribution of dry matter per screen size classification is logarithmic, not linear, giving an arithmetic mean biased to low values. Valid statistical treatment requires converting to the logarithm of particle size and calculating the mean logarithmic particle size. An older and somewhat simpler system of expressing particle size is by modulus of fineness, in which the problem of logarithmic distribution is resolved by using standard screens that form an approximate logarithmic series. The modulus is expressed as an average screen size number that, although related to size, has no dimensional units. This system is somewhat arbitrary and is less satisfactory in detecting nonuniformity in particle population (Van Soest, 1994).

[4] Seeds were incubated in ruminal nylon bags for 24, 48 and 72 h.

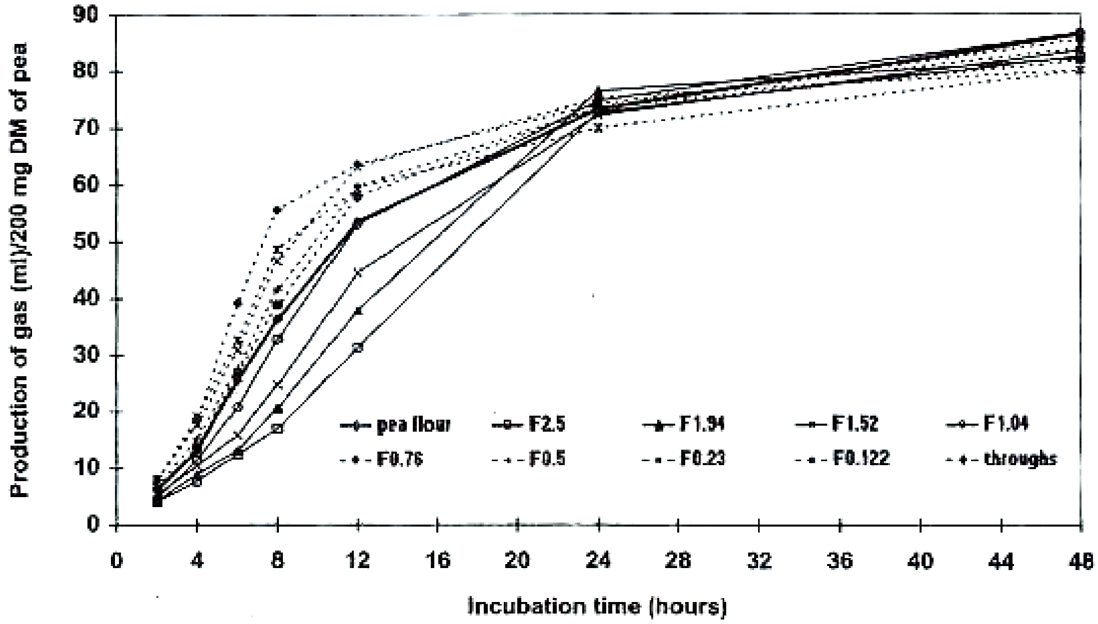

Figure 4. Influence of the size of pea particles on the production of gas following their *in vitro* degradation. F = flour, number indicates mesh size in mm (Maaroufi *et al.*, 1998).

5.3 *In situ methods*

In feed evaluation the feedstuffs evaluated the samples are processed before measuring. According to Michalet-Doreau & Ould-Bah (1992) sample characteristics is of major importance besides bag porosity when using fibre bags. Particularly the grinding fineness is of importance in order to obtain a homogenous sample and also to mimic the effect of mastication. Salivation and especially mastication of ingested feeds increase feed wetting and surface area, and thus solubilization rates and access of microorganisms to the feed components. Further Michalet-Doreau & Ould-Bah (1992) says that it is necessary to measure feed particle size after different grinding to establish the feed particle size in sacco measurements. Then it is of importance to choose the feed grinding for which the mean particle size is near to that of masticated and presented to the rumen. The feed particle size is determined by sieving either dry or wet samples through sets of screens and weighing the material on each screen. The particle size distribution is expressed as DM (or N) cumulative present of particles collected on the different screens. An exponential equation can be fitted to data in order to estimate mean particle size, the screen size of the sieve on which 0.50 of the particle weight would be collected. For concentrates the decrease in particle size during mastication could be considered as negligible, so that the particle size of feed introduced in bags is equal to that of the feed in the diet, at least when the concentrates are given in ground form to the animal. Particle size is also of great importance for the results when incubating fibre bags with feeds in the rumen. Dixon & Hosking (1992) reports of experiments performed on legume grains on measuring DM and total N disappearance from feed samples with this technique. The results shows that the method of preparation of the grain legume samples substantially affected solubilization and fermentation. According to Dixon & Hosking (1992), Freer & Dove (1984) performed an experiment in which the degree of processing of lupin grain was increased from “coarse” (samples passed through a grinding mill without screen) to “fine” (samples ground through a 0.8 mm screen), solubility of DM increased from 0.03 to 0.46 and solubility of N from 0 to 0.74. The results from this experiment suggest that rate of digestion tends to be greater for lupin grain of smaller particle size, the predicted degradability at a fractional outflow rate of 0.02 h^{-1} increased from 0.69 and 0.75 for N and DM respectively for “coarse” samples to 0.95 for “fine” samples (Dixon & Hosking, 1992).

6 Processing

Orskov (1980) claims that processing can only be justified if it aids digestion, and Owens & Heimann (1994) state that the major reasons for particle size reduction in feed manufacturing operations include exposing greater surface area for digestion. Processing is not necessarily physical, and is sufficient if it makes the endosperm accessible for bacteria or enzymes (Orskov, 1980). Only a few grains can satisfactory be fed unprocessed to cattle, including corn (Hale, 1980) and pea (Corbett, 2003 personal communication). Most other feed grains need to be processed with some method, as illustrated in Figure 5 (Hale, 1980; Beauchemin *et al.*, 1994). This is because cattle masticate only as much as is necessary for sufficient salivation to permit swallowing. Therefore the larger grains of peas and corn are more often broken than other smaller sized grains. Dixon & Hosking (1992) reports that when lupin grain was fed as whole seed to cattle, an appreciable proportion (up to 0.26 in dairy cattle) escaped digestion and was excreted in the faeces (Dixon & Hosking, 1992). Furthermore, seeds have a relative high specific gravity and sink in the rumen, decreasing the regurgitation of the grain (Hale, 1980; Beauchemin *et al*., 1994; McAllister *et al.,* 1994). However, it appears that grain legumes preferably should be ground or rolled before fed to cattle (Orskov 1980; Dixon & Hosking, 1992; Van Soest, 1994). The observation that a decrease in digestibility can be attributed entirely to the whole grain recovered in faeces suggests that legume grain need only to be cracked to allow complete digestion by cattle (Dixon & Hosking, 1992). Feed grains vary in proportions of seed coat, strength of seed hull attachment, and grain hardiness, characteristics that can influence the degree of grain damage during mastication. Rapid water uptake with subsequent swelling and softening of the grain could also lead to greater whole grain damage during mastication (Kaiser, 1999). The type and degree of processing are critical in altering digestibility and use of nutrients by the animal (Nocek & Tamminga, 1991). In general, processing is associated with improvements in efficiency of nutrient utilization by ruminal microorganisms and the total tract. Processing can be subdivided into physical, chemical and combined physicochemical methodologies (Nocek & Tamminga, 1991). Most processing methods increase ruminal starch digestion, which usually increases percentage of starch digestion in the small intestine. It may be beneficial to increase the supply of starch to the lower gut and thereby increasing the immediately available energy for the animal (Nocek & Tamminga, 1991; Yu *et al*., 2002). In ruminal fluid, untreated pea is characterized by a slow degradation rate of starch and a rapid solubilization of protein (Corbett, 1997). Hence, after intake, ammonia rises rapidly in the rumen. If there is lack of easily degradable energy in synergy with the increased ruminal ammonia level, deficits in rumen microbial protein synthesis may occur (Tamminga, 1979; Focant *et al*., 1990; Van Soest, 1994).

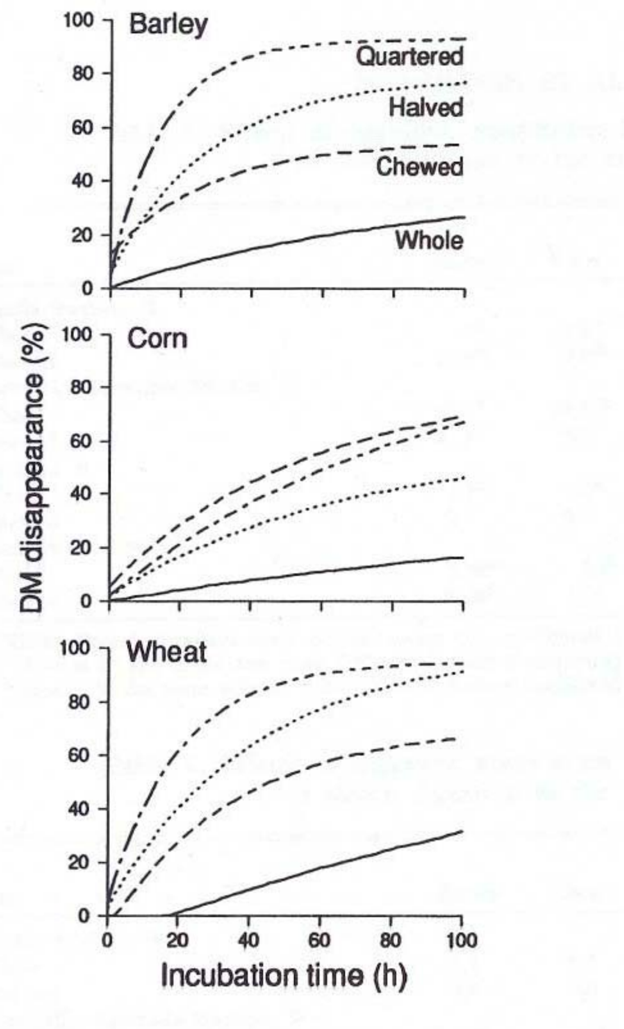

Figure 5. *In sacco* disappearance of DM from whole, chewed, halved and quartered kernels of barley, corn and wheat (Beauchemin *et al*., 1994).

Feed processing can be divided into dry- or wet processing. However, different authors suggest different classification of processing methods, for instance Van Soest (1994) classifies processing into cold, dry heat and hydrothermal processing[5]. In this review, I will use the classifications according to Tait & Beames (1988) as described in Table 1. Some different terms regarding the results after different processes are defined in Table 2.

6.1 Physical processing

6.1.1 Grinding or dry rolling

Grinding is an extremely useful processing method for small farmer-feeder operations since the equipment is versatile and economic. Considerable differences in particle size for different feedstuffs despite the use of the same screen, makes it difficult to compare results between otherwise similar feedstuffs (Michalet-Doreau & Cerneau, 1991). The most commonly used equipment is the hammer mill and the roller mill (Figure 6).

6.1.1.1 Roller mill systems

Roller mills are equipped with rollers in pairs. A single pair mill can be used to crack or crimp feedstuffs. Double pair (two pair high) mills can be used in feed milling operations when two distinctly different grains are processed through one mill[6]. Triple pair (three pair high) mills are used for special applications requiring a significant reduction, or for very difficult to process materials[7] (Owens & Heimann, 1994). Rollers with coarse grooves produce a coarse finished product at high capacities, while finer grooving results in a finer finished product at lower capacities. Roll speed is important, lower speeds are used in cracking, crimping and flaking, and higher roll speeds are used for grinding (Owens & Heimann, 1994)[8]. Magnusson (1980) considers that peas must be pre-rolled before the final crimping to increase the processing rate. To get a successful result it is necessary that the rollers are grooved and that both are driven. An alternative is to grind roughly in a hammer mill (Magnusson, 1980).

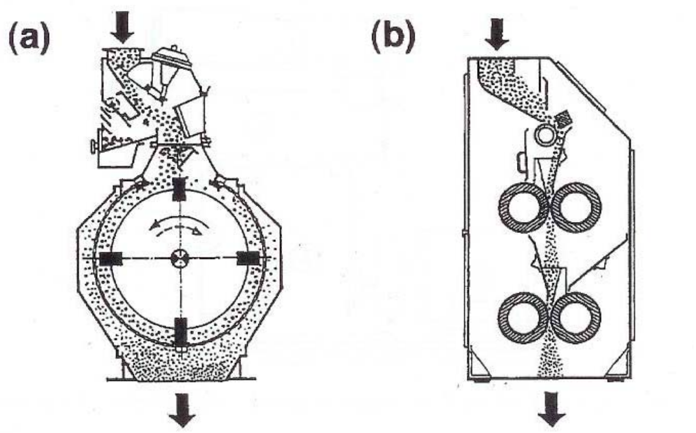

Figure 6. Schematic illustration of a) a hammer mill; and b) a roller mill (Audet, 1995).

6.1.1.2 Cracking/crimping

Cracking and crimping are the most basic roller mill processes for feed grain preparation (Owens & Heimann, 1994). Cracking breaks the kernel of grain into two or more pieces while crimping opens up the seed coat. Corrugation on the rolls must match both the grain to be processed and

[5] Cold: grinding, cracking, rolling and crimping, extrusion and pelleting, ensiling. Methods designed to break the pericarp and expose the endosperm to digestive attack. Some heat may be produced by friction in these methods. Dry heat: popping, micronizing. Hydrothermal methods uses moist heat for 8-25 min with or without pressure and drying after flaking and rolling.

[6] For instance, a machine processing both corn and oats requires one set of coarse grooved rolls to process corn, and one set of fine grooved rolls to efficiently process the oats. A double pair mill equipped with different roll speeds (one roll turning faster than the other) can be used as a grinder to reduce all kinds of friable materials including grains, pelletized products other feed ingredients (Owens & Heimann, 1994).

[7] A triple mill can produce a variety of finished products from different feed stocks such as whole grain, mixed meals, or other combinations. Occasionally, three pair high mills will be used to permit one machine to serve as both a two pair high grinder and a single pair cracking/crimping mill (Owens & Heimann, 1994).

[8] Roll speed differences simply mean that one roll turns faster than the other and are usually described in the form of a ratio for example: 1.5:1.

Table 1. Description and classification of different processing methods.

Process name	Class	Description	Reference
Hammer milling	Dry	The most commonly used equipment for grinding. Screens within the range of 3-6 mm are frequently used. The fineness of grind depends on several factors including type of grain, moisture content, screen size and flow rate.	Tait & Beames, 1988
Rolling	Dry	Produces a product varying from merely cracked to fine ground depending on tolerance setting, type of rollers and factors mentioned under hammer milling.	Tait & Beames, 1988
Expanding	Dry heat (Wet)	Similar to single screw extrusion but with an annular discharge valve, instead of a die. (Steam can be used for heating or injection)	Yu *et al.*, 2002
Extruding	Dry heat	Grain is forced through a smooth cylinder, followed by a corrugated tapered cylinder by means of screw augers. The friction generated results in an exit temperature of approximately 95°C.	Tait & Beames, 1988
Micronizing	Dry heat	The grain is subjected to infrared heat generated from gas heated ceramic tile (microwaves of 3×10^8 to 3×10^{11} Hz)[9]	Tait & Beames, 1988
Popping	Dry heat	Results in rupture of the starch granules within the grain. The degree of popping varies with temperature (≈150°C is often used), moisture content and type of grain.	Tait & Beames, 1988
Roasting	Dry heat	The grain is revolved in a cylinder and lifted by a series of fins thorough jets of flame. Exit temperature similar to those in popping or micronizing.	Tait & Beames, 1988
Acid treatment	Wet	High moisture grain treated with organic acids i.e. acetic and/or propionic acids. Often considered more a preservative method than a processing method.	Tait & Beames, 1988
Alkali treatment	Wet	The grain is either soaked in or sprayed with NaOH to produce a final concentration of 2.5-4 % NaOH depending on the type of grain. Ammonia can be used, but is considered more a preservative method than a processing method.	Barnes & Ørskov, 1982;
Reconstitution	Wet	Moist grain is stored anaerobically for approximately 20 days before feeding with the intent to increase the moisture content to 25-30 %. Response to reconstitution depends on type of grain, moisture content, ambient temperature and storage time.	Tait & Beames, 1988
Soaking	Wet	Short term soaking, about 12-24 h. Historically a common practice, but little evidence of beneficial effects exist.	Tait & Beames, 1988
Steam flaking	Wet heat	More precisely controlled conditions than with steam rolling. The North American process involves steaming for a time sufficient to raise moisture content to around 18 %.[10] The grain temperature is ≈100°C when it reaches the rollers. The British steam flaking process involves cracking the grain, applying water and allowing the moisture to absorb for up to 48 h, followed by steaming under pressure (2.1 kg cm^{-2}, 134°C) for 10-15 min before rolling.	Hale, 1980; Tait & Beames, 1988
Steam rolling	Wet heat	Conditions are highly variable and are frequently not specified, in general grain is subjected to steam for 35 min before rolling.	Tait & Beames, 1988
Exploding	Wet heat pressure	Grain is steamed under high pressure followed by release through a small diameter orifice. Typical conditions are 15 kg cm^{-2} at 200°C for 20 s.	Tait & Beames, 1988
Pelleting	Wet heat pressure	Often considered a hydrothermal process since commercial plants often use steam. Steam increases moisture content to ≈ 17 % and discharge temperature to 60-94°C. Rollers force pre-ground grain through dies with openings of 4-10 mm diameter.	Tait & Beames, 1988
Pressure cooking/toasting	Wet heat pressure	Steaming prior to rolling can be done under pressure.[11]	Tait & Beames, 1988; Goelema, 1999

[9] Van Soest (1994) defines micronizing as exposure for infrared radiation at 150-180ºC for 30-60 seconds followed by flaking through rollers.
[10] Sufficient time for corn is around 12 min, for sorghum at least 24 min (Hale, 1980).
[11] Between 1 and 2 min at about 3 kg cm^{-2} (143°C) is typical for corn, although higher pressures may be necessary for sorghum (Tait & Beames, 1988).

Table 2. Definitions of some terms frequently used in starch and protein processing.

Term	Definition	Reference
Crack	A kernel of grain (pellet etc) broken in two or more pieces, normally done with rolls operating with no differential speeds.	Owens & Heimann, 1994
Crimp	Typically describes slightly flattened small grains such as oats, wheat and barley. Primary objective is to open up the seed coat. Larger grains such as corn and milo can be crimped if some conditioning (steam, high moisture) is employed.	Owens & Heimann, 1994
Crumble	Pellet or extruded feed material broken into smaller bits, pellets are assumed to be crumbled when all are reduced to less than the full pellet diameter.	Owens & Heimann, 1994
Expanding	Subjecting grains to moisture, pressure and heat to gelatinise the starch, examples of expanding processes are extrusion, steam flaking and rolling.	Nocek & Tamminga, 1991
Flaking	Conditioning corn and small grains with heat and/or moisture, then flattened between the rolls. Flaking is assumed to be more severe than crimping.	Owens & Heimann, 1994
Gelatinisation	Complete ruptures of starch granules by a combination of moisture, heat, pressure and in some cases mechanical shear force.	Nocek & Tamminga, 1991
Ground	Substantial reduction in size by processing of feed material between corrugated rolls operating at different roll speeds.	Owens & Heimann, 1994
Retrogradation	Reassociation of starch molecules separated by gelatinisation. Hydrogen bonding between amylose and amylopectin is re-established, although retrograde starch does not regain the native starch character. Heating can to some degree reverse retrogradation.	Nocek & Tamminga, 1991
Rolled	Not application specific and should be avoided.	Owens & Heimann, 1994
Swelling	Exposing starch to water and gradual temperature increase to 55°C cause starch granules to take up water and swell. Irreversible swelling of starch granules occur if more heat is applied (60-80°C).	Nocek & Tamminga, 1991

the finished product[12]. Differential speeds can be used to impart a shearing action for cutting the product and promotes a self-cleaning action between the rolls. When higher moisture (16 – 30 %) grains are processed, differential speeds are often necessary to help keep the rolls clean. Unfortunately, differential roll speeds tend to produce more fines when a very coarse crack or crimp is desired, especially when dry grain being processed.

6.1.1.3 Flaking

The primary purpose of flaking (Owens & Heimann, 1994) is to change the physical form of the grain to increase the surface area, and make the feed more digestible by partial gelatinisation, or loss of bifringence of the starch granules. The degree of gelatinisation is important, 40-50 % is desirable (Hale, 1980) since incomplete gelatinisation will retrograde, resulting in a low quality grain (Van Soest, 1994). True flaking can normally be achieved only if the grain is conditioned before rolling[13] (Owens & Heimann, 1994). In the steam chamber, the moisture content of the grain is increased to approximately 18 % and then run through the rollers to produce a quality control flake. To get a good quality flake, the rolls must be set very close together, decreasing the capacity of the roller mill by 45-50 % of that for dry rolling (Hale, 1980). The purpose of conditioning is to make the grain soft and pliable to produce a tough durable flake[14]. The degree of gelatinisation is chiefly affected by the flake thickness; and flake thickness is, in turn, dependent on the level of conditioning. Owens & Heimann (1994) and Hale (1980) emphasizes

[12] Accordingly, corrugations for processing corn will be coarse (4 to 6 grooves per inch), corrugations for milo or grain sorghum will need finer (6 to 10 grooves per inch), while corrugations for small grains such as wheat, oats, and barley may range even finer (10 to 18 grooves per inch).

[13] Typical conditioning systems include dry heat (Roasters, Jet Sploader, etc.), live steam (atmospheric steam chambers or pressurized steam vessels), or other more exotic processes like micronizing (heating by infrared), popping etc.

[14] If the conditioning is inadequate, the flakes produced will break easily and create objectionable levels of fines.

that any added moisture must be removed if the finished product is to be stored. Another factor affecting utilisation of flaked grains is rupture or release of the starch granules from the protein matrix that surrounds the granule. For example, starch digestibility in reconstituted sorghum grain was high, yet there was no gelatinisation. However, in reconstitution there is a complete disruption of the protein matrix in the endosperm. Flaking either corn or sorghum grain results in increased digestion of the starch in the rumen when compared to dry-rolled grain. Starch digestibility varies with its source, for instance barley starch digestibility in the rumen is extremely high and not affected by processing (Hale, 1980).

6.1.1.4 Grinding

Roller mill grinders will produce more tons/hour at a given horsepower than traditional "full circle" hammer mills when producing the same finished particle size. Because the grind produced by a roller mill is very uniform, the finished product(s) have an excellent physical appearance, with excellent flow and mixing characteristics. This is especially important for mash or meal type feeds, since flow from bins and feeders can be difficult to regulate and segregation and separation may occur in shipping and handling. Furthermore, the product is not significantly heated in the grinding process, which decreases problems related to heat and moisture such as hanging up in bins or spoiling in storage (Owens & Heimann, 1994).

6.1.2 Other high energy processing methods

Popping, micronizing and exploding cause the grain to expand and the endosperm structure of the kernel are totally disrupted. These methods are high energy demanding processes, and not of interest for on-farm processing.

6.1.3 High moisture grain

Grain harvested from the field at 25-30 % moisture can be stored in a suitable structure whole, ground or rolled form. However, most whole grain should be rolled or ground before feeding (Hale, 1980). Furthermore, wet grain demands larger storage facilities than reconstitution or steam flaking. The improvement in feed efficiency is probably due to the fact that in early-harvested grain the starch and protein components are not in the crystalline state as with dry grain. Certainly, the early-harvested grain would require considerably less wetting in the rumen for microbial digestion. According to Kaiser (1999) most of the treatments used to modify the nutritive value of whole grain are applied to high moisture grain, or result in an increase in grain moisture content as a part of the treatment process (reconsitution, see chapter below). The treatment choices are ensiled grain i.e. high moisture grain is stored anaerobically for an extended period resulting in a fermented product, acid-treated grain, alkali-treated or ammoniated grain, as described in Table 1. Generally, high moisture grain is stored anaerobically; only the alkali-treated grain can be kept for longer periods in aerobic conditions. Very few studies concerning effect on peas in airtight storage can be found, however, Åman *et al.* (1990) used airtight storage to preserve barley for feeding chicks. The barley was harvested at moisture contents of 36, 25 and 23% and infected with storage-associated moulds. Part of each lot of barley was also treated with lactobacilli or lactobacilli and yeast. The starch, CP and β-glucan content remained fairly constant while the solubility of these components changed, especially in the barleys with 36% moisture. Treatment with protecting microorganisms did not influence the content or solubility of CP, starch or mixed-linked beta-glucans during storage.

6.1.4 Reconstitution

Reconstitution is a method by which grain at normal moisture storage levels is reconstituted to 25-30% moisture and stored anaerobically for a short period, often only a few days, usually not more than 20 days before feeding (Hale, 1980; Tait & Beames, 1988). Moisture level, storage temperature, storage time and physical properties of the grain are important for proper reconstitution. For instance, sorghum grain must be reconstituted whole, and the moisture level must be at least 25 % for proper reconstitution. Therefore it is difficult to raise the moisture level of stored grain (10-14 %) to 30 % in a short time. It can be accomplished with heated water or under pressure, but these applications are energy demanding, expensive, and increase risk for on-farm applications (Hale, 1980).

The complete mechanism of action by which the process of reconstitution improves utilisation of grain by ruminants is not fully understood. The initial stages of reconstitution appear to be closely related to the early stages of germination. In germination the embryo of the seed secretes gibberelines, which then migrate to the aleurone layer of the seed and result in production of amylases and proteases. The amylases stimulate starch solubilization and the proteases increase protein hydrolysis. The peripheral endosperm, which is adjacent to the aleurone, is affected markedly by enzyme activity. It is this portion of the endosperm that is thought to be relatively indigestible to rumen bacteria and intestinal enzymes. Based on in vitro studies, this aspect of grain modification accounts for the approximately 40% of the total improvement of the utilisation of reconstituted grain noted in feeding trials. In reconstituted sorghum grain, the endosperm is completely disrupted and the protein matrix becomes disrupted and frees the starch granules and protein bodies (Hale, 1980).

Reconstitution holds considerable promise, particularly if the moisture elevation necessary for reconstitution can be accomplished in a short period of time. The major advantage of the method is the low fossil fuel energy input as the processing takes advantage of the production of endogenous enzymes within the grain (Hale, 1980).

6.1.5 Heat treatment

As described in Table 1, several processes involve heat treatment. Dry heat, frictional heat and wet heat affect protein differently (Goelema, 1999). As illustrated in Figure 7, protein is denatured during heat treatment (Van Soest, 1994; Goelema, 1999), stabilising the molecular structure and inducing enzyme-stable bonds to carbohydrates, thereby reducing the availability of the substrate (Van Soest, 1994). It appears that denatured protein is protected from hydrolysis in the rumen, or the degradation rate is decreased. Thereby the proportion of undegraded protein leaving the rumen increases. However, the bonds must be reversible in the intestine, or the total digestibility will be reduced. More extreme processing can destroy the primary structure, degrading the protein.

Effect of extrusion on pea protein and starch characteristics has been well studied for early lactation cows (Petit *et al.*, 1997), as well as *in situ* in heifers (Focant *et al.*, 1990) and in bulls (Walhain *et al.*, 1992) and in sheep (Aufrere *et al.*, 2001). Extrusion of peas increases rumen degradability of starch but had no effect on rumen degradable protein according to Petit *et al.* (1997), but Walhain *et al.* (1992) observed a dramatically reduced CP effective degradability. The DM intake is higher in cows fed extruded peas as compared to those fed soybean meal (SBM), and the milk protein content is higher for cows fed peas, otherwise there is no difference in production of 4 % FCM and milk composition (Petit *et al.*, 1997). Therefore, peas can substitute SBM completely as a protein source in the diet of early lactation cows (Petit *et al.*, 1997). Extrusion of peas at temperatures 140ºC and above has no beneficial effect on protein protection (Walhain *et al.*, 1992) and milk production (Petit *et al.*, 1997). Focant *et al.* (1990) compared ground, steam-flaked and extruded peas in situ in Friesian heifers. Steam flaking had no effect on any of the parameters studied when compared to ground peas. Whilst extrusion decreased N solubility and gelatinised starch content without negative effect on N pepsin solubility and bioavailability, and total volatile fatty acid concentration was increased and ruminal ammonia was decreased. Processing did not affect the duodenal flow of starch. In conclusion, extrusion significantly affected peas giving higher feed N, bacterial N, non-ammonia N and increased flow of all AA to the duodenum (Focant *et al.*, 1990). In sheep, extrusion at 140°C /190°C does not have any significant effect on ruminal N degradation of pea (Aufrere *et al.,* 2001).

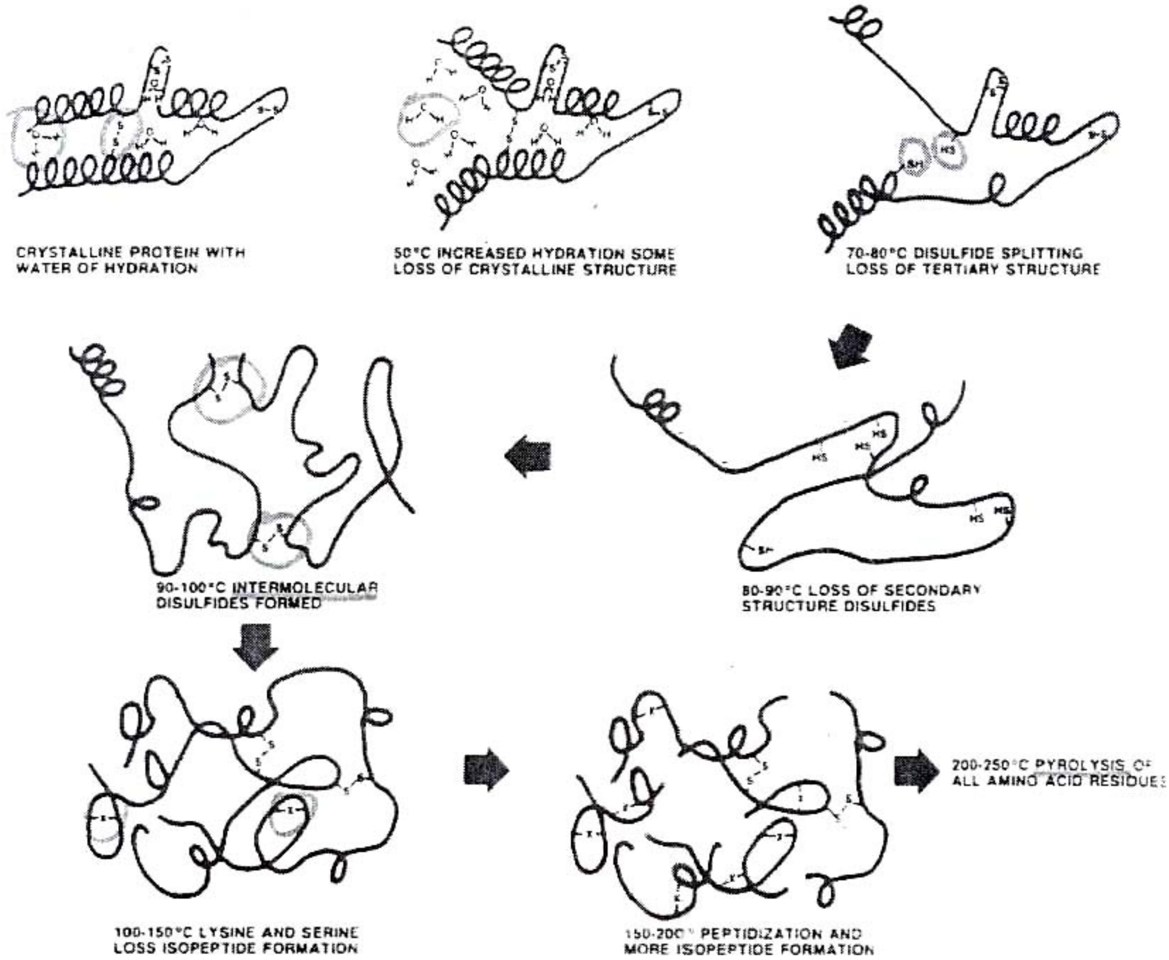

Figure 7. Changes a protein undergoes during heat treatment (Svihus, 2003).

The effect of autoclaving peas on CP composition (Mustafa *et al*., 1998) and rumen degradability (Aguilera *et al*., 1992; Mustafa *et al*., 1998) has been studied *in situ* in cows. Autoclaving peas for up to 30 min will reduce CP degradability without affecting protein quality of rumen-undegradable protein (Mustafa *et al*., 1998). However, Aguilera *et al*. (1992) observed that autoclaving legume seeds decreases the rate of degradation but has minor influence on the extent of degradation. Their conclusion was that autoclaving results in substantial decreases in the value of effective degradability of pea seeds. They especially observed that there was a sharp fall in both the instantaneous soluble fraction and rate of degradation. This results in a large potentially degradable insoluble fraction and a moderate or low rate of degradation and thus is expected to have an effective degradation dependent on changes in ruminal outflow rate (Aguilera *et al*., 1992).

The influence of heat treatment (at 100, 125 and 150ºC for 5, 15 and 30 min) and glucose addition on in situ digestibility of protein in dairy cows has been studied by Ljøkjel *et al*. (2003). The study included both barley and peas and rumen degradation of protein was reduced in both barley and peas. In peas the rumen degradable protein (RDP) decreased with increasing temperature and time, and the lowest RDP was obtained with the harshest treatment (150ºC/30 min). Highest amount of post-ruminally digestible proteins in peas was obtained after treatment at 150ºC for a short time (i.e. 5 min); addition of glucose reduced the RDP of peas. The authors concluded that protein is not an accurate predictor of rumen degradation of individual AA in either barley or peas, since degradation of individual AA varies considerably from total protein in both untreated and treated samples.

The variability in results of heat treatment on pea protein utilisation can in part be explained by the complex interaction of temperature and time as illustrated in Figure 8. In addition, moisture and pressure are important factors affecting the result.

6.2 Chemical processing

6.2.1 Acidic - Ammonia

The role of organic acids is primarily as a preservative, through the control of fungal organisms. There appears to be little effect of acids such as acetic and propionic acid on the physical and chemical properties of cereal grains except for a reduction in the level of α-tocoperol (vitamin E) (Tait & Beames, 1988). Waltz & Stern (1989) studied effects of protection method on protein degradation of soya-bean meal (SBM) by rumen bacteria. Different treatments were included among them untreated and propionic acid. Undegraded dietary N in the effluent was significantly higher ($P<0.05$) for SBM protected by propionic acid than for control and protection by propionic treatment increased ($P<0.05$) total AA flow compared with the control. This implies that acid treatment can have a positive effect in reducing protein degradation in the rumen. Khorasani *et al*. (1989) investigated the effect of acid treatment (i.e. acetic acid, formic acid, or propionic acid) on canola meal. Acid treatment reduced ruminal degradability of canola meal CP and did not depress estimated intestinal CP digestibility.

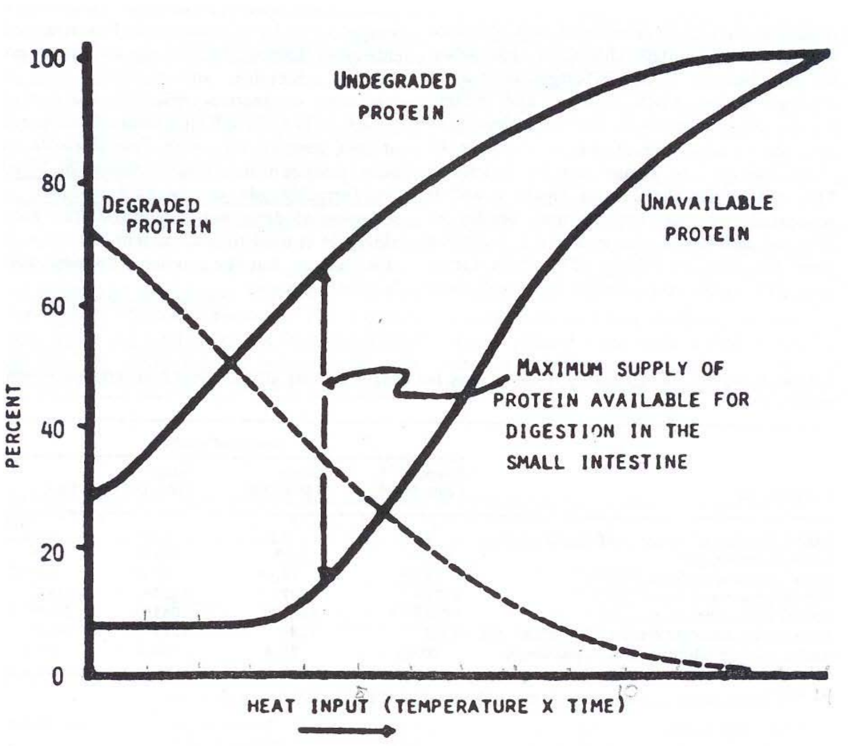

Figure 8. Effect of heat input on protein utilisation (Satter, 1986).

Ammonia treatment effectively reduces mould growth but a free ammonia atmosphere must be retained for this to be effective (Tait & Beames, 1988).

6.2.2 Alkaline – sodium hydroxide

Orskov (1986) present a method of caustic soda treatment of grain to disrupt the fibrous seed coat in grain for cattle. The retention of the structure of the grain and the resulting reduction in degradation rate had the dual effect of reducing ruminitis and parakeratosis, and producing a more uniform supply of VFA for absorption. The change in pattern of fermentation also meant that milk composition remained normal, even with 80% grain in the diet. High moisture grain can also be preserved with caustic soda treatment if anaerobic storage is not available (Orskov 1980, 1986). However, Waltz & Stern (1989) reports that sodium hydroxide not affected ($P<0.05$) CP degradation of SBM.

7 Digestibility and animal production

7.1 Rumen degradation of protein

In the rumen the yield of microbial biomass is related to the amount of substrate available and the energy used for maintenance, which, in turn, is a function of the maintenance requirement and the growth rate (or dilution rate). Energy within cells is used for either growth or maintenance, and maintenance energy can be defined as the energy required maintaining cells in a live state. Important maintenance costs for the bacteria are motility, cellular turnover, and production of cellular molecules, active transport, inefficient phosphorylation, uncoupling and lysis of cells. The maintenance energy requirement is dependent on the bacterial growth rate with the slower growth rates requiring proportionally more maintenances energy than faster growth rates, consequently cell yields are lower at slower growth rates (Dewhurst *et al*., 2000). Furthermore, major changes in microbial species may occur when grain legume supplements replace barley grain supplements. For instance, dairy cows fed lupin grain have much greater concentrations of protozoa than those fed barley (Dixon & Hosking, 1992).

Dixon & Hosking (1992) point out that since negligible digestion of grain legume seeds occurs until the seed coat is disrupted by mechanical means such as rumination, presumably there may be a long delay before whole grain legume components are digested. Consequently, even though grain legume components are readily fermentable on exposure to rumen digesta, lags in the beginning of digestion are likely to mean that ingested supplements of whole grain legumes will be fermented over a considerable period following ingestion (Dixon & Hosking, 1992).

Rumen protein degradation follows the scheme: protein → oligopeptides → dipeptides → amino acids → ammonia. The degradation of amino acids to ammonia is an extracellular, but cell associated, process. The rate-limiting step in protein degradation is the degradation of oligopeptides to dipeptides from the N terminal (Hvelplund & Weisbjerg, 2000). Protein degradation of legume seeds in the rumen involves two steps, as described by Goelema (1999), being hydrolysis of the peptide bond by protease and peptidases and decarboxylation and/or deamination of AA. The first step results in release of peptides and AA, while the end products of the second step are VFAs and branched chain fatty acids, CO_2 and NH_3. Proteolysis in the rumen results from the proteolytic enzymes from bacteria, protozoa and fungi. Bacterial proteases in the rumen are mainly cysteine endoproteases, but metallo- and serine proteases are also present. Protozoa also exhibit proteolytic activity, cystein and asparatate proteases, whereas rumen fungi have an extracellular metalloprotease. As a result of proteolytic digestion, polypeptides are released; these are then broken down into dipeptides and amino acids. Some of the amino acids are incorporated into microbial protein, but the main part of the amino acid-N is converted to NH_3 by deamination. Part of the NH_3 is reincorporated into microbial protein, but much is lost by diffusion across the rumen wall. The rate and extent to which protein degradation occurs will depend on the type and concentration of enzymes and on the number of susceptible peptide bonds and their accessibility (Yu *et al*., 2002). The secondary and tertiary structure of proteins will influence the accessibility of proteases to specific peptide bonds, thereby affecting the rate and extent of ruminal protein degradation (Calsamiglia *et al*., 2000).

Particle size changes N degradability in peas. Michalet-Doureau & Cerneau (1992) showed that N degradability of peas ground with a hammer mill increases when smaller screens are used i.e. smaller particles (< 2.5 mm) have a larger rapidly degradable fraction than larger particles (> 2.5 mm). Furthermore, when hammermilled peas are given at high levels to dairy cows, rumen pH values and ammonia-N concentrations remain at risk-free levels concerning depressions in rate of fibre digestion or intake of cereal hay. The positive effect on rumen pH is associated with the relatively slow starch degradation of peas. Furthermore, mean intake of oaten hay increases when cows are fed pea rather than barley grain (Valentine & Bartsch, 1987). Dixon & Hosking (1992) suggests that digestibility of hulls from grain legumes are much higher than for cereal grains, reflecting the low lignin content of this material. The authors refer to trials performed by Edwards *et al*. (1973) and Rowe and Hargreave (1988) with digestibilities of 0.60-0.62 for organic matter, N and fibre components of faba beans and lupin grain. When studying N degradation in the rumen in relation to different processing methods it is important to consider the influence of mastication on whole seeds. In *in vitro* and *in situ* methods, peas cannot be incubated without prior processing. Some processing such as grinding is necessary to reduce the variation in degradation. Furthermore, for better comparison with the *in vivo* situation some simulation of the mastication is necessary (Michalet-Doreau & Cernau, 1991).

7.2 Digestive interactions between feedstuffs

The proportion of roughage in the feed ration appears to have an effect on the digestibility of grains. In the case of lactating dairy cows, the roughage proportions are frequently sufficiently high to permit a high digestibility of ground grains (Hale, 1980). A greater proportion of cornstarch escaped degradation in the rumen if fibrous roughages formed part of the diet (Orskov, 1986). With rations containing 50% roughage, the digestibility of dry-rolled sorghum grain was higher than on rations containing 98% (Hale, 1980). Consumption of roughage can vary considerably depending on the method of processing of the cereal component of the feed ration (Orskov, 1980).

7.3 Feeding of dairy cattle

Peas have a low bypass protein content compared to common protein supplements such as canola meal and soybean meal (Corbett, 1997). This makes it difficult to formulate rations for high producing cows utilising large amount of peas. For late lactation cows, peas can completely replace soybean meal in the diet (Corbett, 1997). According to Thomke (1979) up to 0.3 of DM can be peas in concentrate mixture without negative effect on intake, and can replace concentrates as soybean meal (0.09) and rapeseed meal (0.04) and grains. During the first eight weeks of lactation, consumption of concentrate was at least 9 kg per day, significantly higher for individual cows with a yield over 30 kg fat corrected milk. There were no negative effects on fertility and no changes in milk fat content compared to the other concentrate mixtures (Thomke, 1979). If dairy cows are fed concentrates that include 0.4 of DM peas or more, milk production decreased compared to cows fed ordinary concentrate (Magnusson, 1980).

Cows may increase production when fed rapeseed or rapeseed-pea mix supplement instead of ordinary supplement or pea supplement, although peas alone had no effect on production (Khalili *et al*., 2002). However, the study used only eight cows in a duplicated 4 x 4 Latin square design, which may be inadequate for finding real significances (Engstrand, 2003 personal communication). In another study, Syrjälä-Qvist *et al.* (1981) determined that peas are almost equivalent to soybean meal as a protein source, concluding that in silage and hay based feeding the proportion of peas in concentrate mixture can be at least 0.35 and the daily pea ration can amount to 3-4 kg without any harmful effects on milk production. Christensen & Mustafa (2000) suggest that dairy cows can consume 2-3 kg daily as concentrate ingredient in place of barley grain and protein supplements. Cattle find peas palatable, which may compensate the observation that only 0.22 of the pea protein, will pass undegraded from the rumen (Thomke, 1979; Corbett, 1997; Christensen & Mustafa, 2000). Ruminal fermentation characteristics and digestibility of nutrients is changed when soybean meal and barley grain is substituted with peas in concentrate mixture, suggesting changes in site and end products of digestion. However, no significant effect on production parameters was observed, and this study used only four dairy cows of varying ECM yield in a 4 x 4 Latin square which may be inadequate for finding real significances (Khorasani *et al*., 2001). Furthermore, Corbett *et al.* (1995) suggest that peas can be substituted for soybean/canola meal as a protein source for high-producing dairy cows. In the study they compared 25% field peas as the major protein source in concentrate with standard concentrate using principally soybean and canola meal as protein source. Yield of ECM was higher for cows in early lactation fed pea-based concentrates, while no change was observed for mid- and late lactation cows. However, milk fat percent was significantly higher for early- and mid lactation cows fed the pea supplement (Corbett *et al.*, 1995).

8 Discussion and conclusion

It is obvious that more studies on processing of threshed peas and effects on protein and starch stability need to be performed. Only few production experiments on dairy cows have been performed where peas have been fed (Thomke, 1979; Syrjälä-Qvist *et al.,* 1981; Corbett, *et al.*, 1995; Khorasani *et al.,* 2001; Khalili *et al.,* 2002). Furthermore, even fewer (Petit *et al*., 1997) studies have been performed evaluating the effect of processing on feed intake and production parameters in dairy cows. This is the main reason why grains other than peas have been included in this discussion. Corn is a grain more frequently and more extensively studied than peas and with similar size and shape as peas. Therefore, results from studies performed on corn concerning processing effects, such as particle size after grinding, can be used to formulate theories concerning processing effects on peas. However, one must keep in mind that corn is chemically very different from peas, with the protein component being much more resistant to invasion of rumen microbes than is the case in peas (McAllister *et al.*, 1990; 1994). Also, mean particle size after grinding is always greater for corn than for peas (Michalet-Doureau & Cernau, 1991). Soybeans are also well studied, and some results can be used for discussions concerning peas, since both grains are rich in protein. As with corn, one must be careful interpreting the result from soybean studies applied to peas. The difference between barley, corn, soybean and peas in N content of different granulometric fractions is illustrated in Figure 9. Another problem

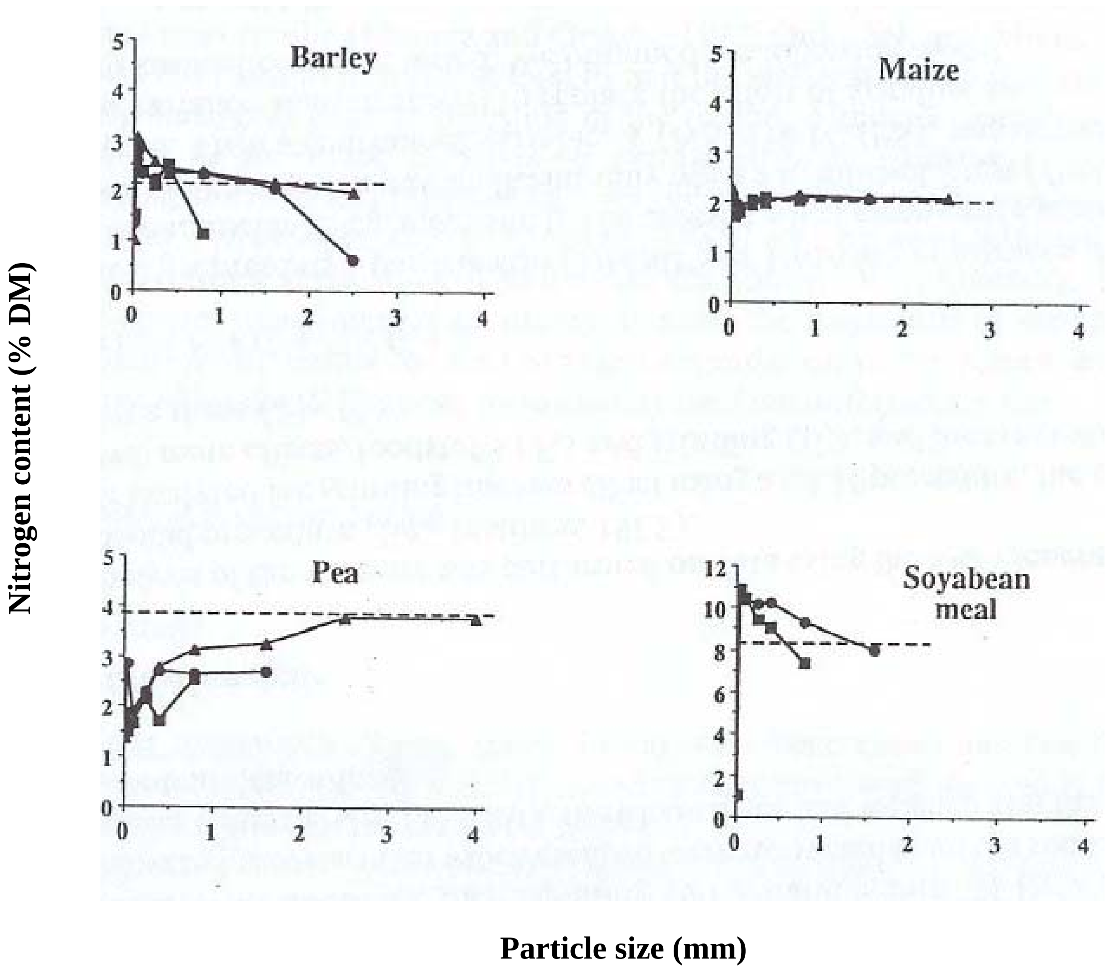

Figure 9. Evolution of nitrogen content in the different granulometric fractions in relation to the grinding fineness: ■, 0.8 mm screen; •3 mm screen; ▲, 6 mm screen; _ _ _ N content of initial feed. The granulometric fractions correspond to the percentage of material remaining on the specific sieve sizes (Michalet-Doreau & Cerneau, 1991).

in interpreting and comparing earlier results is the often incomplete method descriptions where authors have left out details concerning heat treatments or don't determine particle size or specify essential details (i.e. screen) on the mill. This may influence the fact that feed processing not yet is considered science. Another aspect is that feed processing has been practised a relatively short time (Svihus, 2003 personal communication).

The general conclusion in this review is that particle size is important. Poppi *et al.* (1980) concluded that a particle size of 1.2 mm is optimal for fastest passage through the rumen to the intestine. Siciliano-Jones & Murphy (1991) concluded that functional specific gravity (FSG), which is important for rumen passage rate, could be affected by feedstuff, particle size, and *in vitro* fermentation. Results from their study, including corn, suggest that FSG of feedstuffs may be manipulated by processing. Furthermore, when peas are ground, the smaller particles are low in N (Michalet-Doureau & Cernau, 1991) but high in starch (Maaroufi *et al*., 1998). With increasing particle size, protein content increases and starch content decreases. The largest particles are hull particles and thereby very low in both protein and starch (Maaroufi *et al*., 1998). Interesting is also the fact that processing sometimes may be unnecessary. Peas can be assumed to be similar to corn in the way that they will be affected by chewing. Beauchemin *et al.* (1994) noted that unprocessed corn effectively could be fed to ruminants because chewing both during eating and during rumination extensively d amaged the pericarp of the kernel. According to Corbett (1997), peas do not survive chewing intact. Grinding most easily changes particle size, and it appears that the best results will be achieved if the mill used can be set to grind in a homogenous manner resulting in a feed with homogenous particle size distribution. In that aspect roller mills are preferable to hammer mills (Owens & Heimann, 1994). Farmers can easily buy dried peas at a good price and fairly easily grind them on-farm. The simpler processing needed the better. Dry peas can be dehulled before grinding. Some authors have discussed this in detail (Dandanell-Daveby, 1997; Bayourthe *et al*., 2000; Maaroufi *et al*., 2000). from ground mixed feeds, since the shell part tends to separate from the rest of the feed (Maaroufi *et al*., 2000). The separation will create a heterogeneous feed, increasing with storage time. Probably separation of particles is not significant if peas are milled and fed on a daily basis. Since no results are available from production trials made on cattle fed peas processed coarse or fine, only speculations over the possible effects can be made based on studies of soybean, soybean meal and corn. Tice *et al.* (1993) examined if coarseness of roasted soybean affected digestibility and performance of lactating cows. Treatments compared were whole, cracked or ground. Roasting increased the undegraded protein of the soybeans but neither milk yield nor composition was affected by particle size. Netemeyer *et al*., (1980) concluded from a similar study on soybean meal that altering particle size (i.e. 1500 and 250 μm) does not affect milk yield, rumen ammonia, and blood urea in Holstein cows. Furthermore, Wilkerson *et al.* (1997) investigated the effects of harvesting and processing methods on the value of net energy of corn grain for lactating Holstein cows. Yields of milk and milk protein increased when high moisture corn replaced dry corn, and when ground corn replaced rolled corn. The differences in milk yield and composition were the result of improved NFC, CP, and presumably starch digestibilities. Reis *et al*. (2001) concluded that the difference in corn particle size must be large, if pronounced animal responses in milk production and composition is to be detected. If differences in production parameters are to be detected, it is not enough to examine particle size alone. Rather it seems that a combination of treatments such as particle size, preservation method (i.e. acid treatment) and developing stage (i.e. water content) of the grain will give detectable changes in production parameters as milk yield and milk composition (Galyean *et al*., 1981).

Another relatively simple processing method for dried peas is reconstitution before feeding. The main drawback is the difficulty to determine time of soaking needed to achieve appropriate moisture levels in the peas. There are no complete studies concerning reconstituted dried peas fed to cows, and experiments need to be performed to determine appropriate conditions for this processing method. Dairy cows should be able to masticate peas with higher moisture content, whether they are reconstituted or wet-stored. For that reason, special processing of wet peas may not be necessary. However, it may be beneficial for palatability to crush wet-stored peas before feeding (Bertilsson, 2003 personal communication). Crushing wet peas can be done in ordinary mills (Larsson, 1988), but the sieve should be excluded, since the wet pea mash probably causes it to clog. If the sieve is excluded, grinding wet peas should result in a heterogeneous feed of flake-like peas, which probably are more palatable for dairy cows. An important matter to consider is the possible effect of the wet-storage conditions on protein and starch stability. Again, the on-farm practical application is important.

The aim of processing threshed peas as it is discussed in this paper is to decrease protein degradation in the rumen. To achieve this, starch availability and degradation must also be considered. A conflict is evident, since the physical processing that is expected to stabilise protein or increase its passage through the rumen at the same time will increase the rate and extent of starch ruminal digestion. Giving the microbes easier access to starch may increase their capacity to synthesize microbial protein. There are two opinions about the degradability of pea starch; some authors consider it slowly degradable (Robinson & McQueen, 1989 referred in Corbett *et al*., 1995; Corbett, 1997; Bayourthe *et al*., 2000) while other studies consider it to be rapid degradable (Goelema, 1999; Yu *et al*., 2002). However, as with other pea characteristics, it has not been very well investigated, and more studies need to be performed. Considering the protein stability, it is important to understand how protein sources differ in their susceptibility to breakdown in the rumen. Usually, proteins readily soluble in the rumen are considered to fully degrade to ammonia N. However, Mahadevan (1979) demonstrated that some insoluble proteins were hydrolysed more rapidly than soluble proteins by the protease in *Bacteroides amylophilus*. On the other hand, Aufrere *et al*. (2001) showed that proteins from lupin and pea differed in degradation rate. Interestingly, part of the rapid degraded protein could remain in the rumen and contribute to the non-ammonia N proportion. This was especially noted for lupin protein, and to a smaller extent for pea protein (Aufrere *et al*., 2001). Satter (1986) concluded that protein solubility can be a simple and useful technique to monitor treatment effects on a given protein source, but it cannot be relied upon to predict degradation across a diverse group of feeds. Pea protein is generally considered easily degradable, with rumen degradability between 79 – 88%, thus little feed rumen bypass protein remains for digestion in the small intestine (Yu *et al*., 2002). This is the main reason why we want to stabilise the protein, decreasing its rumen degradability. It is equally important to understand something about protein quality (Satter, 1986), including amino acid composition and availability of the undegraded protein to formulate hypotheses for decreasing rumen degradable protein. Pea protein has a high content of lysine and threonine (Corbett, 1997; Dandanell-Daveby, 1997) but a low total content of sulphur containing AA. Both methionine and cystein are considered first and second limiting, or co-limiting, AA in peas. For secretion of milk protein, a combination of peas and cereals results in improved protein quality in the mixture, since lysine is the first limiting amino acid in cereals. Rapeseed meal has a high content of methionine and cystein (Chapoutot & Sauvant, 1997; Khalili *et al*., 2002) and should be excellent as feed combined with peas.

On farm level processing of feed must be practical, economical and improve the nutritional value of the feed as well. The choice of processing method should be adapted to these prerequisites. Most methods that involve heat treatment or steaming are expensive, dangerous, or both, excluding them from on-farm applications (controlling heat treatment mean in most cases handling 10 bar pressure, Perez, 2003 personal communication). In the choice between wet and dry methods, it is easier to harvest at an appropriate time and acid treat or dry the peas rather than utilise a wet processing method. The simplest wet method appears to be reconstitution, which can be performed on-farm in reasonable volumes. Furthermore, most farmers have a roller mill or a hammer mill to process their grain as concentrate. These mills can be used to grind peas, both wet and dry, although they may need some adaptation. Grinding dry peas is seldom a problem; it is a matter of determining particle size and adjusting the mills accordingly. Hammer mills are more common in Sweden, but are of restricted use when grinding wet peas since the sieve can clog if the water content is too high. The cut point for this is not determined, but it definitely will not work to grind peas with more than 25 % water content (Larsson, 1988; Morén, 2003 personal communication). The roller mills are more reliable for grinding wet peas, if the rollers have appropriate groove dimensions and, preferably, two separately adjusted rollers (Larsson, 1988). If dry peas are rolled, as opposed to ground, the product differs widely in particle size, from very coarse to very fine (Larsson, 1988). The difference in particle size distribution after grinding with roller mill or hammer mill is illustrated in Figure 10. Particle size distribution is dependent on pressure, roller gap, water content and flow of the product that is going to be rolled (Ensminger & Olentine, 1978). It appears that a target water content around 16-18 % is best for roller milling, as illustrated in Figure 11, with least risk for technical complications in combination with maximal particle size differences depending on mill adjustments (Hale, 1980; Larsson, 1988; Tait & Beames, 1988). Also, this water content makes the pea pliable enough for cows to crush them during mastication. Palatability is important for dairy cows, and they are probably reluctant to eat whole, dry peas and even whole, wet peas may have a decreased palatability (Bertilsson, 2004 personal communication). A simple crushing of the peas may increase palatability.

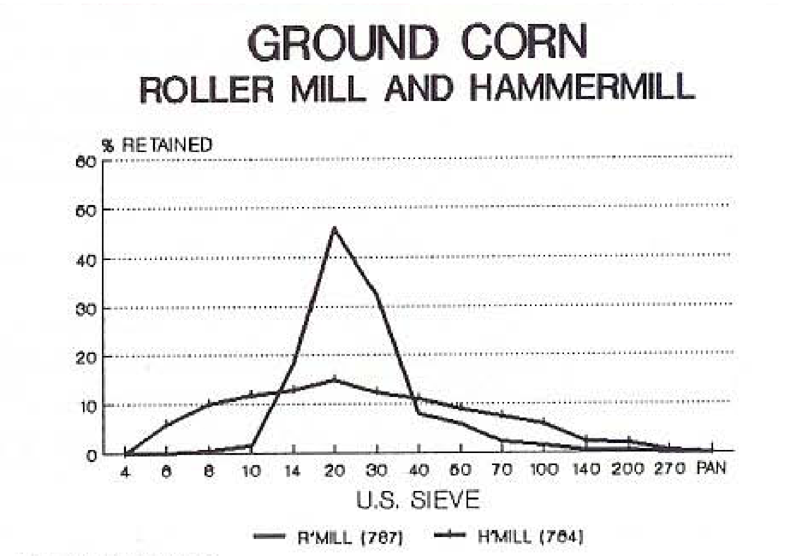

Figure 10. Comparison of corn ground in a roller mill (R'MILL) and hammer mill (H'MILL) to similar finished mean particle size. Distribution of particle size with different sieve sizes (Owens & Heimann, 1994).

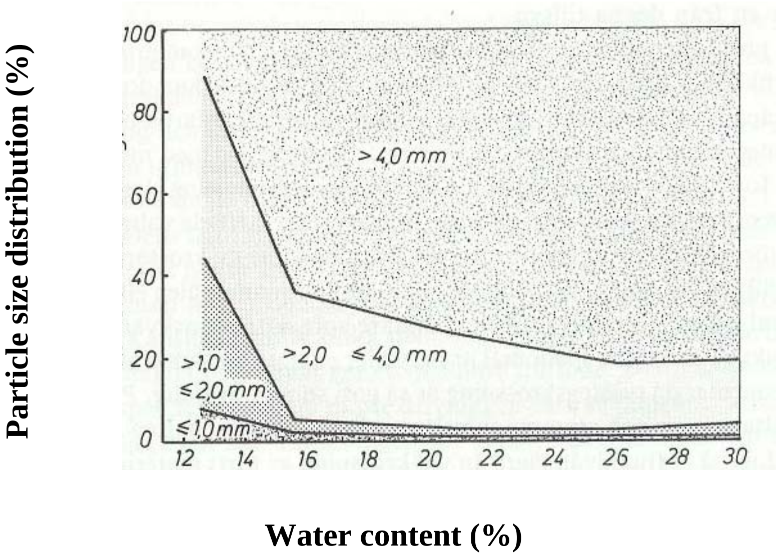

Figure 11. Particle size distribution after rolling of grains with different moisture contents. Y-axis: particle size distribution (%); X-axis: water content (%) (Larsson, 1988).

When examining effect of treatment on rumen protein stability and production parameters, it is difficult to find differences when studying treatments separately (Galyean *et al.*, 1981; Reis *et al.*, 2001). Therefore, combinations of treatments should be studied, such as acid treatment and grinding or reconstitution and grinding. However, it is still necessary to discriminate between the different treatment effects (Goelema, 2003, personal communication), which can be difficult. Since particle size appears to be important, this treatment should be included. According to

Poppi *et al*. (1980) a particle size of 1.2 mm is optimal, and this should be included together with at least one other particle size significantly different from 1.2mm.

On the whole, it can be concluded that for ruminants, as far as physiological constraints and metabolic constraints are concerned, both with grain in sole and in mixed diets, the least amount of mechanical processing necessary to give acceptable digestibilities is best for the animals in terms of health, cost and animal performance (Orskov, 1986).

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