ABSTRACT

This research was carried out at CoRFiLaC in collaboration with the CoRFiLaC research group and technical staff.

The understanding of how cows chew their feed might be one fundamental previous step necessary to study if, and how feed particle size could alter efficient feeding, milk production and composition and animal health. Our main objective was to learn some rules, of how feed particle size is reduced during the ingestive mastication in dairy cattle, in order to get an idea about rumen mat consistency from diet particle size and intake. We measured lengths distributions of feed and respective bolus particles which are potentially contributing to rumen mat formation, estimated an approximate dry matter proportion of this sample fraction, and evaluated differences in chewing behaviour between dry and lactating cows.

Poppi et al. (1985) reported that particles retained on a 1.18 mm screen, using a vertical sieving technique, are highly resistant to passage from the rumen in cattle. As vertical sieving techniques divide particles by their widths rather than their lengths and Mertens (1984) reported constant lengths to widths ratios varying from 3.4:1 for corn silage to 10:1 for alfalfa and bermudagrass hay, we concluded that particles resistant to passage out of the rumen might have lengths probably not shorter than 5 mm. We focussed on the analysis of particle lengths ≥ 5 mm. Analyses were performed using an image analysis technique. A mean length (ML) was calculated considering only particle lengths ≥ 5 mm. In order to determine the mass of particles ≥ 5 mm, we found that a screen with 1.6 mm openings, using a horizontal wet sieving technique, separated best the particles ≥ 5 mm from those of smaller dimensions. The dry proportion of samples on that screen was called PROP_1.6.

We performed two experiments. In the first experiment, 6 rye grass hay treatments differing in particle lengths, one corn silage, one grass silage and one TMR sample were fed to four dry and four lactating, rumen fistulated, dairy cows after rumen were emptied, and boli, rumen mat and feces were sampled. Rye grass hay treatments were as follows: rye grass cut at 50 mm lengths and dried to hay, long rye grass hay, chopped rye grass hay retained on the Penn State Particle Separator (PSPS) screens of 19 mm, 8 mm, 1.18 mm and on the bottom pan. In the second experiment we selected 10 total mixed ration (TMR) samples from Sicilian dairy farms. We divided TMRs into fractions by sieving them through four sequential sieves of 19 mm, 8 mm, 2.5 mm and 1.18 mm. All fractions and the unprocessed TMRs were fed to three dry dairy, rumen fistulated cows, after rumen were emptied, and boli were sampled. Feed (with exception of the long rye grass hay, rye grass cut at 50 mm lengths and dried, chopped rye grass hay retained on the 19 mm and the 8 mm screen), bolus, rumen mat and feces were sieved through a 1.6 mm screen to obtain PROP_1.6,

particles retained were separated, imaged and ML was calculated. Dry matter (DM) and contents of crude protein (CP) and neutral detergent insoluble fiber (aNDF) were determined in the feed samples.

The longest ML of a TMR particle the cows were able to swallow was about 18 mm. This was nearly twice the longest ML of rye grass hay bolus particles, which could be swallowed at ML $\leq 10 – 11$ mm.

As a difference to TMR particles, all rye grass hay fractions probably stimulated chewing to some extent. Even the smallest rye grass hay particles retained on the PSPS bottom pan were apparently reduced in PROP_1.6 although this fraction is not defined physical effective (Kononoff and Heinrichs, 2003). Ingestive chewing reduced PROP_1.6 of the unprocessed TMRs. Eating reduced also PROP_1.6 of TMR fractions retained on screens with openings of at least 2.5 mm, but not PROP_1.6 of the TMR fractions passing that screen. Eating reduced ML of TMR fractions retained on screens with openings of at least 8 mm, but neither ML of particles which had passed the 8 mm screen, nor ML of the unprocessed TMRs were reduced significantly ($p > 0.05$). The sum of TMR residues on the two upper PSPS screens with 19 and 8 mm openings might underestimate TMR physical effective fiber (pef), while the sum of residues from all three PSPS screens might overestimate TMR pef. We suggest the use of a sequential sieve set containing a 19 mm, a 8 mm sieve and an additional screen with 2.5 mm openings, instead of the additional screen with 1.18 mm openings, for diet evaluation on the farm.

Feed and bolus PROP_1.6 were highly correlated ($R^2 = 0.94$, 65 observations), when unprocessed TMRs and TMR fractions were fed, with $y = 0.79x + 0.03$ and y being bolus and x feed PROP_1.6. Rye grass hay particles and most silage samples were apparently chewed more intensely and having lower bolus PROP_1.6. Feed and bolus ML were highly correlated ($R^2 = 0.86$, 47 observations), when unprocessed TMRs and TMR fractions were fed and feed ML ≤ 20 mm, with $y = 0.76x + 2.08$ and y being bolus and x feed ML. Most silages apparently fitted this regression, too. When rye grass hay was fed and feed ML ≤ 20 mm, feed and bolus ML were correlated with $R^2 = 0.43$ at 21 observations, with $y = 0.24x + 6.13$ and y being bolus and x feed ML. Rye grass hay and TMR particles with ML > 20 mm were apparently chewed to constant lengths, with feed particle size not being related to bolus particle size. Chopped rye grass hay particles with ML > 20 mm were particles retained on the 19 and the 8 mm PSPS screen, whereas TMR with ML > 20 mm were retained only on the 19 mm screen. Only particle size of feeds with ML under this threshold might be able to influence parameters such as rumen retention time, intake and rumen degradation of feed, if these parameters were related to bolus particle size.

Rye grass hay was chewed more intensely compared to TMR particles. Rye grass hay particles were dry, while TMR treatments contained 36 – 48% water. Rye grass hay particles contained 12 – 14 (% DM) CP and 54 – 59 (% DM) aNDF, whereas CP and aNDF of TMRs were 14 – 24 and 20 – 48 (% DM), respectively. During eating, chemical parameters influenced more reduction of ML of longer compared to shorter particles, but reduction of PROP_1.6 was more affected in the shorter particles. For each % decrease in sample DM bolus ML increased approximately 0.2 mm, under the particular condition where feed particles ML ranged between 14.7 and 43.7 mm, CP content ranged between 12.8 and 13 (% DM) and aNDF content ranged between 47.5 and 51 (% DM).

Chewing behaviour of dairy cows was not altered by physiological stage nor by the interaction of treatment feed by physiological stage. Bolus PROP_1.6 and ML from dry cows were not different from lactating cows. Even though dry and lactating cows received different diets, PROP_1.6 and ML of rumen mat and feces were alike. Approximately 26% and 36% dry matter of rumen mat was retained on a 1.6 mm screen in dry and lactating cows, respectively. Rumen mat particles retained on that screen and ≥ 5 mm, had ML of 8.6 mm in dry and 10.3 mm in lactating cows. There was a trend for higher PROP_1.6 of fecal particles from dry cows compared to lactating cows ($p = 0.105$), but ML of 7.4 and 7.9 in dry and lactating cows, respectively, were not statistically different.

The lengths of the longer particle fraction from TMR, which was retained on a 1.6 mm screen, were reduced to a higher extent during rumination compared to the longer fraction of ingested particles from rye grass hay. Only approximately 38% or less of TMR DM is constituted by particles potentially retained in the rumen. However, these particles might need a longer time for rumination compared to chewed hay particles.

In both, dry and lactating cows, proportions of rumen mat particles > 4 and > 6 mm, respectively, at individual lengths, retained on a 1.6 mm screen, were highly correlated to proportions of bolus particles. Rumen mat ML can be estimated from lengths distribution of bolus particles retained on a 1.6 mm screen.

Diese Arbeit ist meiner Mutter Heidi gewidmet.

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- Particles are broken into half during one chew.

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I assumed that the frequency of chews needed before a certain feed can be swallowed depends on the longest particles in the mouth.

I made further the following assumptions in order to simplify the cases and highlight the principles:

- Particle lengths are proportional to volume.

- Particles are broken into half during one chew, maximum length of particles to be swallowed, maximum length of particles passing to feces.

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CHAPTER 1

General Introduction

History of Feed Particle Size Evaluation in Nutrition of Dairy Cows

Research regarding possible effects of feed particle size in livestock production systems started probably when industry began to pellet forages. The Artificial dehydration of forage increased chemical quality of forage because it allowed the harvest and conservation at an early and immature stage, independently from climatic conditions. The first device for artificial drying of forage, mentioned in literature, was developed already in 1894 by an English farmer named Neilson. The Institute of Agricultural Engineering of the University of Oxford conducted a series of experiments on the artificial drying of hay crops in 1924 and 1925. During the following years several studies were performed in regard to exposure time and temperatures to use during the artificial drying procedure and the chemical quality of the forage product (Huffman, 1939). Pelleting and wafering processes were often combined to dehydration systems because reduced volume decreased costs of storage and shipping. Usually wafers consisted of highly compressed forage made from long or coarsely chopped material. On the other hand, to obtain pellets, forage was finely ground and compressed. At first, researchers saw an opportunity for a possible grain replacement by this new feed resource and started experiments in regard. Woodman et al. (1930), using dried grass protein cake, replaced all of the concentrate mixture and observed satisfactory milk and fat production. Farmers purchased finely ground forage because they were told that in this form forage was able to replace grain. Hope et al. (1950) found that milk production declined when 15, 30 or 45 per cent of the grain ration was replaced with a finely ground, dehydrated, cereal grass-legume mixture. Blosser et al. (1952) compared the value of dehydrated alfalfa in the ground, chopped, and pelleted forms when substituted for 30% of the grain ration. A slight advantage in milk yield was found for the dehydrated alfalfa pellets compared to the ground alfalfa, but yields for the chopped and dehydrated alfalfa were not different.

Farmers started to feed pellets and apparently, pelleting improved the value of fibrous feed for certain classes of livestock. Jensen and McGinnis (1952) demonstrated an advantage in pelleting laying diets containing high levels of alfalfa. Schneider and Brugman (1950) pelleted a ration containing 10 per cent alfalfa for growing fattening pigs and found that this ration produced significantly greater gains than the same ration unpelleted. Eaton et al. (1952) reported that calves fed artificially dried and pelleted hay gained more weight and ate more hay than calves fed field-cured field-baled hay or artificially dried and ground hay. Paladines et al. (1964) reported that pelleted or finely ground, high-roughage rations improved the rate and efficiency of gain in growing fattening ruminants. Beardsley (1964) compiled data in a review of the effects of pelleting on intake, live weight gains and feed efficiency for several different forages fed to calves or yearlings and showed that, calculated on an individual comparison basis and averaged, pelleting a long hay increased the daily feed intake by 25%, increased daily gain by 98% and decreased feed required per unit of gain by 36%.

However, when finely ground or pelleted forages were fed to milking cows, the results on production were controversial. Some authors observed increased intake and production when pelleted forages were fed. Ronning et al. (1959) fed pelleted or chopped alfalfa hay to milk producing cows and observed that pelleting increased dry matter intake (DMI) and fat corrected milk (FCM) 4%, and that butterfat was unaffected. Brooks et al. (1962) fed Coastal bermudagrass either baled or ground and pelleted. Cows fed the pelleted Coastal bermudagrass produced more milk and FCM, had higher forage intake, gained more weight and milk with higher fat content than those fed the baled hay. Other authors found negative responses on production parameters. Milk fat was often reduced when fine ground and / or pelleted forages were fed. The production of milk with a low fat test by dairy cows fed a finely ground forage was first reported by Powell (1939). Subsequently, numerous trials with ground or pelleted forage have been conducted to test this phenomenon. In the first of two trials conducted by Porter et al. (1953), milk yield was increased and there was a slight decrease in the butterfat percentage of milk, at the same time, when ground and pelleted alfalfa hay was fed. In the second trial, a real drop in butterfat percentage was observed but in this case, the consumption of roughage was much lower, apparently due to the hardness of the pellet used. Depression of fat % was also observed by Van Soest (1955) and Ensor et al. (1959) when finely ground hay was liberally fed. Jones et al. (1958) reported a drop in butterfat percentage from 4.1% on baled alfalfa hay to 4.0% on pelleted alfalfa hay, with the difference being statistically significant. When forage was ground through 0.64 to 0.80 cm and through 0.95, 1.9, and 2.5 cm screens, slight depression in milk fat was observed by Appleman and Addis (1960) and by Haenlein et al. (1961), respectively.

The next step for research was to focus on possible explanations of these phenomena. Moore (1964) reassumed results from literature of these years in a symposium on forage utilization. The purpose of his presentation was to illustrate how the nutritive value of forage might be affected by its physical form. Cited from Moore (1964): “Compared to normal feeding practices when long or chopped hay is fed, the feeding of ground or pelleted forage results in more rapid prehension with less mastication. This results in less salivation with less secretion of buffer salts into the rumen. Since the feed particles are fine, there is less rumination and probably less stimulation of saliva secretion. More rapid bacterial fermentation results with a rapid production of organic acids. There is an increased concentration of volatile fatty acids (VFA) and a consequent lowering of pH further intensified because of a lesser amount of buffer salts from saliva being present. The lowered pH may also increase the rate of absorption of organic acids from the rumen. Because the ground and pelleted forage is finer in particle size than long or chopped hay, this also results in an increased rate of passage from the reticulo-rumen which in turn lowers the amount of digestion which can take place in the rumen as well as the total digestibility of the dry matter (DM), particularly the cellulosic or crude fiber fractions. Because of the increased rate of passage of ground and pelleted forage from the rumen, more space becomes available for more feed which in turn results in greater consumption of feed DM." Feed particle size might influences surface area of feed particles to be attached by rumen microorganisms, chewing and saliva production, intake and rumen retention time. All these parameters can affect microbial composition and growth in the rumen, production of volatile fatty acids and digestibility of feeds. Elliot and Loosli (1959), Palmquist and Ronning (1961) and King and Hemken (1962) measured a decline in milk fat percent that occurred with the feeding of ground, pelleted roughages and the changes in molar ratios of VFA in favour of propionate relative to acetate. Baumgardt (1967) concluded that maximum efficiency of milk production should be achieved on rations which result in a ruminal acetate / propionate ratio of about 2.75. Blaxter and Graham (1956) and Rodrigue and Allen (1960) reported reduced digestibility, especially of the fiber fraction, the finer the forage was ground. However, limited digestibility was explained only several years later when microbiological measurements in rumen fluid were performed. Steward (1977) and Russell et al. (1980) showed that cellulolytic ruminal bacteria are sensitive to even modest declines in pH. Crawford et al. (1980) reported that protozoa in continuous cultures were maintained only at higher solids retention times of approximately 30 h and declined continuously when solids retention times were set at 22 and 14 h. All digestibilities tended to increase with increasing solids retention time and liquids dilution rate. However, most digestibilities reached a plateau at 22 h at dilution rates of 0.11 and 0.15 / h. Increasing SRT to 30 h resulted in little or no increase at these dilution rates.

Several authors proposed changes in feeding regimen in order to moderate milk fat depression caused by pellet feeding. Palmquist et al. (1964) postulated that feeding hay pellets and grain at separate times provided a more even supply of rumen fermentation products for milk fat synthesis. Feeding pelleted forage four times rather than twice daliy (O'Dell et al. 1968, 1964) and feeding supplemental baled hay or corn silage to twice-daily-fed animals (O'Dell et al. 1964) prevented declines in milk fat. O'Dell et al. (1968) indicated that the critical grind size to effect milk fat depression was 0.64 cm. Chalupa et al. (1970) proposed that small quantities of supplemental conventional forage (corn silage or baled hay) could alleviate the milk fat depression caused by feeding pellets as the sole forage.

Interests in regard of particle size of individual forages changed somewhat as soon as total mixed rations became the widespread adopted feeding system of dairy cows in the United States. First reports concerning the so called complete rations appeared in the mid 60's. Until the late 50' pasture was dominant forage for most dairy herds, followed by hay and silage. Cited from Coppock et al. (1981): "In the late 50's and early 60's, an important discovery was made concerning the ability of lactating dairy cows to respond to additional concentrates. As the average herd size increased in the 1960's there was greater emphasis on labor efficiency. There seemed to be a uniform tendency to feed all concentrates in the milking parlors, and parlors with associated feeding equipment were designed accordingly. As production increased and greater mechanization further reduced the time cows spent in the parlor, cows were not able to consume their required concentrate. Some farmers added concentrate to the forage fed outside in a bunk in addition to parlor grain feeding. However, this often aggravates the problem of providing a correct protein-energy ratio across the complete production spectrum of the herd." As a consequence, magnetically, transponder activated feeders or electronically controlled feeding systems were developed. Cows which ought to receive additional concentrate had a magnet, transponder or a cadmium plated key on the neck chain. Computer controlled feeders allowed individual concentrate feeding of each cow (Coppock et al. 1981). Another strategy to avoid grain feeding in the milking parlor was the feeding of cows with a complete or total mixed ration (TMR). It is defined as a quantitative mixture of all dietary ingredients, blended thoroughly enough to prevent separation and sorting, formulated to specific nutrient content, and offered ad libitum. This system was used much earlier for other livestock species, because dairy nutritionists felt that it was difficult to apply because of the wide range in energy requirements within a herd, and the consequent need for grouping of cows. Dairy farmers in the United States are feeding total mixed rations since the mid 60's (Olson 1965, McCoy et al. 1966) and development of computerized diet formulation started about the same time (Howard et al. 1968, Chandler and Walker 1972). Nocek et al. (1986) compared feeding with TMR to computer controlled feeder systems. The authors reported that the computer controlled feeder system was an effective method to allot grain in a freestall housing situation according to individual cow production. However, in the United States most dairy farmers adopted the TMR feeding. Silages in the TMRs were able to dilute and mask the flavour of unpalatable ingredients such as urea. Diet formulation for groups of cows might have been easier than diet formulation of individual animals. The feeding system with TMR allowed the use of a wide range of concentrates and by-products at the same time, and diet formulation was not restricted to few components as it might have been the case with computer controlled feeder systems. Long forage was not fed to cows in lactation anymore, because all ingredients were blended in the mixer wagon. In regard of particle size, the questions for farm management became how long forages should be chopped to produce good quality silages and how long feed ingredients should be blended in the mixer wagon to obtain optimal diet particle size. Diet particle size depended on both, initial particle size of feed ingredients, especially the forages, and mixing time. Farmers needed to consider that silages were further reduced in size during the mixing of the diet in the mixer wagon. The challenge for research was to define that diet particle size that was most efficient for nutrition of dairy cows.

Many research studies focussed on the understanding of the regulation of feed intake. Research Troelsen and Bigsby (1964) developed an artificial masticator to simulate particle breakdown in the rumen. Artificial mastication was carried out on samples from 14 hays that had been fed to sheep. Particle breakdown was highly correlated to feed intake. Van Soest (1966) suggested that the cell wall structure of plant materials is responsible for the volume occupied in the rumen by forage, even if partially digested and devoid of contents. Smith and Waldo (1969) proposed a procedure to determine particle size breakdown in the gastrointestinal tract of ruminants. The authors suggested the use of neutral detergent as decribed by Van Soest (1966) prior to a sieving procedure to allow particle size analysis of pelleted forages without the use of mechanical forces to disintegrate the pellets.

Balch (1950), Mertens (1973) and Waldo et al. (1972) have shown that digestibility is a function of the kinetics of digestion and passage. Intake of forages is related to fiber digestion because it is limited by the rate of disappearance of material from the digestive tract (Castle, 1956; Conrad et al.,1964; Crampton, 1957; Mertens, 1973; Thornton and Minson, 1972 ; Waldo, 1969). Based on these two concepts, Mertens and Ely (1979) developed a first dynamic, mathematical model which described the disappearance of forage fiber from the digestive tract of ruminants considering the kinetics of passage, particle size reduction and digestion. Cited from Mertens and Ely (1979): "The objective of many forage evaluation programs in animal science and agronomy is to assess forage quality for chemical and physical characteristics of the feed. Although many factors have been suggested and evaluated as determinants or indicators of forage quality, most have been discarded or found to be of limited use when used as the sole index of forage nutritive value. This suggests that accurate assessment of forage quality must include the interactions of the animal and its microorganisms with the chemical, morphological and physical properties of forages and the end products resulting from their utilization. Since it may not be feasible to measure and evaluate all relevant factors and interactions involved in forage quality in a single experiment, it was concluded that modeling and simulation can offer an excellent opportunity to delineate the role of animal and plant characteristics in forage fiber digestion. A model of forage fiber digestion could provide information about digestibility and intake."

Short diet particles are likely to increase intake but when roughage becomes limited in the diet because of the inclusion of grains or finely chopped forages, metabolic disorders often occured, even though the diet was adequate in all known nutrients. Lack of coarse material in the rumen reduced chewing activity (Balch et al, 1955; Sudweeks et al., 1980), reduced rumen motility diminished muscle tone (Colvin et al., 1978; Nocek and Kesler, 1980; Nocek et al., 1980). Some of the disorders resulting from low roughage intake were the fat cows syndrome (Morrow, 1976; Clark and Davis, 1980; Fronk et al., 1980), abomasal ulcers (Bide and Dotward,1975; Julien and Conrad, 1977), acidosis (Brent, 1976), rumen parakeratosis (Nocek and Kesler, 1980), displaced abomasums (Breukink and deRuyter, 1976), polioencephalomalacia (Brent, 1976), laminitis (Brent, 1976), ketosis (Fronk et al., 1980) and, in dairy cows, reduced milk fat percentage (Balch et al., 1955; Chalupa et al., 1970; Latham et al., 1974; Thomas, 1975; Murdock and Hodgson, 1979; Erdman et al., 1980). These diseases were not correlated directly to forage particle size itself but more to forage quantity and chemical composition of the diet. Only years later, forage particle size itself has been evaluated to possibly affect diseases such as left displaced abomasums (Shaver, 1990; Dawson et al., 1992, Shaver 1997) or subacute rumen acidosis (SARA) and laminitis (Stone 2004). Krause et al. (2002a, 2002b) fed lactating cows the same amount of fine or coarse alfalfa haylage with the same amount of either dry or high moisture corn. As expected, rumination and total chewing times were greater in the diets containing long haylage (Krause et al., 2002b), whereas effective ruminal digestibility of diet DM tended to be higher (P = 0.08) in the diets containing high-moisture corn (Krause et al.,2002a). Diets containing finer haylage particles and high-moisture corn reduced mean ruminal pH, the minimum daily pH, and both the time and area (time∗amount) below pH 5.8 compared with diets containing coarser haylage and dry ground corn (Krause et al.,2002b). The results from this study indicated that ruminal pH is influenced both by dietary components affecting chewing and salivary buffer secretion, and by those affecting ruminal carbohydrate fermentation. Forage particle size might be an important coparameter to influence occurrence and gravity of all diseases which are correlated to rumen pH and volatile fatty acid concentration and composition, because of its ability to regulate chewing activity, saliva flow to the rumen and buffering capacity. In the 70's and 80's, researchers knew from Balch et al. (1955) that chewing time and milk fat percentage of dairy cows were reduced as grain replaced long hay in the diet and that chewing time was reduced as particle size decreased. Chewing time became an indicator of roughage value. Sudweeks et al. (1975) studied effects of forage and concentrate types and amounts on chewing time and derived roughage indexes for those forages and concentrates. However, chewing changed not only with forage quality (Welch et al. 1969), total DM, and forage intake (Sudweeks et al. 1980), but with particle length as well. In consequence, Santini et al. (1983) proposed to use adjusted forage intakes to predict roughage indexes. The adjusted forage intake was calculated by multiplying actual intakes of forages by mean particle length of the forage.

There was a need for a better description of particle size reduction including particle size distribution in feed, digestive tract and feces (Mertens and Ely, 1979). The concept of a critical particle size has been used in the development of models of digesta flow (Hungate, 1966; Baldwin et al., 1977; Ulyatt et al., 1976; Mertens and Ely, 1979). Particles that appear in feces have escaped the rumen and can be used to indicate the size of particles that do not need or stimulate chewing. Important studies have been performed in the 80's to define this critical size. Poppi et al. (1985) concluded that particles retained on a 1.18 mm sieve had a high resistance to passage from the rumen of both cattle and sheep. Cardoza (1985) measured the particle size of feces from dairy cows fed 40 different combinations of forage and concentrate. He observed that <5% of fecal particles were retained on sieves with 3.35 mm apertures and that the median particle size of feces for dairy cows was retained on sieves with apertures of 0.4 to 1.18 mm. Mertens (1997) concluded that particles passing a 1.18 mm screen readily pass out of the rumen and provide little stimulus for chewing and defined only particles retained on this screen to be physical effective. There were already some previous concepts of effective fiber or roughage replacement values that could be used quantitatively to formulate rations that would maintain the production of milk fat. These effective fiber values were based on different standards, such as cottonseed hulls (Harris 1984), hay (Gleaves et al., 1973; Milligan et al. 1981), or alfalfa silage (Clark and Armentano, 1993; Swain et al., 1994). Mertens (1986) suggested that the role of physical characteristics of feeds would be elucidated more clearly if the differences in chemical fiber (NDF) among feeds were removed. He suggested a system for assessing the roughage value of feeds based on a theoretical standard (long grass hay containing 100% NDF). Mertens (1986) standardized the effectiveness values that had been proposed previously (Clark and Armentano, 1983; Harris, 1984; Swain et al., 1994) so that they would be on the long grass standard scale and used those values as roughage value adjustment factors that could be multiplied times NDF. However, nowadays, the term "physical effective fiber" or "physical effective NDF (peNDF)" usually refers to the concept presented by Mertens (1997). He proposed a laboratory method to assess physical effective NDF, which included the determination of the feed dry proportion on a 1.18 mm screen using a vertical sieving technique. The proportional dry residue had to be multiplied by the NDF content of the sample in order to obtain physical effective NDF. This parameter is used in current nutrition models, such as CPMDairy Version 3.0.8

(Cornell University, Ithaca, NY; University of Pennsylvania, Kennett Square, PA; and William H. Miner Agricultural Research Institute, Chazy, NY).

About the same time Mertens published his concept, Lammers et al. (1996), proposed a simple tool to use on the farm to assess feed particle size. The standard method for determining the particle size distribution of chopped forages was standard S424.1 of the American Society of Agricultural Engineers, ASAE (ANSI, 1993). The screens of the ASAE device had nominal openings in the screens of 19.0, 12.7, 6.3, 3.96, and 1.17 mm from the top to the bottom screen, respectively. It was designed for chopped forage material only. Sample drying and sieving procedure were expensive and time consuming. Lammers et al. (1996) designed a simplified separator with two screens and a bottom pan. The hole sizes were selected to match the expected distribution of feed particles based on results of samples in the ASAE device. Screens were needed to characterize the larger particles that were of interest and to separate the sample into measurable fractions. Because the larger particles were more important, the top screen was selected to measure the larger particles, and the bottom screen was selected to separate the remaining portion nearly equally. These hole sizes also gave two points that were far enough apart to increase the reliability of the slope of the particle size distribution line. The diameters of the hole sizes of the screens were 19 and 8 mm for the top and bottom, respectively, dividing the sample into three portions: material greater than 19 mm in length remaining on the top screen, material between 19 and 8 mm in length on the middle screen, and material less than 8 mm in length on the bottom pan. The authors proposed a horizontal, manual sieving technique on the wet material, which could be used directly on the farm, avoiding a previous sample drying procedure. The sieving equipment was called Penn State Particle Separator (PSPS). Even though the original apparatus was widely accepted by nutritionists, most of a TMR's concentrate (typically formulated at 40 to 60%), passed through the 8.0 mm sieve. As a result, an additional sieve containing a pore size of 1.18 mm was developed and pretended to more accurately describe the smaller particle fraction of TMRs (Kononoff et al., 2003a). The authors selected the pore size of 1.18 mm for this additional sieve relying on the 1.18 mm screen which was recognized to retain particles which don't pass easily from the rumen (Poppi et al., 1985). However, the authors didn't consider the fact that PSPS method provided a horizontal sieving technique, separating particles more likely by their lengths, whereas Poppi et al. (1985) used a vertical technique, where particles were more likely separated by their widths. The authors should have probably selected a screen with bigger openings in order to separate particles which are more likely to be retained in the rumen from the fraction which contains the smallest particles presumably passing to feces. In the Penn State Technical Bulletin, DAS 02-42 (Heinrichs and Kononoff, 2004) several recommendations are reported: "If corn silage is the sole forage, at least 8 percent of the particles should be in the upper sieve of the separator, compared to a minimum of 3 percent when corn silage is not the sole forage. The chop length of corn silage must balance good packing and fermentation with extremely short, pulverized forage. This means 45 to 65 percent of the silage material should remain on the middle sieve and 30 to 40 percent on the lower sieve of the separator. If the last screen is used for corn silage, no more than 5 percent should be recovered in the bottom pan. As corn silage makes up a greater proportion of the ration, more material should remain in the middle two sieves and less in the top sieve and bottom pan. Up to 20 percent of haylage should remain on the upper sieve. The middle sieve should contain 45 to 75 percent of the haylage and the lower sieve 20 to 30 percent. As with corn silage, no more than 5 percent of the material should be retained on the bottom pan. Guidelines for TMRs for high producing dairy cows are 2 to 8 percent of the particles in the upper sieve, 30 to 50 percent in the middle and lower sieves, and no more than 20 percent in the bottom pan." The PSPS, with or without the additional screen is widely used today and not only as a on farm tool, but also for research. However, it is probably not a good tool to describe mean particle length and has some more limitations when used for research. Cited from Mertens (2005): "Separation of undried feeds with gentle shaking is often incomplete because small particles adhere to large particles and this is especially true for small starch particles. In addition, the particle size distribution of undried forages and total mixed rations can be biased if larger and smaller particles have different DM concentrations." Mertens (2005) recommended a vertical sieving procedure on dry material using a set of 9 sieves and a bottom pan. He required complete separation, which is defined by the shaking time needed to reach the maximum plateau weight of residue in the bottom pan. To minimize bridging of material on sieves, an adequate sample size should be used, no sieve should contain more than 25% of the total sample and additional sieves in the geometric progression should be added to meet this restriction. The top sieve should not retain significant material because it is impossible to calculate the average size of its retained particles because there is no sieve above it through which the particles have passed. If less than 2% of the material is retained on the top sieve, it is acceptable to estimate the size assuming a geometric progression of sieves. Similarly it is impossible to calculate the average geometric mean size of particles in the pan because the pan has an aperture of zero. However this problem is not as serious as for the top screen. The disadvantage of a vertical sieving technique is that it separates particles by the width. In consequence, the calculated mean size represents mean width and has to be converted to length. Igathinathane et al. (2009) confirmed length-based separation inconsistency as a definite feature of mechanical sieving. They concluded that the passing of particles slightly smaller than twice the opening dimension cannot be avoided. The maximum deviation observed in particle lengths was in excess of 17 times with respect to opening dimensions of standard sieves. Mertens et al. (1984) reported that the length of sieved corn silage particles were about 3.4 times their width. The ANSI (1993) method used horizontal shaking and the diagonal dimension of the square opening (which is √ 2 times the square dimension) for determining mean particle length. Mertens (2005) suggested to multiply the mean particle size by 4.8 to approximately estimate the mean particle length. On the other hand, vertical sieving allows complete separation. Complete separation isn't possible with a horizontal procedure as it is provided by the PSPS method. Some particles, which are longer than the sieve aperture will always fall through. The longer the sieving time the higher the probability that particles are divided by their width as well. Square or circular openings don't prevent "falling through" or "nose diving" effect of lengthier particles passing through smaller sized sieve openings. In consequence, sieves with thicker walls were designed (ASABE Standards S424.1, 2007) to help restrict the easier passing of lengthier particles. However, Womac et al. (2007) observed this length-based separation inconsistency in particles size analysis of knife-milled ground corn stover, switchgrass, and wheat straw even, using ASABE design sieves (ASABE Standards S424.1, 2007) with thicker walls. Their measurements of particle length with a digital caliper were roughly five times greater than the geometric mean dimensions calculated from sieving results. Based on their results, they emphasized that sieve analyses of irregular shaped particles offer only relative comparisons, and that actual dimensions of particles retained on sieves are not necessarily represented by sieve opening sizes above and below the sample. A horizontal technique can't allow bouncing of particles, because then again separation would be more by particle width rather than length. In order to obtain repeatable results the shaking must follow a very strict and detailed procedure. According to Kononoff at al. (2003a) the sieve has to be shaken horizontally five times in one direction, then rotated one fourth turn, and again shaken five times. The procedure has to be repeated for eight sets of five replications for a total of 40 shakes. One shake is considered as a forward and backward motion over a distance of 17 cm. Estimates of particle mean lengths calculated from particle residues on 2 or 3 screens only, are probably of poor accuracy anyway, especially when high residues on the top screen are measured.

A computer vision based image processing method can be considered as an alternative or even a replacement for sieve analysis. The square or circular openings of standard sieves, in the strictest sense, only allow "width-based separation" of particles. That is, these openings truly restrict particles of width larger than the sieve opening dimension irrespective of particle orientation with respect to the openings (Igathinathane et al., 2008a). Igathinathane et al. (2009) determined the effect of number of virtual sieves by simulation with respect to sieveless analysis involving all distinct particles. The number "10" indicated the approximate number of sieves that can be handled generally in a single nest of mechanical sieve shakers. Only a close match to the results from sieveless analysis was obtained from sieving results that used more than 50 simulated sieves (75 and 100 sieves). However, image analysis have some restrictions as well. The two basic requirements of computer vision digital image based size and size distribution analysis are an input image and an image processing algorithm. Devices for image acquisition include digital cameras, charge-coupled device cameras (Visen et al., 2004) or flatbed scanners (Shahin et al., 2006; Igathinathane et al., 2008a,b, 2009). For better results with image preprocessing, clear contrast between particles and background is essential. The definition of length and width of a irregular shaped particle, as it might occur with forage particles, might not be an easy decision. Particle separation might be the biggest impediment for a precise particle size determination, especially when samples containing very small particles are examined. Arrangement of the particles with respect to one another other is another important aspect. A singulated arrangement of particles (disjoint particles without overlap or touching one another) makes the preprocessing algorithm simpler avoiding the need for specialized singularization algorithms like watershed or successive erosion and dilation (Shahin and Symons, 2005). Manual separation is labor intense and analysis of a reduced sample size might lead to lost in precision when samples are analyzed which contain particles heterogeneous in size and which are not well mixed. The definition of a sampling procedure and a minimum sample to analyze might become the crucial requirements for correct particle size analysis using image processing methods.

Effects of Feed Particle Size on Diet Utilization and Cow Performance Reported in Literature

A new aspect of feed particle size in regard of dairy cows' nutrition emerged since TMR feeding has been adopted. Several available ingredients are mixed together at individual proportions in order to obtain necessary nutrient composition which ensures the optimal nutrition of the dairy cow. Each animal ought to eat each feed ingredient at the same proportion the ingredient goes to the diet. In consequence, the diet needs to be mixed very well and any type of feed sorting from the animal side should be avoided. The sorting of animals leads to unbalanced nutrition not only of the animals which sort but also of those which don't sort. Feed sorting has been related to left displaced abomasum (Shaver, 1997), to ruminal acidosis and laminitis (Stone, 2004). In general, all diseases and responses in terms of production which are attributed to over or under nutrition of feed components can occur when cows sort for individual ingredients of the diet. If ingestion was selective large diurnal variation in acid production may result in ruminal disorders, which contribute to a rapid depression of DMI (Krause and Oetzel, 2006). Sorting behavior can be assessed by measuring particle size distributions and amounts of offered and refused feed. Sorting activity for each feed fraction retained on individual screens was calculated as the actual intake of each fraction expressed as a percentage or proportion of the predicted intake of that fraction (Leonardi and Armentano, 2003; Zebeli et al., 2009). Studies in which feed sorting has been considered are listed in table 1.1. In 8 out of 10 studies sorting behavior was related positively to feed particle size, in only 2 studies feed particle size was not significantly related to sorting. The longer the particles were the more sorting occurred. In most studies, cows selected in favor of the smaller particles, but not always. In 5 out of 7 studies, cows sorted against the long particles, but in 2 of the studies, the cows seemed to prefer the longer particles. Qualitatively, the cows are able to sort feedstuffs in their diets and usually the really eaten diet contains more concentrate and fine particle forages than the distributed diets. The major constituent of fine particles in the diet is grain of low particle size which is easily fermented by rumen microbiobia. The difference between bunk contents before and after distribution can reveal a lack of physical structure for the animals, at least during the first meal after distribution, when cows first eat fine particles. Moreover, sorting behaviour can largely vary among cows, and in a free-stall barn, where sorting cows are free to move to minimally sorted TMR, this sorting behaviour could be enhanced (Leonardi and Armentano, 2003). Adding long particle hay to the diet can be inefficient for chewing stimulation on these cows (Armentano and Leonardi, 2003). Bhandari et al. (2008) fed a diet containing both alfalfa silage and oat silage, which were varying in chop length. There was no effect of diet particle size on chewing time and rumen pH, but rumen pH was low for all diets, even for the diets containing longer chopped forage. The duration of the rumen pH below 5.6 was greater than 120 min / d for all diets. Hence, it could not be excluded that these diets did not induce subacute ruminal acidosis (SARA). Also, the milk fat percentages of all diets were low and an inversion of the milk fat percentage and milk protein percentage occurred for all diets, which suggested SARA (Kleen et al., 2003; Stone, 2004). The authors explained the onset of SARA despite apparently adequate dietary NDF and particle size distribution by sorting of the cows against long feed particles in favour of short feed particles. On the other hand, cows with induced acidosis and given free choice alfalfa hay or pellets preferentially ate hay (Keunen et al., 2002), which was interpreted by the authors as an attempt to attenuate acidosis. Yang and Beauchemin (2005, 2006a, 2006b) observed similar cow behaviour, where cows selected longer rather than the shorter particles. The authors concluded that dairy cows may intentionally select long feed particles to meet their need for physically effective fiber when ruminal pH is low due to low intake of peNDF. How should diet particle size be to avoid sorting behaviour? Leonardi et al. (2005) reported that particles longer than 26.9 mm may be selected against by some cows. The authors suggested to achieve adequate mean particle length with the least amount of particles longer than 26.9 mm and the greatest amount of particles between 26.9 and 9 mm to obtain a uniform response across the entire herd. Asadi Alamouti et al. (2009) observed increased sorting of the diet against particles > 19mm, and in favour of those < 8 mm ($p < 0.05$), when diets with long forage particles were fed. It might be difficult to exactly define possible diet particle size at which sorting behaviour is reduced to a minimum. However, if cows selected against particles > 19 mm, and particles smaller that size which are retained on the 8 mm PSPS screen are still considered to stimulate chewing, than the farmer should probably adapt chop lengths of forages for silage and mixing times of diet ingredients in the mixer wagon to obtain TMR particles which contain only very small amounts to be retained on the top PSPS screen and most possible particles to be retained on the second PSPS screen with 8 mm openings.

Since decades research studies have been performed to test influence of feed particle size on milk yield, composition and animal health. There is some evidence that feed particle size could alter intake, saliva flow to the rumen and buffer capacity of the rumen, as well as digestibility. Smaller particles might require less chewing, and less saliva might be produced. Smaller particles might be reduced at a shorter time to a size which allows passage through the reticulo-omasal orifice and in consequence cows might be able to eat more, as free space in the rumen increases. Increased intake might allow longer particles to pass through the reticulo-omasal orifice. Luginbuhl et al. (1990) found mean rumen mat and fecal particle size increased when steers had higher levels of intake. Deswysen and Ellis (1990) measured longer particles in the dorsal sac and feces of heifers with higher intake. The authors of the latter study observed that higher voluntary intake was positively related with duodenal DM digesta flow per opening of the reticulo-omasal orifice. Explanation of digestion might be more complex. It probably depends on particle surface in the rumen plus retention time. Smaller feed particles in the rumen have bigger surface areas and may promote microbial attachment and nutrient degradation (Bowman and Firkins 1993). Nutrient availability in the rumen determines microbial composition and growth. On the other hand, smaller rumen particles are more prone to pass out of the rumen than bigger ones (Poppi et al 1985). In other words, smaller rumen particles might deliver nutrient faster to rumen microbes, but microorganisms have less time for consumption. The opposite concepts apply to longer rumen particles. However, the longer particles might be able to trap smaller ones, and increase rumen retention time also for particles which would be small enough to pass out from the rumen easily. The chewing itself, and the buffer flow in consequence, might alter absorption of VFA through the rumen wall and might influence digestion as well. Cellulolytic ruminal bacteria are very sensitive to pH and cannot survive at low pH. The understanding of how feed particles are chewed and of what size ingested particles are might be important to comprehend whether and how feed particle size can influence cow performance, milk composition and animal health.

In table 1.2, 36 research studies are listed in which feed particle size has been evaluated to influence several parameters, such as chewing time, rumen pH, total tract digestibility, DMI, milk yield and composition. Forage and diet particle sizes which were tested varied between studies as well as the forage type itself. Evaluated forages consisted in alfalfa hay, silage and haylage, and corn, oat and barley silage. In 18 studies, where eating time, in terms of minutes per kg DMI, has been measured, 8 studies reported positive correlation of particle size (eating lasted longer the longer the particles were), and 10 reported no significance. In 20 studies, where effects of diet particle size on rumination time, in terms of minutes per kg DMI, have been investigated, 15 were positively correlated to feed particle size and 5 were not significant. In 15 trials, where diet particle size effects on DM and organic matter (OM) total tract digestibility have been evaluated, 8 reported no significant effect, 4 reported DM and 5 OM digestibility to be higher when longer particle diets were fed, and 3 and 2 reported the opposite effect on DM and OM, respectively. In 16 studies, where NDF total tract digestibility has been measured, 5 reported no particle size effect, in 9 studies digestibility was higher when longer particles were fed and in 2 the opposite observations were made. In 11 experiments, where effects on CP total tract digestibility have been evaluated, 5 found no effect, 4 reported higher and 2 lower digestibility when longer diets were fed. Starch digestibility has been evaluated in 10 experiments, and in none of the studies effects of diet particle size were reported. Out of 28 studies, where rumen pH has been determined, 9 reported higher and 2 lower pH, when longer particles were fed, and in 17 studies no effect of diet particle size on rumen pH was observed. DMI was measured in 35 trials, in 13 lower and in 3 higher intake was observed when diet contained longer forages, 19 found DMI not altered by feed particle size. Particle size effects on milk yield have been evaluated in 36 studies. In 33 no effect was observed, 1 found increased and 2 decreased milk production when diets with longer particles were fed. In 36 studies changes in milk fat % were determined, with 24 showing no particle size effects, 10 reporting increased and 2 decreased fat %. Milk protein % and lactose % have been evaluated in 36 and 17 experiments, respectively. Protein % was affected by feed particle size only 5 times positively and 4 times negatively. Reduced milk lactose % was related to feeding of longer particles only in 1 study, in 16 studies no feed particle size effect has been observed. In the investigated studies, diet particle size apparently had most effects on rumination time / kg DMI (with 75% positive responses), followed by NDF total tract digestibility (56% positive responses), eating time / kg DMI (44% positive responses), DMI (37% negative responses) and milk fat % (28% positive responses). However, results of the cited literature are often contrasting. Nutrient requirements of the animals, as well as mass, chemical composition and physical from of the nutrients fed might interact and define as a whole concept the abovementioned responses. Threshold size of feed particles to influence these parameters might vary in base of chemical nutrient requirement and feeding, and effects of chemical nutrient feeding might vary in base of chemical nutrient requirement and feed particle size feeding (Asadi Alamouti et al., 2009; Teimouri Yansari and Primohammadi, 2009; Soita et al., 2005; Krause and Combs, 2003; Krause et al., 2002a, 2002b). Until today, particle threshold sizes to alter cow performance and health are not yet defined. Tafaj et al. (2007) attempted to quantitatively summarize and discuss data from 25 published experiments to evaluate effects of particle size of the forage portion of TMR on digestion, DM intake and milk production in high-yielding early lactation dairy cows. The authors concluded that dietary forage particle size alone did not affect feed intake, milk production or milk fat content in early lactating cows (median 81 days in milk). In contrast, rumen pH positively correlated with particle size of forage of TMR ($p < 0.05$). The acetate to propionate ratio correlated positively to NDF content ($p < 0.05$), but not to dietary particle size. Furthermore, a positive linear relationship occurred between forage particle size and chewing time, ruminating time and the NDF digestibility ($p < 0.05$). Poor response to forage particle size in this study might be, at least in part, attributed to inconsistent particle size determinations of individual experiments. In some studies vertical sieving techniques were performed using 5 sieves with sizes according to the ASAE method S424.1, in others the PSPS horizontal method with 2 or 3 screens was used. A forage particle mean length was estimated and related to response parameters. The calculation of mean lengths included all particle fractions, also that small particle fraction that is prone to pass out of the rumen easily. Lengths of particles which are more likely to be retained in the rumen could define rumen retention time, rumen fill and feed intake. In contrast, lengths analyses of particles which have dimensions similar to faecal particles don't make much sense, because these particles are less likely to contribute to rumen mat formation and they would pass out of the rumen independently from their lengths. The mean length with these very short particles included, might not represent a sensible tool to detect possible particle size effects, because theoretically this sample part supposedly has the opposite response compared to the longer particles remaining in the rumen. Particles of fecal dimensions, contrary to the particles retained in the rumen, could cause increased intake, they should not stimulate regurgitation, and they might cause a drop of ruminal pH, depression of fiber digestion and milk fat (%) in consequence (table 1.3). Another aspect which might explain, at least in part, poor response to feed particle size, might be the fact that feed particle size and not masticate or digesta particle size has been related to response variables. Differences of diets in particle size must not necessarily be maintained after diets have been chewed and ingested. Only in very few studies masticates and / or ruminal digesta particle size was measured additionally to feed particle size and production data (Fernandez et al., 2004; Beauchemin et al., 2003; and Kononoff and Heinrichs, 2003). Fernandez et al. (2004) fed whole plant corn silage chopped either to coarse or to fine particles. Mean size of the bolus particles and the proportion of bolus particles > 2 mm was not altered by forage chop length.

The authors reported neither an effect of forage particle size on eating and rumination time / kg DMI, nor on total tract digestibility, milk yield or composition. The only response to forage particle size observed was pH, which actually was higher when shorter particle diets were fed (table 1.2). Kononoff and Heinrichs (2003) fed one of 4 diets, which were chemically identical but included alfalfa haylage of different particle size; short, mostly short, mostly long and long. Mean particle size of digesta was affected by forage particle size ($p = 0.06$), but the proportion of digesta particles to be retained on a 1.18 mm screen was not. Both, eating and rumination time / kg DMI were higher when diets with longer forage particles were fed, DMI was lower and so was total tract digestibility of DM, OM, crude protein (CP) and NDF (table 1.2). Beauchemin et al. (2003) had either chopped or ground alfalfa hay in the diet. Masticate particle mean length was affected with $p = 0.12$ and rumen mat mean particle length with $p = 0.07$. Proportion of masticates particles retained on a 1.18 mm screen was significantly different ($p < 0.01$) when either chopped or ground alfalfa hay was fed, proportion of rumen mat particles was not. Only rumination time, not eating time / kg DMI was affected by forage chop length, DMI and production was not altered, and digestibility was not measured in this study (table 1.2).

Factors Affecting Chewing Behaviour of Cows

There is still little knowledge about how dairy cows chew their feeds in terms of particle size reduction. Lee and Pearce (1984), Shaver et al. (1988), Nelson (1988), Bailey et al. (1990), Schwab et al. (2002), Rinne et al. (2002), Kononoff and Heinrichs (2003), Beauchemin et al. (2003), Pan et al. (2003), Fernandez et al. (2004) and Acosta et al. (2007) measured feed and respective masticates particle size in cattle. Kennedy (1985), Waghorn et al. (1989), Kovács et al. (1997), Tomoko Oshita et al. (2004), Zebeli et al. (2007) determined feed and respective rumen mat particle size in cattle. One big problem to summarize these data is probably the interpretation of particle size description of feed and bolus due to the variation in sieving techniques and sieve sizes used. In addition, as discussed earlier, the prediction of particle size from sieving procedures has some limitations. One more difficulty to compare results of these studies consisted in the fact that tested feeds were often fed within a TMR and analysed bolus or rumen mat contained particles of all diet ingredients and not only the forage particles which varied in lengths.

Another problem might be the interpretation of chewing data from different animals, different physiological stages and body weights. Body weights of the animals tested varied from 267 kg in Nelson (1988) up to 886 kg in Fernandez et al. (2004). De Boever et al. (1990) listed productive potential, ingestive capacity, chewing efficiency and / or body weight to be possible causes for individual variations of chewing activity. The authors reported that animals with higher intake capacity needed less time to eat and ruminate and that rate of eating or rumination was positively correlated to body weight. Peréz-Barberìa and Gordon (1998) explained that chewing effectiveness or particle size reduction was related to bite force and tooth morphological features such as occlusal surface area, occlusal contact area cutting enamel edges and enamel features. These authors reported that body mass and age of the animal might be related to bite force and tooth wear.

Tested animals in the above listed studies were either steers (Lee and Pearce, 1984; Kennedy, 1985; Nelson, 1988; Kovács et al, 1997; Pan et al., 2003) or dairy cows varying in physiological stage from cows in early and peak lactation (Kononoff and Heinrichs, 2003; Fernandez et al, 2004) to mid-lactation (Schwab et al., 2002; Beauchemin et al., 2003), late lactation (Rinne et al., 2002; Zebeli et al., 2007; Acosta et al., 2007) and dry (Tomoko Oshita et al., 2004). There is some evidence from literature, that chewing activity of dairy cows might be influenced by physiological stage. High-producing dairy cows might need to compensate higher nutrient requirement and increased intake, by chewing diets more intensely during ingestion to reduce necessary rumination time and promote passage. Okine et al. (1991) and Le Liboux and Peyraud (1998) observed increased eating time / kg DMI when feed intake increased in non lactating and milking dairy cows, respectively. In opposite to these studies, other authors observed shorter rumination times in cows with high intakes and / or production even though these cows apparently chewed their feeds less intensely during ingestion. Grant and Albright (1995) reported that high-producing dairy cows are more aggressive eaters, spending less time eating and ruminating per unit of intake. Shaver et al. (1986) reported shorter rumination time / intake of NDF and shorter total chewing / DMI in cows at early and middle lactation compared to dry cows. These cows were fed the same diet, with decreasing intake from early lactation to the dry period, containing alfalfa hay and grain at a ratio of 60:40%.

Only 9 (Shaver et al, 1988; Bailey et al.,1990; Schwab et al., 2002; Kononoff and Heinrichs, 2003; Beauchemin et al., 2003; Pan et al., 2003; Fernandez et al., 2004; Zebeli et al., 2007) out of the 15 above listed research studies evaluated influence of feed particle size on bolus or rumen mat particle size. The other trials dealt with the comparison of forage species or plant maturity. Forage species and conservation (fresh, hay, silage or haylage, straw) as well as chemical composition of tested feeds varied between the studies. Physical structure as well as chemical composition might effect chewing and particle size reduction. Cited from Peréz-Barberìa and Gordon (1998): "Thickness of cell walls, orientation of vascular bundles, morphology of leaves, and the number and thickness of sclerenchyma bundles have been related to the shape and size of particles produced by chewing and the energy required in the process (Akin and Burdick, 1981; Vincent, 1982; Wilson et al., 1989; Mtengeti et al., 1995; Wright and Illius, 1995; Wright and Vincent, 1996). Ingestive chewing and rumination effort varies with feed type, parts and physical presentation of the plant

(Pearce and Moir, 1964; Trudell-Moore and White, 1983; Lee and Pearce, 1984; McSweeney and Kennedy, 1992; Brouk and Belyea, 1993; Dryden et al., 1995) and increases with feed maturity (Poppi, Minson and Ternouth, 1981b; Ulyatt, Reid & Carr, 1982). Changes in anatomical morphology of plant leaves in relation to maturity (thicker sclerenchyma or cell walls) result in increased difficulty of their physical breakdown. Marked differences in toughness are found between plant species (Wright and Illius, 1995). For example, leaves of legumes are less tough than grasses, and within grasses, tropical species are tougher than temperate species (Wilson and Kennedy, 1996). Percentages of NDF, acid detergent fibre and acid insoluble lignin should reflect, to some degree, the toughness of the plant." Mertens (1997) showed, summarizing chewing data from other research studies, that, although the variation in chewing among long forages is related primarily to differences in NDF concentration, chewing per kg of NDF increased as the NDF in long forages increased. Asadi Alamouti et al. (2009) evaluated effects of partial replacement of neutral detergent soluble fiber from pelleted beet pulp, for starch from ground barley or maize grain, in diets on chewing activities. Eating time not rumination time / kg DMI was higher the more beet pulp was fed. Kowsar et al. (2008) measured changes in chewing behaviour of dairy cows when chopped alfalfa hay in the ration was gradually replaced by corn silage. Diet DM and acid detergent fiber decreased the higher the corn silage content in the diets was. Eating and rumination time / kg DMI was not affected by corn silage content in the diet even though the diet which contained only alfalfa hay without any corn silage had apparently the shortest particles. The alfalfa hay diet without any corn silage had the lowest percentages of particles retained on the top and the middle screen of the Penn State Particle Separator (PSPS) with openings of 19 mm and 8 mm, respectively, and highest residues on the lower screen with openings of 1.18 mm. Vincent, (1983) reported that drying of leaves of Lolium perenne and Phleum pratense reduced their water content and increased the tensile strength of intervening cells, although the force required to fracture across the veins was almost independent of water content. However, there is some evidence that water content in the diet might alter chewing behaviour as well. Beauchemin et al. (2003) evaluated ratio of alfalfa silage to alfalfa hay of diets on chewing activity. The diet which contained more alfalfa silage had the longer particles, but required less eating time / kg DMI. Teimouri Yansari and Primohammadi (2009) evaluated the effects of two methods of alfalfa feeding, dry and reconstituted, on chewing. Hays were reconstituted 24 h before feeding by placing the required amount of dry hay into an industrial container and adding slowly water at ambient temperature to the hay during mixing to achieve a theoretical DM content of 350 g/kg. Diets containing the reconstituted hay had shorter eating and rumination times / kg DMI.

Research Objectives

The understanding of how cows chew their feed might be one fundamental previous step necessary to study if, and how feed particle size could alter efficient feeding, milk production, milk composition and animal health. Our main objective was to learn some rules, of how feed particle size is reduced during the ingestive mastication in dairy cattle, in order to get an idea about rumen mat consistency from diet particle size and intake. Our first issue was to measure particle size reduction during the eating of grass hay at various chop lengths (chapter 2). Grass hay has been chosen because we wanted to analyze that kind of feed first that is presumably chewed most intensely by the cows. We have excluded straw, because straw is not commonly used for the nutrition of dairy cows. In that first experiment, one corn silage sample, one grass silage sample and one TMR sample have been analysed additionally, in order to get an initial sense of how chemical composition of feeds might influence chewing behaviour. A detailed particle size study of masticates from various individual forages at individual lengths would have been very time consuming. For the next trial, we decided, to evaluate particle size reduction of TMR fractions, because this is actually the most commonly used feed. This feeding system occurred first in the mid 60' in the United States and nowadays it has been adopted by many dairy farms in North America and Europe. It is defined as the practice of weighing and blending all feedstuffs into a complete ration which provides adequate nourishment to meet the needs of dairy cows. It can contain several different forages as well as numerous by-products and concentrates. The TMR fractions, which we have tested, were obtained by a sequential sieving procedure. The fractions differed not only in particle size but also in chemical composition, as especially the shorter particle fractions contained more grain (chapter 4). Differences in chewing behavior between dry and lactating dairy cows are treated in chapter 3.

Table 1.1. Effect of diet particle size on sorting behaviour and preference of cows.

Reference	Feed[a]	Cow behaviour	
		Sorting	Preference[b]
Asadi Alamouti et al. (2009)	Alfalfa hay	+	short
Teimouri Yansari et al. (2004)	Alfalfa hay	+	
Teimouri Yansari et al. (2004)	Alfalfa hay	+	
Kononoff and Heinrichs (2003)	Alfalfa haylage	ns	
Bhandari et al. (2008)	Alfalfa silage		short
Bhandari et al. (2007)	Alfalfa silage	+	
Krause and Combs (2003)	Alfalfa silage	+	short
Yang and Beauchemin (2006a and b)	Barley silage		long
Zebeli et al. (2009)	Corn silage	+	short
Bhandari et al. (2007)	Corn silage	+	
Couderec et al. (2006)	Corn silage	ns	
Yang and Beauchemin (2005), Beauchemin and Yang (2005)	Corn silage		long
Kononoff et al. (2003b)	Corn silage	+	
Bhandari et al. (2008)	Oat silage		short
Leonardi et al. (2005)	Oat silage	+	short
	Total[c]	10	5 short, 2 long
	ns	2	
	+	8	
	-	0	

[a] Diet feed component differing in particle size.

[b] Short = sorting in favour of short particles; long = sorting in favour of long particles.

[c] Total = number of research papers where parameter was measured; ns = number of research papers where sorting was not significantly correlated to diet particle size;
+ = number of research papers where sorting increased with more longer particles.
- = number of research papers where sorting increased with more shorter particles.

Table 1.2. Effects of diet particle size on dry matter intake (DMI), milk yield and composition, on ruminal pH, chewing time and total tract digestibility in literature.

Reference	Diet PS[a]	Feed[b]	DMI	Milk yield	Milk composition: Fat	Milk composition: Protein	Milk composition: Lactose	Ruminal mean pH	Chewing time: Eating h/d	Chewing time: Eating Min / kg DM	Chewing time: Rumination h/d	Chewing time: Rumination Min / kg DM	Total tract digestibility[c]: DM	OM	CP	NDF	Starch
			---- kg / d ----		---------- % ----------								----------------- % -----------------				
Grant et al. (1990a)	1	AH	ns	ns	+	ns		+	+		+	+					
Grant et al. (1990b)	1	AS	ns	ns	+	$+^{t}$		$+^{t}$	ns		+						
Schwab et al. (2002)	1	CS	-	ns	ns	ns	ns	ns	ns	$+^{e}$	ns	$+^{e}$	ns	ns		$+^{e}$	ns
Clark and Armentano (2002)	1	AS	ns	ns	ns	ns			ns	ns	+	+					
Leonardi et al. (2005)	1	OS	-	-	ns	$-^{t}$		ns	+	+	$+^{t}$	+					
Onetti et al. (2003)	1	CS	-	ns	ns	ns		+									
Beauchemin et al. (2003)	1, 2	AH	ns	ns	ns	ns	ns	+	ns	ns	+	+					
Bhandari et al. (2007)	1, 2	AS	ns	ns	ns	ns		ns									
Bhandari et al. (2007)	1, 2	CS	-	ns	ns	ns		-									
Kononoff and Heinrichs (2003)	1, 2	AHL	-	ns	ns	ns		ns	+	+	ns	+	-	-	-	-	
Yang et al. (2001)	1, 2	AS AH BS	ns	ns	+	+	ns	ns					ns	ns	ns	ns	ns
Teimouri Yansari and Primohammadi (2009)	1, 2	AH	-	ns	-	+	ns		+	+	+	+	ns	ns	+	+	

Table 1.2. Continued

Reference	Diet PS[a]	Feed[b]	DMI	Milk yield	Milk composition: Fat	Milk composition: Protein	Milk composition: Lactose	Ruminal mean pH	Chewing time: Eating h/d	Chewing time: Eating Min / kg DM	Chewing time: Rumination h/d	Chewing time: Rumination Min / kg DM	Total tract digestibility[c]: DM	Total tract digestibility[c]: OM	Total tract digestibility[c]: CP	Total tract digestibility[c]: NDF	Total tract digestibility[c]: Starch
			---- kg / d ----		---------- % ----------								----------------- % -----------------				
Bhandari et al. (2008)	1, 2	AS	ns	ns	ns	ns		ns	+[¥]		ns						
Bhandari et al. (2008)	1, 2	OS	-	ns	-	-[t]		ns	ns		ns						
Teimouri Yansari et al. (2004)	1, 2	AH	ns	ns	+	-	ns	+	+	+	+	+	ns	ns	ns	+	
Yang and Beauchemin (2005), Beauchemin and Yang (2005)	2	CS	ns	ns	ns	ns	ns	ns	+	ns	+[t]	ns	+	+	+	+	ns
Couderec et al. (2006)	2	CS	-[t]	ns	ns	ns	ns										
Einarson et al. (2004)	2	BS	-	ns	ns	ns	ns	ns									
Kononoff et al. (2003)	2	CS	-	ns	ns	ns		+[t]	ns	ns	ns	+[t]					
Rustumo et al. (2006)	2	AHL CS	ns	ns	+[¥]	ns	ns	ns									
Yang and Beauchemin (2006a and b)	2	BS	ns	ns	ns	-	ns	ns	ns	ns	+	+[t]	-[t]	-[t]	ns	-[t]	ns
Yang and Beauchemin (2007a and b)	2	AS	ns	ns	ns	ns	ns	+	ns	ns	ns	+	ns	ns	+	+	ns

Table 1.2. Continued

Reference	Diet PS[a]	Feed[b]	DMI	Milk yield	Milk composition: Fat	Milk composition: Protein	Milk composition: Lactose	Ruminal mean pH	Chewing time: Eating h/d	Chewing time: Eating Min / kg DM	Chewing time: Rumination h/d	Chewing time: Rumination Min / kg DM	Total tract digestibility[c]: DM	OM	CP	NDF	Starch
			---- kg / d ----		---------- % ----------								----------------- % -----------------				
Yang and Beauchemin (2009)	2	AS	ns	ns	ns	ns	ns	+	ns	ns	ns	ns					
Zebeli et al. (2009)	2	CS	-	ns	ns	ns	ns										
Bal and Bal (2010)	2	AH	ns	ns	ns	ns											
Alamouti et al. (2009)	2	AH	ns	ns	ns	ns	-	ns	ns	ns	ns	ns	$+^{t}$	$+^{t}$	ns	ns	
Soita et al. (2005)	2	CS	ns	ns	ns	ns											
Krause and Combs (2003)	3	AS	+	-	+	ns	ns	ns	+	+	+	+	$+^{t}$	+		ns	ns
Bal et al. (2000)	3	CS	ns	ns	ns	ns	ns	ns					ns	ns	ns	+	ns
Woodford and Murphy (1988)	4	A	+	+	+	ns		ns	ns	nm	+	+	ns	ns		+	
Jaster and Murphy (1983)	4	AH	-										+		+	+	
Fisher et al. (1994)	4	AS	-[d]	ns	+	ns	ns	ns		ns		ns					
Beleya et al. (1989)	4	AH	ns	ns	ns								-		-	ns	
Colenbrander et al. (1991)	4	AS	+	ns	ns	+			ns		+						
Fernandez et al. (2004)	4	CS	ns	ns	ns	ns		-	ns	ns	ns	ns	ns	ns		ns	ns
Le Liboux and Peyraud (1998)	4	DHA		ns	+	ns		ns	+	+	+	+		+		+	ns
Le Liboux and Peyraud (1999)	4	DHA	ns	ns	+	+		+	+	+	+	+		+		+	ns

Table 1.2. Continued.

Reference	Diet PS[a]	Feed[b]	DMI	Milk yield	Milk composition: Fat	Milk composition: Protein	Milk composition: Lactose	Ruminal Mean pH	Chewing time: Eating h/d	Chewing time: Eating Min / kg DM	Chewing time: Rumination h/d	Chewing time: Rumination Min / kg DM	Total tract digestibility[c]: DM	Total tract digestibility[c]: OM	Total tract digestibility[c]: CP	Total tract digestibility[c]: NDF	Total tract digestibility[c]: Starch
			---- kg / d ----		---------- % ----------								----------------- % -----------------				
		Total[f]	35	36	36	35	17	28	23	18	23	20	15	15	11	16	10
		ns	19	33	24	26	16	17	13	10	9	5	8	8	5	5	10
		+	3	1	10	5	0	9	10	8	14	15	4	5	4	9	0
		-	13	2	2	4	1	2	0	0	0	0	3	2	2	2	0

[a] Method of diet particle size determination:

1 Vertical sieving with a 1.18 mm screen in the sieve set
2 Horizontal sieving using the Penn State Particle Separator
3 Mean geometrical particle size
4 No particle size measurements of TMR

[b] Diet feed component differing in particle size.

[c] DM = dry matter, OM = organic matter, CP = crude protein, NDF = neutral detergent insoluble fiber.
AH = alfalfa hay; AS = alfalfa silage; CS = corn silage; OS = oat silage; AHL = alfalfa haylage; BS = barley silage; A = alfalfa haylage and alfalfa pellets; DHA = dehydrated alfalfa.

[d] only in primiparous cows, $p < 0.10$, not significant in multiparous cows.

[e] only one of two contrasts were significant, $p < 0.10$.

[f] Total = number of research papers where parameter was measured; ns = number of research papers where parameter was not significantly correlated to diet particle size;
+ = number of research papers where parameter response on diets with longer particles was positive;
- = number of research papers where parameter response on diets with longer particles was negative

ns = not significant;
+ = positive response on diets with longer particles, $p < 0.10$;
- = negative response on diets with longer particles, $p < 0.10$.

[ŧ] $p < 0.15$
[¥] $p < 0.18$

Table 1.3. How could feed particle size influence milk production?

Effect	"Short particles" Similar to fecal particles		"Long particles" Longer than fecal particle	
	Mass	Particle length	Mass	Particle length[1]
Contribution to rumen mat formation	-	no	+	+
Stimulation of regurgitation	-	no	+	+
Intake	+	no	-	-
Ruminal pH	-	no	+	+
Fiber digestion	-	no	+	+
Milk fat %	-	no	+	+

[1] Longer particles have increasing effect +,
longer particles have decreasing effect -.

CHAPTER 2

How Do Dairy Cows Chew their Feed?
Part I:
Particle size analysis of feed and ingested bolus particles from rye grass hays with different particle lengths distributions, from a grass and a corn silage sample and from a sample of a total mixed ration

INTRODUCTION

In many research studies feed particle size has been tested to be a possible parameter capable to influence milk yield, composition and animal health. Smaller feed particles in the rumen have bigger surface areas and may promote microbial attachment and nutrient degradation (Bowman and Firkins 1993). Nutrient availability in the rumen determines microbial composition and growth. On the other hand, smaller ruminal particles are more prone to pass out of the rumen than bigger ones (Poppi et al 1985). In other words, smaller ruminal particles might deliver nutrient faster to ruminal microbes, but microorganisms have less time for consumption. The opposite concepts apply to longer ruminal particles. However, the longer particles might be able to trap smaller ones, and increase ruminal retention time also for particles which would be small enough to pass out from the rumen easily. The understanding of how feed particles are chewed and of what size ingested particles are might be important to comprehend whether and how feed particle size can have influence on cow performance, milk composition and animal health. In literature, reported effects of feed particle size are often contrasting. For example, in most of the studies, milk yield did not change when diet particle size was varied. However, Leonardi et al. (2005) and Krause and Combs (2003) showed decrease in milk yield, with $p < 0.01$ and $p = 0.08$, respectively, when diet particle size increased. Woodford and Murphy (1988) reported higher milk yield ($p < 0.05$) when diet contained alfalfa haylage instead of alfalfa pellets. In many studies (Bal and Büyükünal Bal 2010, Zebeli et al. 2009, Yang and Beauchemin 2009) no effects of diet particle size on milk fat has been reported. Some authors (Teimouri Yansari et al. 2004, Krause and Combs 2003 and Le Liboux and Peyraud 1999) observed milk fat (%) to be impaired ($p < 0.05$) when diets with shorter particles were fed. Others (Teimouri Yansari and Primohammadi 2009 and Bhandari et al. 2008) reported increased milk fat (%), $p < 0.001$ and increased fat yield (kg/d), $p = 0.09$, respectively, when diets with shorter particles were fed. Some of these contrasting results, might be, at least in part, explained by the fact that feed particle size and not masticate or digesta particle size has been related to response variables. Differences of diets in particle size must not necessarily be maintained after diets have been chewed and ingested. Only in very few studies masticates and / or ruminal digesta particle size was measured additionally to feed particle size and production data (Fernandez et al. 2004, Beauchemin et al. 2003 and Kononoff and Heinrichs 2003).

There is still little knowledge about how dairy cows chew their feeds in terms of particle size reduction. Particle size measurements of a sample should probably include an estimate of how much of the sample is not prone to pass rapidly out of the rumen as well as an analysis of distribution of particle lengths of that part. Lengths of particles which are more likely to be retained in the rumen could define rumen retention time, rumen fill and feed intake. In contrast, lengths analyses of particles which have dimensions similar to fecal particles don't make much sense, because these particles are less likely to contribute to rumen mat formation and they would pass out of the rumen independently from their lengths. The calculation of a mean length including these very short particles might confound a possible particle size effect, because theoretically this sample part supposedly has the opposite response compared to the longer particles remaining in the rumen. Particles of fecal dimensions, contrary to the particles retained in the rumen, could increase intake, they should not stimulate regurgitation, and they might cause a drop of ruminal pH, depression of fiber digestion and milk fat (%) in consequence.

The objective of our study was to measure lengths distributions of feed and respective bolus particles which are potentially contributing to rumen mat formation and estimate the dry matter proportion of this sample fraction. We wanted to start our analyses with feeds which probably require most chewing but which are commonly used in dairy rations at the same time. We selected 6 rye grass hay samples differing in particle lengths distribution. Additionally, we also tested a corn and a grass silage sample and a sample of a total mixed ration (TMR).

MATERIALS AND METHODS

The experiment has been conducted at CoRFiLaC, which is a dairy research center located in Ragusa in the Southeast of Sicily, in Italy, and funded by the Sicilian region.

Treatment Feeds and Chemical Analysis

Treatments included 6 rye grass hays which had different particle lengths distributions, one grass silage which was a mix of rye grass and triticale, one corn silage and one TMR sample. The TMR was the basal diet of our fistulated animals in lactation. First cutting, rye hay, harvested in June and stored in 250 kg round bales, was used to produce five of six rye grass hay treatments in this study. The first treatment was the long hay itself which was the forage source of our fistulated dry cows. We provided 4 more hay treatments by processing one bale through a horizontal mixer wagon for approximately 12 minutes and separating the chopped material into four sizes by processing it through a Penn State Particle Separator (PSPS) with three screens (19 mm - upper, 8 mm - middle, and 1.18 mm - lower) and a bottom pan. The residues on each sieve and bottom pan were collected to produce hay treatments. Approximately 100 g of chopped hay from the mixer wagon was placed on the top screen of the PSPS, shaken three times on each side for three full turns. We continued the sieving procedure until sample size was sufficient for our trial. We provided a final rye grass hay treatment by cutting fresh rye grass by hand at a constant length of 50 mm and drying the chopped forage to hay. The fresh grass was cut at pre-bloom maturity, 0.75 to 1 meter in height from the field used to produce the baled hay.

Chemical analysis consisted in the determination of dry matter (DM), organic matter (OM), neutral detergent fiber (aNDF), and crude protein (CP). Feeds were dried overnight at 105°C to obtain DM and ashed in a muffle furnace at 550°C for 4 hours to obtain ash content and OM. We analysed aNDF content according to Mertens (2002) using sodium sulfite and heat stable α-amylase (Sigma-Aldrich, Steinheim, Germany). Nitrogen content was determined by a standard Kjeldahl procedure with Cu^{2+} as a catalyst, and multiplied by 6.25 to obtain CP. We also determined the silage pH.

Animals and Feeding Protocol

We used four dry, mature and four lactating Holstein cows in the study. Cows were ruminally fistulated. Both rations, for dry as well as for lactating cows, were formulated according to CPMDairy Version 3.0.8 (Cornell University, Ithaca, NY; University of Pennsylvania, Kennett Square, PA; and William H. Miner Agricultural Research Institute, Chazy, NY) to meet nutrient requirements of the dry and the milking cows, respectively, of the farm where our fistulated animals were located and sampling occurred.

The dry animals were fed a ration composed of ad libitum rye grass hay from the cutting used for the study and 3 kg of concentrate (18% CP, 22% aNDF, 8.5% ash, containing corn meal, soybean meal, wheat bran, minerals and vitamins). Grain was offered once a day from a common feed manger. The lactating animals were 139 ± 73 days in milk (DIM) and produced in average 30.9 ± 10.3 kg per day and cow. Milk fat and protein were 3.2 ± 0.7% and 3.2 ± 0.1%, respectively. These cows were fed a total mixed ration (TMR) which contained 12.3% rye grass hay, 12.9% mixed grass silage, 20.9% corn silage, 28.9% corn and barley grain, 3.7% wheat bran, 16.6% soybean meal (44% CP), 1.1% Soypass® and 3.6% mixed supplement of vitamins, minerals, urea, fat and amino acids. Ingredient, chemical composition of diets, intake and milk production are reported in table 2.1. Animals were housed in a communal pen with free access to hay or TMR and water.

Sampling of Feces, Rumen mat and Bolus

Sampling occurred at two periods. We sampled in each period from two dry and two lactating cows. In period 1, lacating cows were in average 202 DIM and proded 37.8 kg / d / cow. In period 2, lactating animals were in average 76 DIM and produced 24.1 kg / d / cow.

Prior to experimental sampling, animals were moved to individual tie stalls, feed was with held for 4 hours, and a fecal sample from each cow (approximately 500 g) was collected into a polyethylene bag, sealed and placed on ice.

We removed rumen cannulas and emptied rumen digesta. We collected the more solid material in up to six sequential 10 liter buckets. A sample of rumen digesta (approximately 250 g wet material) from each bucket was collected for particle size analysis, sealed and placed on ice. Following rumen evacuation, approximately 300 g of each hay treatment or 1000 g of silage or TMR was offered. The sequence of treatments was random for each individual cow. Feed intake of each treatment for each cow was determined, by weighing the offered feed and refusal. We additionally counted the chews during ingestion. The cows were allowed to swallow two boli within a treatment prior to sampling three boli for particle size analysis. Boli were obtained by manual collection through the rumen cannula at the rumen-reticular oesophageal orifice when cows were observed to swallow. The three boli were composited in one polyethylene bag, sealed, labelled with cow ID and treatment number and placed on ice. Residual feed was removed from the feed manager and the next treatment offered. Boli collection procedures were followed for each treatment until all treatments had been offered to each cow. Excess boli for each treatment were removed from the rumen prior to the delivery of the next feed treatment.

Particle Size Analysis

The objective of this study was to analyse particle lengths of that sample part that is more likely to contribute to rumen mat formation. Poppi et al. (1985) found particles retained on a 1.18 mm screen highly resistant to passage from the rumen in cattle. Cardoza (1985) showed that the median fecal particles from dairy cows are retained on sieves with apertures from 0.4 to 1.2 mm. Mertens (1997) concluded that particles passing a 1.18 mm screen readily pass out of the rumen and provide little stimulus for chewing and defines only particles retained on this screen to be physical effective. The mass of physical effective particles is determined by vertical sieving. This method separates particles by their widths rather than their lengths. Minimum width of sample retained is defined by dimension of the opening. In screens with square openings, the particles could theoretically fall through the diagonal, which would be approximately 1.7 mm in case of screens with square openings of 1.18 mm side length. The ANSI (1993) method used horizontal shaking and the diagonal dimension of the square opening (which is √ 2 times the square dimension) for determining mean particle length. Mertens et al. (1984) have reported constant particle width to length ratios depending on the forage source. Ratio of width to length for alfalfa and bermudagrass hay particles was about 10:1 and for corn silage about 3.4:1. Mertens (2005) suggested to multiply the mean particle size by 4.8 to approximately estimate the mean particle length. In conclusion, physical effective particles are probably not shorter than about 5 mm. This is the reason why we decided to analyse particle lengths of particles ≤ 5 mm.

Image analysis allows a much more detailed description of particle lengths distribution compared to sieving procedures. Precision of particle lengths description by sieving depends on the number and size of the screens used as well as on the technique itself. What is retained on the sieves is always a mixture of particles of different dimensions, even if a very large number of screens are used. On the other hand, image analysis also has some limitations. The biggest limitation is probably the sample size. Calculation of individual particle dimensions are automated, but particles have to be separated by hand. We decided to eliminate particles < 5 mm by sieving, prior to image analysis, in order to speed up particle separations and to increase possible sample size. We needed to select that screen size that retained all particles ≥ 5mm and removed as many particles < 5 mm as possible at the same time, in order to estimate most precisely the mass of particles ≥ 5mm. We decided, after some previous tests, to use a 1.6 mm screen for the present study. There were up to 10% of fecal particles left on this screen and each sample analysed had some particles < 5 mm.

Figure 2.1 reassumes roughly particle size analysis. All samples were sieved twice through a 1.6 mm screen with exception of the long rye grass hay, rye grass cut at 50 mm length and dried to hay, chopped rye grass hay retained on a 19 mm screen, and chopped rye grass hay passing a 19 mm screen, but retained on a 8 mm screen. Samples were weighed on the screen. Screen and sample were immersed in water at room temperature, the screen was moved four times 90° clockwise followed by 90° anticlockwise. After each move the screen was lifted gently. We repeated the whole procedure 12 times. One sieved sample was placed in the oven and dried to a constant weight at ≤ 60°C. We also determined sample DM under the same drying conditions, and calculated proportional dry matter retained on a 1.6 mm (PROP_1.6) screen based on both measurements. The other sieving residue was subjected to image analysis according to Licitra et al. (2005). Image analysis was performed directly on 2.5 – 3 g of rye grass cut at 50 mm length and dried to hay, chopped rye grass hay retained on a 19 mm screen, and chopped rye grass hay passing a 19 mm screen, but retained on a 8 mm screen. Long rye grass hay, and chopped rye grass hay passing a 1.18 mm screen but retained on the bottom pan were not imaged. Samples were soaked in acetone, NDF solution and tert-butanol to ease particle separation, and then dyed with Safranin solution to improve image processing. Wet particles (3 – 3.5 g) were separated and distributed in 24 cm x 36 cm quadrants on a white nylon mesh (0.39 mm x 0.77 mm pores). Pictures were taken using a digital camera (Nikon Coopix E 990, model no. 4112962, 3.34 Megapixel, Nikon Corp., Tokyo, Japan) mounted 50 cm from the surface of the mesh screen. The flash was turned off and pictures were recorded at a resolution of 2,048 x 1,536 pixels. Images were analysed using the image processing toolbox in Matlab® (Version 6.0.0.88, 2000, the Mathworks, Inc. Asheboro, NC) set to the local threshold technique.

Statistical Analysis

Statistical analyses were carried out with SAS (Version 9.1, SAS Inst. Inc., Cary, NC). The GLM procedure of SAS was used to test for differences between the chemical composition of treatment feeds.

Particle frequency by 1 mm lengths was calculated from the Matlab image analysis. Particle mean length (ML) of particles retained on the 1.6 mm sieve and ≥ 5 mm, standard error and statistical tests were determined using the SAS LIFETEST and LIFEREG procedures. The distribution of 1 minus the cumulative frequency proportion as a function of length (l) was calculated. This distribution follows a failure time curve, as the cumulative distribution function (CDF) is related to the survival function as 1-S(t), where S(t) is the survival distribution function evaluated at time t, with length, l, substituted for t. The CDF represented the probability that a length did not exceed length l. The PROC LIFETEST, method Kaplan-Meier (KM), was used to estimate ML. The difference in survival curves was tested using the univariate test statement in PROC LIFETEST. In order to test for differences in mean lengths and control for cow, survival curves were also examined using PROC LIFEREG. Nonparametric estimates of the CDF function and estimates of ML were best fit using a loglogistic distribution. Separation of mean survival time (i.e. ML) was performed with treatment and cow as class variables; cow and treatment were included as independent variables for boli; treatment alone was included in the model for the hay treatments.

The SAS MIXED procedure was used for testing differences in PROP_1.6, ML and chews / g DM ingested. Differences in PROP_1.6 within feed and bolus samples were tested with cow as repeated subject and the covariance matrix set as VC. Independent variables were treatment, production level and sampling period. Differences in ML within feed and bolus samples were tested with cows set as repeated subject. The covariance matrix were set as ARH(1) and AR(1), respectively. Independent variables for feed ML tests were treatment, production level and sampling period, for bolus ML tests treatment, production level, treatment * production level and sampling period. Differences in chews g DM ingested were tested with cows as repeated subject. Covariance matrix was set ARH(1) and independent variables were treatment, production level and treatment*production level.

RESULTS AND DISCUSSION

Table 2.2 shows the chemical composition of the treatment feeds. Rye grass hay treatments had all similar contents of ash (10 – 12% DM), grass silage had a lower ash content (10% DM), the TMR and the corn silage had the lowest ash content (6 – 8% DM). Highest CP was measured in the TMR sample (17% DM), followed by the chopped rye grass hays retained on the third screen and bottom pan (14% DM), by the other rye grass hay treatments (12% DM) and by the silage samples (9% DM). Contents of aNDF was lowest in the TMR (38% DM). The corn silage and the grass silage sample contained 48 and 53 (% DM) aNDF, respectively. The third screen and bottom pan residues of the rye grass hay samples had an average content of 54 (% DM) aNDF. The rye grass hay treatments with the longer particles had aNDF contents ranging from 57 – 59 (% DM). In the hay treatments CP content increased as particle size decreased. A greater proportion of leaf fragments might have been retained on the lower screen and bottom pan. The one rye grass treatment where fresh grass was cut to 50 mm lengths and subsequently dried to hay had similar chemical characteristics compared to the baled hay used for the other treatments. Silage and TMR DM was 29% and 51%, respectively.

The figures 2.2 present the variability of particle lengths distribution within and between treatments from image analysis. The long hay and the hay passing all the screens and retained on the bottom pan were not imaged. We measured some of the long hay particles by hand and found the typical particle to be approximately 600 mm long. The hay particles retained on the bottom pan were powdery. The residual particles retained on the 1.6 mm screen were visually much shorter than fecal particles retained on the same sieve and very difficult to separate by hand. Figures 2.2.a, b and c represent rye grass cut at 50 mm lengths and dried to hay, the chopped rye grass hay and retained on the upper PSPS screen and the chopped hay which had passed the upper PSPS screen but was retained on the middle screen, respectively. These three treatments were not sieved through the 1.6 mm screen before particles were separated and images were taken, because we supposed that all treatment particles were longer than 5 mm anyway. However, the image analysis shows that these treatments contained particles < 5 mm. Rye grass forage cut at 50 mm lengths and dried to hay had in average 14% of particles < 5 mm, chopped hay retained on the upper PSPS screen 23% and chopped hay retained on the middle PSPS screen 11%. Excluding particles < 5mm, for the treatment cut to 50 mm length, the majority of particles (≥ 1%), ranged from 40 to 57 mm. The chopped hay retained on the upper PSPS screen had a wider range of particles lengths compared to the latter treatment and the maximum amount of particles (≥ 1%), varied between lengths of 26 and 64 mm. The majority (≥ 1%), of rye grass hay particles retained on the middle PSPS screen had lengths between 11 and 40 mm. Rye grass hay retained on the lower PSPS screen had particles up to 18 mm lengths, the grass silage had particles up to 23 mm lengths, the corn silage up to 18 mm lengths and the TMR sample particles up to 19 mm lengths, considering the particles ≥ 1%. We observed a discrete variability of particle proportions at individual lengths within treatment feeds. The TMR sample, the silages, and the chopped rye grass hay sample retained on the upper PSPS screen appeared more heterogeneous compared to the other treatments.

Figures 2.3 show the mean reduction of particle lengths during ingestive mastication. Particle % relative to total imaged particles, at individual lengths was plotted for both, treatment feed and respective bolus. Mean distribution within 3 mm intervals and means of eight animals were plotted. Bolus particle % was less than feed particle % for particles ≥ 20 mm, 20 mm, 14 mm, 8 mm and 11 mm, for rye grass hay cut at 50 mm lengths, for chopped rye grass hay retained on the upper PSPS screen, for chopped rye grass hay retained on the middle PSPS screen, for chopped rye grass hay retained on the lower PSPS screen and for the grass silage, respectively. The rye grass hay cut at 50 mm lengths and the chopped hay retained on the upper PSPS screen apparently had the same bolus particle lengths distribution, regardless the distributions of particle lengths of the original feeds. There was apparently little difference in lengths distributions of imaged particles from corn silage, and TMR and the respective bolus particles. Curves of feed and bolus particles of the corn silage and TMR sample overlap more than one time.

In figures 2.4 proportions of imaged bolus particles are plotted upon respective proportions of imaged feed particles at equal length. These figures show both, reduction in particle lengths and correlation between feed and bolus particles. Figures 2.4.a, b and c show feed and bolus distributions from rye grass hay cut at 50 mm length, chopped rye grass hay and retained on the upper PSPS screen and chopped hay retained on the middle PSPS screen, respectively. Less than 1% of imaged bolus particles from rye grass hay cut at 50 mm length, but 63% of the imaged hay particles were longer than 34 mm. We estimated an $R^2 = 0.46$, if we correlated feed and bolus particle proportions from this treatment, within a range of particle lengths of 4 - 34 mm, by a linear regression. Less than 1% of imaged bolus particles from chopped rye grass hay retained on the upper PSPS screen, but 58% of the imaged hay particles were longer than 31 mm. We estimated an $R^2 = 0.14$, if we correlated feed and bolus particle proportions from this treatment, within a range of particle lengths of 4 - 31 mm, by a linear regression. Less than 1% of imaged bolus particles from chopped rye grass hay retained on the middle PSPS screen, but 28% of the imaged hay particles were longer than 27 mm. We estimated an $R^2 = 0.60$, if we correlated feed and bolus particle proportions from this treatment, within a range of particle lengths of 4 – 27 mm, by a linear regression. There was apparently little correlation between proportions of imaged feed and bolus particles at individual lengths, and for these three hays bolus particle lengths distributions of particles ≥ 5 mm could not be estimated accurately from feed lengths distributions. Chopped rye grass hay retained on the lower PSPS screen was the hay treatment with shortest particles imaged. Less than 1% of imaged bolus and only 6% of imaged feed particles from chopped rye grass hay retained on the lower PSPS screen were longer than 18 mm (figure 2.4.d). In opposite to the hay treatments with longer particles, there was a high correlation ($R^2 = 0.99$) between imaged feed and bolus particle proportions of individual lengths, within a range of particle lengths of 9 – 18 mm. There were higher proportions of imaged bolus particles relative to imaged hay particles at lengths in the range of 1 – 8 mm, and lower proportions for particles between 9 – 18 mm, with $y = 0.7847x - 0.0064$, and y and x being imaged bolus and feed particle proportions, respectively. Less than 1% of imaged bolus particles from grass silage were longer than 38 mm (figure 2.4.e). There were more bolus particles relative to feed particles at lengths within 1 – 8 mm, and more feed relative to bolus particles, at lengths within a range of 9 - 38 mm. Grass silage bolus particle proportions (y) were highly correlated with feed particle proportions (x), within the particle range of 9 – 38 mm ($R^2 = 0.99$), with $y = 0.9055x - 0.0014$. Particle lengths proportions of corn silage and TMR particles at individual lengths both were apparently not different from proportions of the respective bolus particles (figures 2.4.f and g). The longest swallowed bolus particles with proportions ≥ 0.01, of corn silage and TMR were approximately 32 and 45 mm.

Figure 2.5 shows the cumulative distributions of bolus particles retained on a 1.6 mm screen. The fatter lines are the silages and the TMR sample, and the thinner lines the rye grass hay samples. Apparently, all bolus particles, with exception of the chopped hay retained on the lower PSPS screen with the smallest openings of 1.18 mm, had very similar distributions of particle lengths. The bolus from chopped rye grass hay retained on the lower PSPS screen had the shortest particles, bolus from corn silage and the other rye grass hay treatments were intermediate in lengths and bolus from grass silage and TMR contained the longest particles. Rye grass hay was apparently chewed up to bolus with a constant distribution of particle lengths, independently from distributions of feed particle lengths, as long as hay particles were long enough to be retained on the middle screen of the PSPS with 8 mm openings. There might be a threshold of maximum length of particles which can be swallowed by the cow. Treatments with the major part of their particles being longer than that threshold length might be chewed as long as necessary to obtain particles which can be swallowed. Particle size of these feeds might determine the duration of the eating time, but bolus particles might have very similar distributions of particle lengths independently from initial lengths and rumination time might be constant.

Figure 2.6 shows that we are actually overestimating the mass of particles ≥ 5 mm with our current method. Up to 65% of particles, referred to the total number of particles, retained on the 1.6 mm screen were < 5 mm. We observed higher proportions of particles < 5 mm the less particles were retained on that sieve. The error in mass estimation was highest in fecal and rumen mat samples and lowest in the treatment feeds with the longer particles. As an alternative to the current method, we could have considered all particles retained on the 1.6 mm screen for a mean length calculation. However, we have observed that rumen mat and fecal samples contain 64 to 74% and 80 to 92% of dry matter, respectively, which passes the 1.6 mm screen. Most of the fecal particles, but also a large amount of rumen mat particles are < 5 mm. These smaller particles in the rumen might have not yet passed to feces, because they are not dense or heavy enough to pass to the reticulum, or because they are trapped by the longer particles. They are probably less contributing to rumen mat formation and should not be considered for the mean length calculation. We also could have substituted the 1.6 mm screen by another sieve with openings > 1.6 mm but then we would have compromised our decision to analyse lengths of all particles ≥ 5 mm. Especially in the analysis of treatment feeds with longer particles there was risk to overestimate mean length of particles ≥ 5 mm using a sieve with bigger openings. A precise mass estimation of particles ≥ 5 mm, might require a variation of screens with differing openings depending on dimension of particles to analyse. Screens with larger openings might be needed to separate exactly particles ≥ 5 mm in fecal or rumen mat samples compared to feed samples with long particles, but it would be probably rather difficult to define individual sieve sizes to use for individual samples.

We could approximately estimate an error in terms of numerical proportion of particles < 5 mm, retained on the 1.6 mm screen, from the dry matter proportion of particles retained. The extent of overestimation of the mass of particles ≥ 5 mm is probably less severe relative to the numerical proportion of particles < 5 mm measured on the screen. The shorter particles supposedly have also smaller weights compared to the longer ones. If particle size reduction during mastication was measured some of the error might be reduced by the fact that in both measurements, feed and bolus, overestimation occurred. For future analyses, some of the error might be reduced by a more severe sieving technique.

Feed and bolus PROP_1.6 and ML and chews / g DM are reported in table 2.3. We did not measure PROP_1.6 of long rye grass hay, of rye grass cut at 50 mm length and dried, of chopped rye grass hay retained on the upper PSPS screen and chopped rye grass hay retained on the middle PSPS screen. We are assuming that ≥ 80% DM of these treatments would have been retained on the

1.6 mm screen, if measured, because 80% of DM of the hay treatment with finer particles which were from the lower PSPS screen, was retained on the 1.6 mm screen. Bolus PROP_1.6 indicate that the grass hay cut at 50 mm lengths and dried was probably more intensely chewed relative to long rye grass hay and chopped rye grass hays retained on the upper and middle PSPS screens. This treatment required the highest number of chews / DM. The hay treatment with the smallest particles retained on the PSPS bottom pan and the TMR sample had similar feed PROP_1.6, but the hay apparently has been chewed more intensely relative to TMR. There was little reduction in PROP_1.6 and ML of silages and TMR during ingestive mastication. Rye grass hay was apparently chewed up to a constant bolus ML of 10 – 11 mm, independently from distributions of feed particle lengths, as long as hay particles were long enough to be retained on the middle screen of the PSPS with 8 mm openings, and having a minimum ML of 25 mm. Only the bolus particles of hay retained on the 1.18 mm PSPS screen with shorter ML of 10 mm, had a shorter bolus ML of 8 mm. The findings of our experiment are similar to observations made by Shaver et. al. (1988). In the latter study, mean particle lengths of masticate particles and particle distribution on sieves were not different for chopped and long alfalfa hay, however pelleted hay had smaller mean length and more particles collected on small sieves. In literature, several authors found bolus particle size not affected by feed particle size. Pan et al. (2003) measured particle size distribution of boli of orchard grass stems cut at 10 and 2 cm and ground to pass through a 1 cm sieve. Particle size distribution of boli captured via an esophageal fistula was very similar among the treatments. Bailey et al. (1990) found mean particle length of boli unchanged when long or chopped timothy hay was fed. Schwab et al. (2002) and Fernandez et al. (2004) analysed masticate particles from whole plant corn silage of different lengths. In both studies, no effect of forage particle size on bolus mean particle length was observed. Others authors found reduced bolus particle size when feed particle size was reduced. Beauchemin et al. (2003) measured a trend ($p = 0.12$) for reduced mean bolus particle length when diets contained ground versus chopped alfalfa hay. Kononoff and Heinrichs (2003) observed a trend ($p = 0.06$) for shorter digesta mean particle length when diets with shorter alfalfa haylage particles were fed. Results form the current study suggest, that the ML of 10 – 11 mm might represent the maximum ML of rye grass particles the cows were able to swallow. The threshold ML of rye grass hay particles to effect bolus particle length might be < 25 mm. These particles were passing a 8 mm screen. Only particle size of grass hays with ML under this threshold might be able to influence parameters such as rumen retention time, intake and rumen degradation of feed, if these parameters were related to bolus particle size. A sieving equipment to better describe particle size reduction of chopped grass hay particles during eating, should probably contain some additional sieves with openings < 8 mm. If rye grass hay particles passing the 1.18 mm were not physical effective, then these additional sieves should have openings > 1.18 mm and < 8 mm. However, the rye grass hay particles passing the 1.18 mm screen might be very small in size and might contribute little to rumen mat formation, but during ingestion, they still were reduced in size and stimulated chewing (table 2.3). According to Mertens (1997), physical effective particles stimulate chewing. However, the stimulation of rumination, rather than chewing, in general, could be a more appropriate definition of physical effectiveness of feed particles.

Chemical parameters might affect chewing intensity during ingestive mastication. Rinne et al. (2002) measured shorter rumen particles when maturity of ensiled grass increased. Nelson 1988 found that forage maturity and chemical composition was related to fragmentation of forage during ingestive mastication. Apparently longer particles can be swallowed from TMR compared to rye grass hay, because the water content and the lower aNDF might allow bending of feed particles. Only approximately 38% or less of TMR DM is particles potentially retained in the rumen. However, these particles are longer than compared to the chewed hay treatments and might need a longer rumination time.

In literature, instead of masticates or digesta particle size determinations, usually eating and rumination times are measured additionally, when effects of feed particle size are evaluated. Eating time / DM might give an idea of how much feed particle size is reduced during ingestive mastication. The more the feed lengths exceed the maximum length of a particle which can be swallowed by the cow the longer eating time should be. If all feed particles had lengths shorter than the maximum length of particles which can be swallowed, then eating time should not vary with particle size. Eating time depends probably on both feed particle size (Teimouri Yansari and Primohammadi 2009, Teimouri Yansari et al., 2004, Leonardi et al., 2005) and chemical parameters (Krause and Combs 2003, De Boever et al., 1993a). In the current experiment, particle lengths distributions of rye grass hay treatments were different, but similar in most of the respective boli. In consequence chews /g DM should represent feed particle size reduction during mastication. In our study, there was some trend for more chewing when feeds with longer particles were fed. Long hay and chopped rye grass hay retained on the upper PSPS screen were chewed more intensely compared to chopped hay retained on the middle and lower PSPS screen. However, we expected the long hay to be chewed much longer compared to the chopped hays. Chews / g DM of long hay and chopped hay retained on a 19 mm screen were not significantly different. In our study other criteria rather than particle size might have more influenced chews / g DM. Chopped rye grass hay retained on the lower PSPS screen and grass silage had similar PROP_1.6. The hay had a shorter particle ML but was chewed more intensely compared to the silage. Rye grass hay cut at 50 mm length and chopped rye grass hay retained on the upper PSPS screen had similar particle ML but the latter hay was chewed less intensely. The rye grass sample cut at 50 mm length and dried were similar in aNDF content compared to the other rye grass hay treatments, but particles were very rigid compared to samples from the baled hay and needed more intense chewing. The hay treatment with the shortest particles, retained on the PSPS bottom pan, was chewed more intensely relative to chopped hay retained on the middle and lower PSPS screen. These treatments were similar in CP and aNDF contents, but the hay with the smallest particles was very powdery and feed might have needed higher insalivation in order to be swallowed. In our study, TMR and corn silage were not reduced in particle size during ingestive mastication, even though some chewing activity has been observed. We might have observed some jaw movements in our study which were actually not chewing. The results relative to chewing activity in the current study differed somewhat from our expectations. One reason for this deviance might have been the fact, that we observed chewing activity only over a very short time period, which was the time necessary to collect the bolus samples.

Eating and rumination time describe a very complex process of events. The figures 2.7 show in three hypothetical examples how feed particle size might effect ingestive chewing, rumination, rumen fill and intake. I assumed that the frequency of chews needed before a certain feed can be swallowed depends on the longest particles in the mouth. I also made some further, less realistic, assumptions to illustrate easily some possibilities how feeds could be chewed for ingestion. I assumed particle lengths to be proportional to volume, and that particles are broken into half during one chew. In figures 2.7.a and b the two feed examples have different mean size. In figure 2.7.a this difference might be less pronounced compared to 2.7.b. Different proportions of feeds of different lengths might be mixed together in figure 2.7.a. Eating time might be the same, because the number of necessary chews might depend on the maximum length of particles which were picked up. In consequence, ruminating time and rumen fill would be reduced and intake increased in the feed that had the smaller particle mean size. In figure 2.7.b the difference in lengths between the two feeds is greater compared to the latter example. Eating time might be shorter in the feed with shorter compared to the feed with longer particles, but rumination time, rumen fill and intake might not necessarily be influenced. This example could fit with the hay treatments tested in our experiment. Hay treatments with ML varying from 25 up to approximately 600 mm had all the same bolus ML. Figure 2.7.c illustrates how particle distribution rather than mean length could have effect on eating and rumination time. Both feeds have the same particle mean length but one feed has a very narrow distribution and the other feed a wide distribution of particle lengths. The feed with the narrow distribution might be chewed less during ingestion, because maximum particle length is shorter compared to the other feed, but it might require a longer rumination time.

Rustas et al. (2010) fed long or chopped whole crop barley silage to dairy steers. Eating time / DM intake was reduced when chopped silage was fed relative to long but rumination time / DM intake was not affected. De Boever et al. (1993b) measured eating and rumination time / forage DM intake of corn silages chopped at 4, 8 and 16 mm lengths. Eating time / forage DM intake was lower when silage chopped at 8 mm compared to 16 mm was fed, but rumination time / forage DM was not changed. When silages chopped at 4 and 8 mm lengths were fed, eating time was not altered, but rumination time was higher when the silage with longer particles was fed.

Allen (1997) reported a critical particle size for total chewing time. Size of feed particles which were retained on sieves with an aperture size of 3 mm had smaller impact on total chewing time compared to feed particles passing that screen. The results of the present study suggest that, feed particle size could affect eating time, in the rye grass hay treatments with particles retained on a 8 mm screen, but rumination time is more likely to be independent. In contrast, the hay particles passing that screen might be able to alter rumination time but not eating time. Rumination time has supposedly a higher impact on total chewing compared to eating time. In consequence, hay particles passing the 8 mm PSPS screen might be able to influence total chewing more compared to the particles retained on that screen. The difference of the critical sieve aperture size, 8 mm compared to 3 mm, could be explained, at least in part, by differences in sieving techniques used.

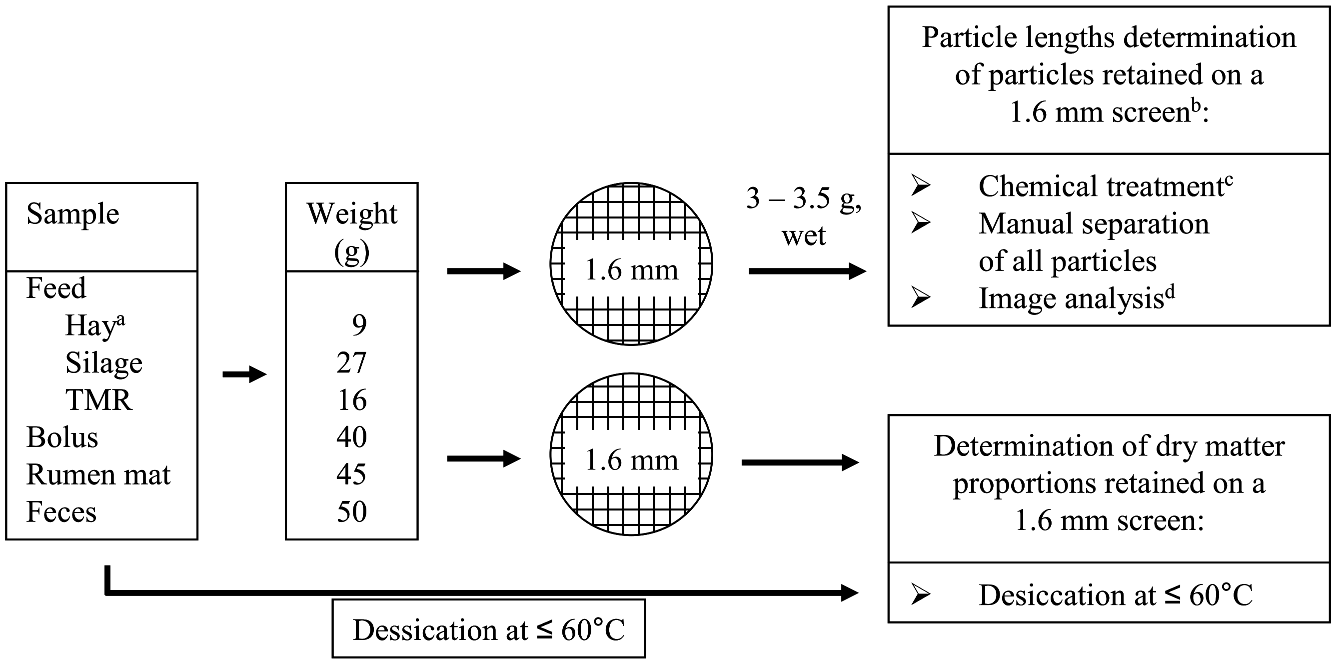
CONCLUSIONS

Rye grass hay treatments with ML varying from 25 up to approximately 600 mm had all the same bolus ML of 10 – 11 mm, independently from feed ML. The ML of 10 – 11 mm might represent the maximum ML of rye grass hay particles the cows were able to swallow. Feed and bolus particle size are not necessarily correlated. Only for feeds with shorter particles, the chopped rye grass hay retained on the lower PSPS screen with openings of 1.18 mm, the grass, the corn silage and the TMR, lengths distribution of feed particles were highly correlated to respective bolus particles within a certain range of particle lengths. The threshold ML of rye grass hay particles to effect bolus particle length was within a range of 10 - 25 mm, which were particles either retained on the 8 mm screen or particles which had passed this screen and were retained on a 1.18 mm screen. Only particle size of rye grass hay with ML under this threshold might be able to influence parameters such as rumen retention time, intake and rumen degradation of feed, if these parameters were related to bolus particle size. A sieving equipment to better describe particle size reduction of chopped grass hay particle during eating, should probably contain some additional sieve with openings smaller than 8 mm. If rye grass hay particles passing the 1.18 mm were not physical effective, then these additional sieves should have openings > 1.18 mm and < 8 mm. However, the rye grass hay particles passing the 1.18 mm screen might be very small in size and might contribute little to rumen mat formation, but during ingestion, they still were reduced in size and stimulating chewing. The stimulation of rumination, rather than chewing, in general, could be a more appropriate definition of physical effectiveness of feed particles.

Chemical parameters might alter chewing intensity during ingestive mastication. Higher water content and a lower aNDF content of TMR particles compared to rye grass hay particles might allow bending of particles and the swallowing of longer particles in consequence.

Figure 2.1. Determination of sample particle size – Horizontal wet sieving of the sample through a sieve with 1.6 mm openings,

- determination of particle lengths distribution of sample retained, with particular attention on particles ≥ 5 mm and
- sample dry matter proportion retained.



[a] Chopped rye grass hay particles were sieved through the Penn State Particle Separator to provide treatment feeds. Only samples with particles passing a 8 mm screen but retained on a 1.18 mm screen were sieved. Image analysis on the other chopped hay treatments was performed directly on 2.5 – 3g of dry sample. Hay samples passing a 1.18 mm screen but retained on the bottom pan were only sieved but not imaged. Long hay samples were neither sieved nor imaged.

[b] Sample mean lengths were calculated considering particles ≥ 5 mm.

[c] Samples were soaked with acetone, tert-butanol and neutral detergent to ease particle separation and dyed with Safranin solution to improve image processing.

[d] described by Licitra et al. (2005).

Figures 2.2. Variability of particle lengths distribution within and between treatments – *Image analysis* of treatments (a – g).

Figure 2.2.a. Rye grass particles cut at 50 mm length and dried to hay.

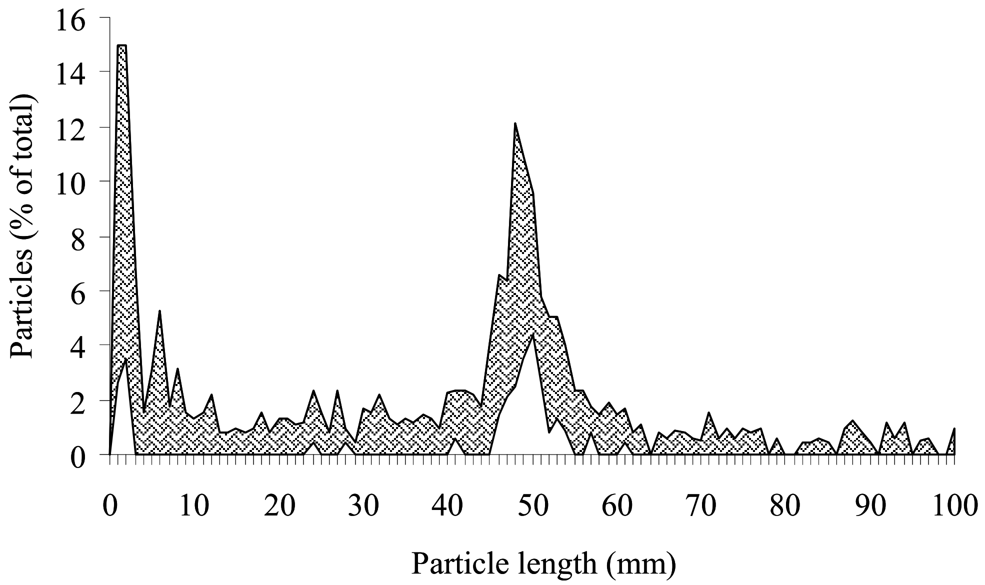


Figure 2.2.b. Rye grass hay particles retained on a 19 mm screen.

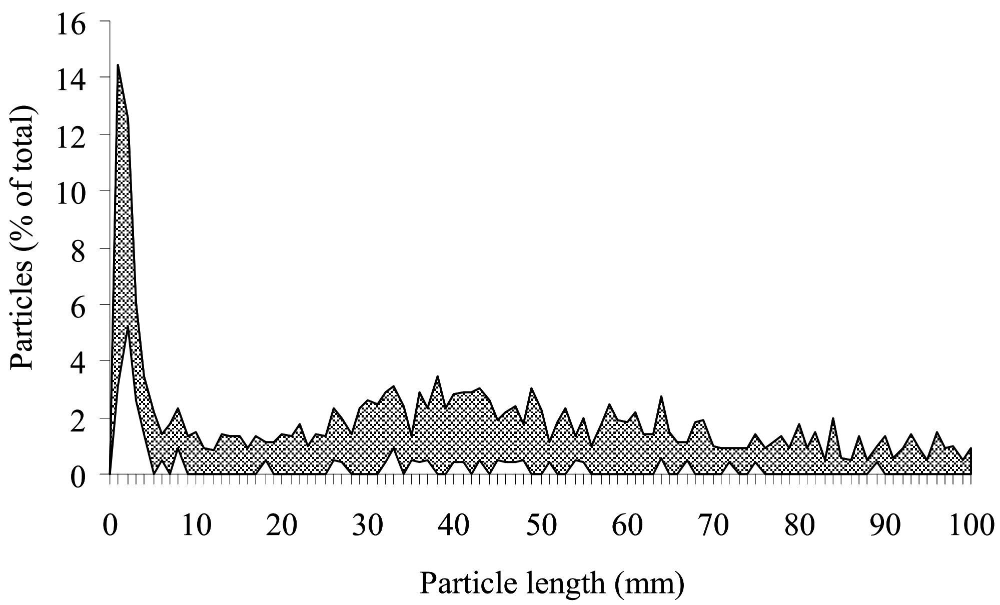


Figure 2.2.c. Rye grass hay particles passing a 19 mm screen but retained on a 8 mm screen.

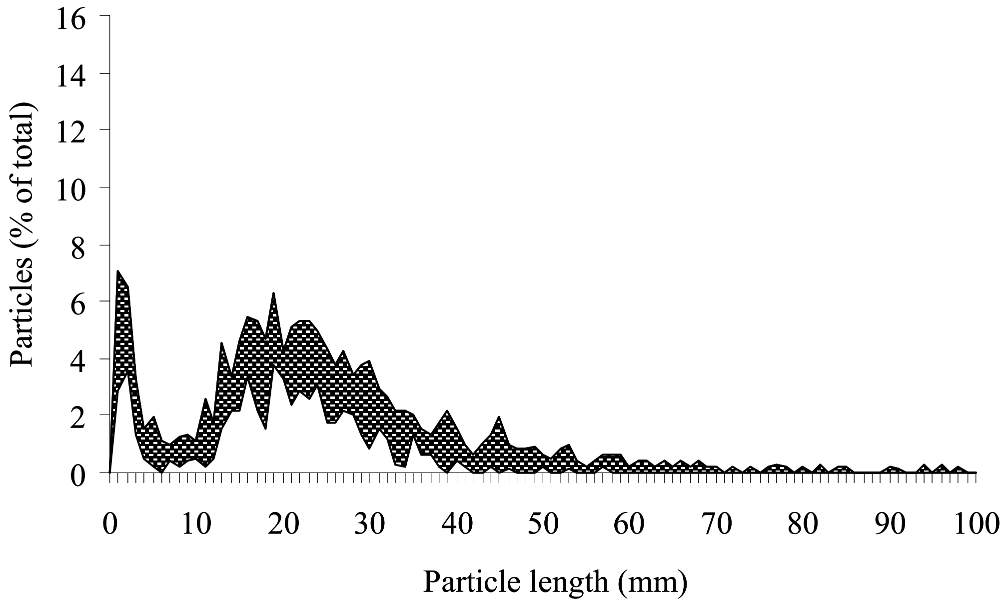


Figure 2.2.d. Rye grass hay particles passing a 8 mm screen but retained on a 1.18 mm screen - *Image analysis* after elimination of small particles by sieving treatment feed through a 1.6 mm screen.

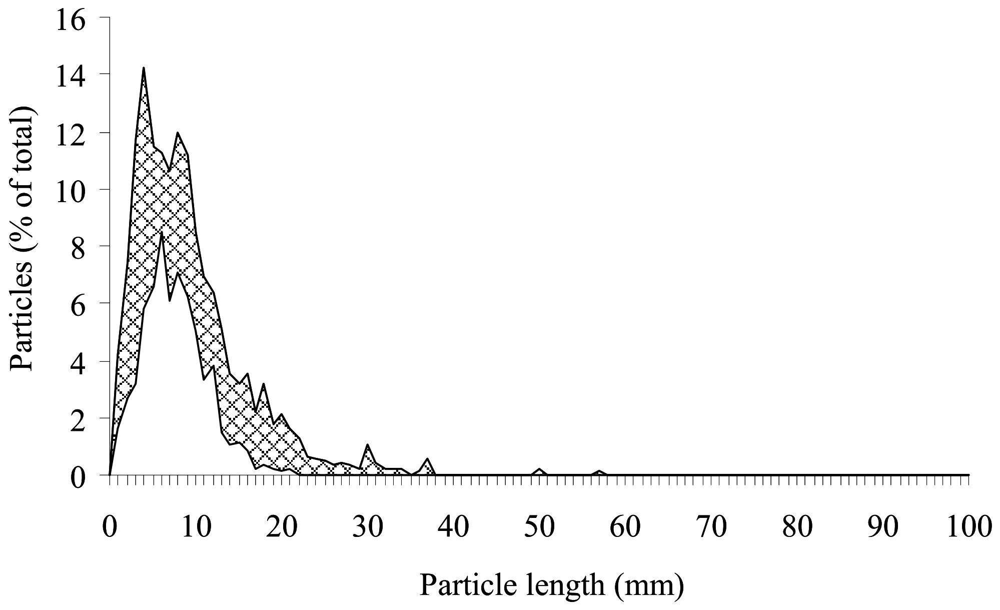


Figure 2.2.e. Grass silage - *Image analysis* after elimination of small particles by sieving treatment feed through a 1.6 mm screen.

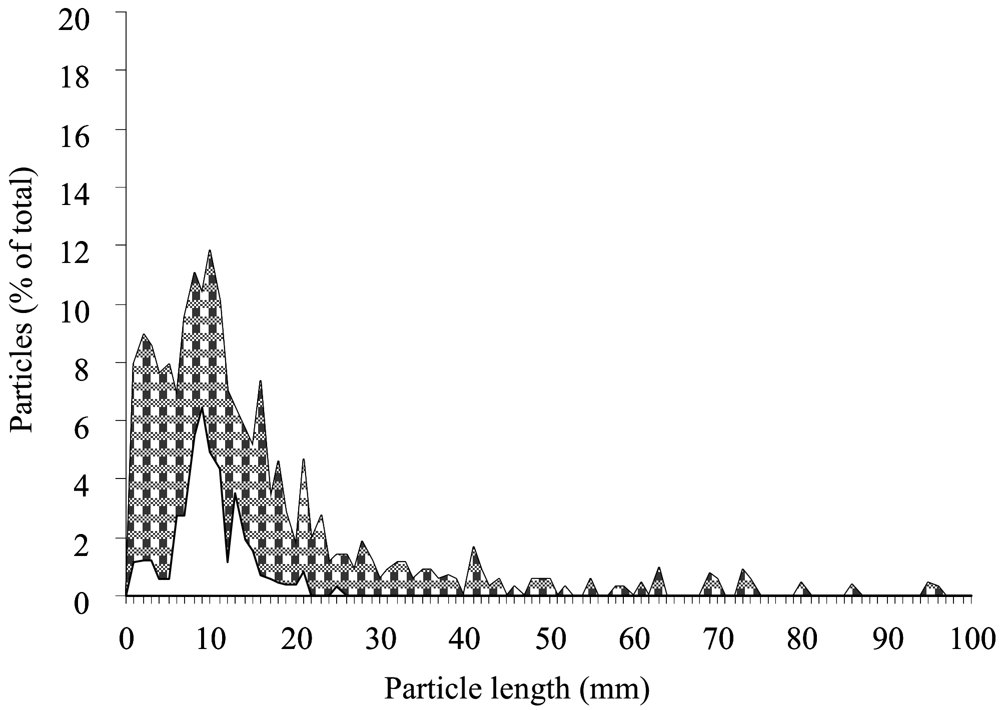


Figure 2.2.f. Corn silage - *Image analysis* after elimination of small particles by sieving treatment feed through a 1.6 mm screen.

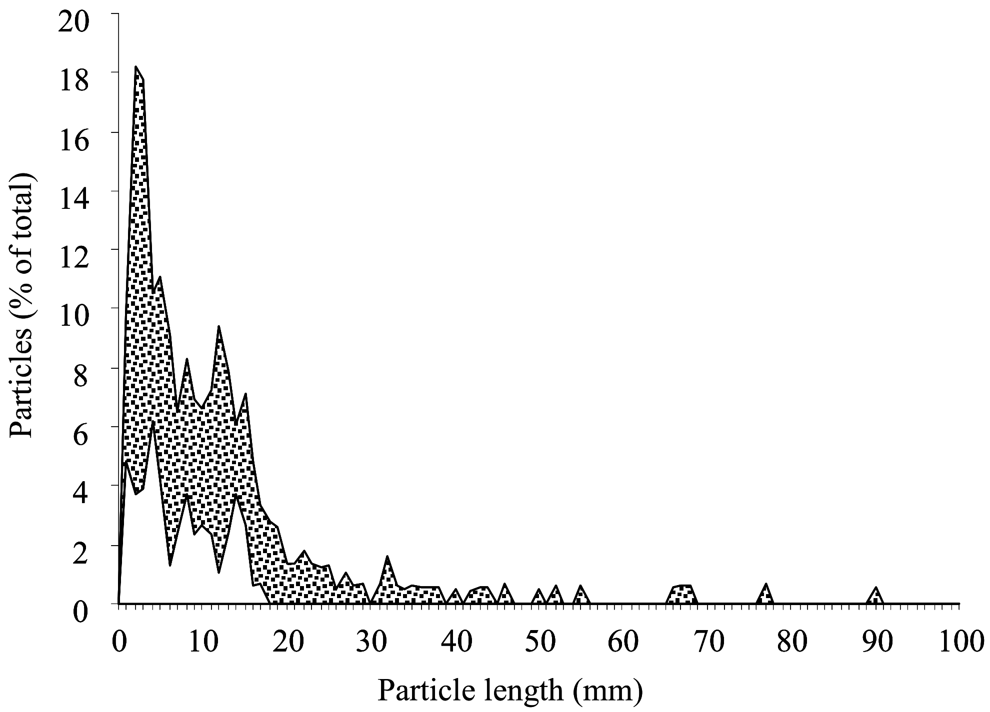
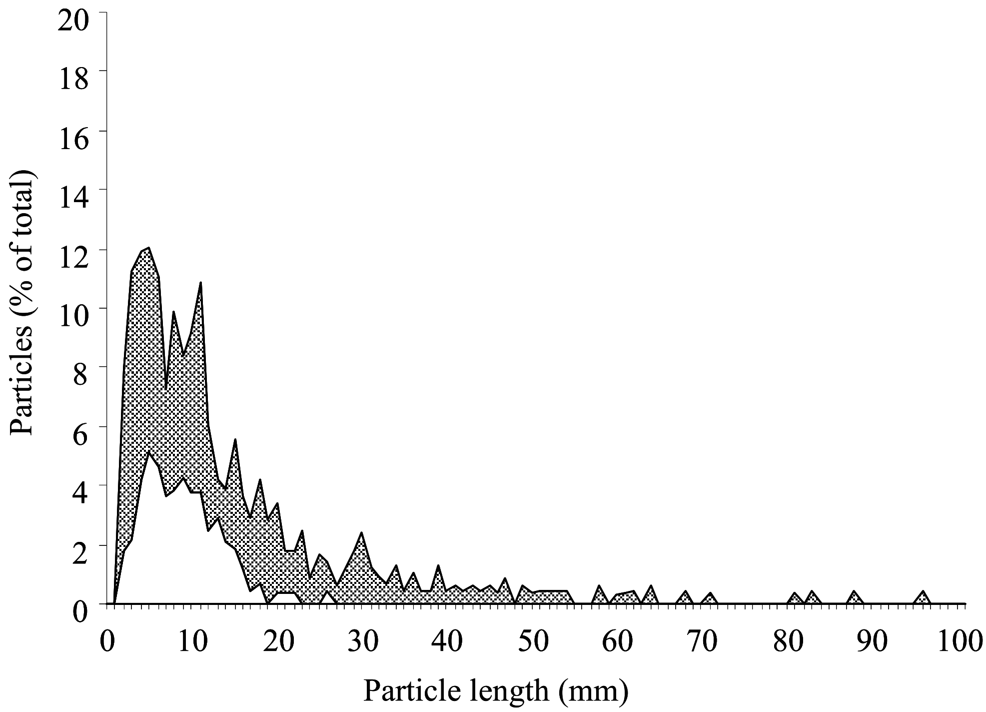


Figure 2.2.g. ▩TMR - *Image analysis* after elimination of small particles by sieving treatment feed through a 1.6 mm screen.



Figures 2.3. Mean* reduction of particle lengths during ingestive mastication (a – g) – *Image analysis* of treatment particles (d – g) and respective boli (a – g) after elimination of small particles by sieving through a 1.6 mm screen.

* Mean distribution within 3 mm intervals and means of eight animals were considered.

Figure 2.3.a. ▩ Difference in particle lengths distribution between rye grass particles cut at 50 mm length and dried and their respective bolus particles.

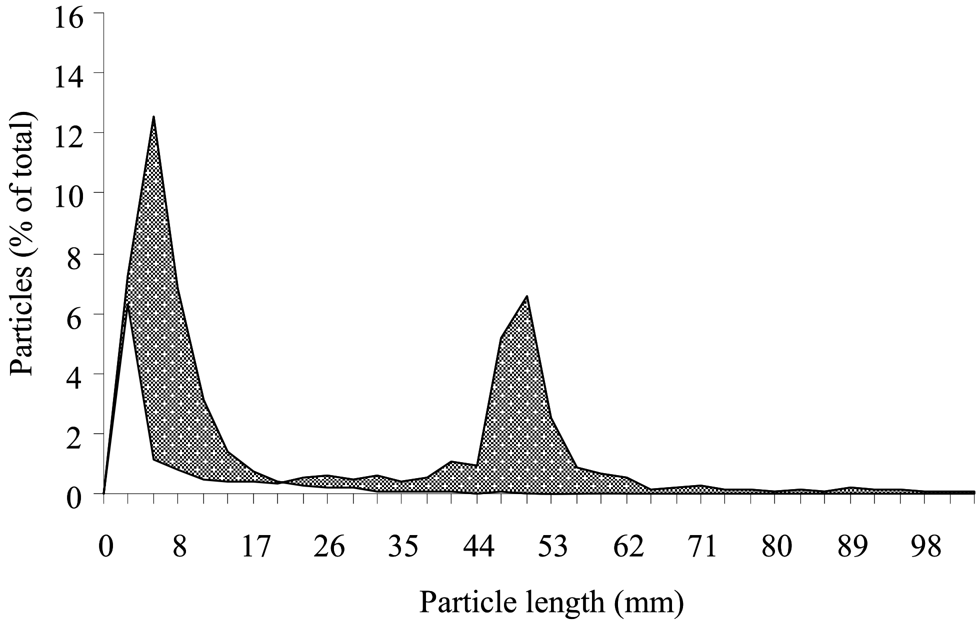


Figure 2.3.b. ▩ Difference in particle lengths distribution between rye grass hay particles retained on a 19 mm screen and their respective bolus particles.

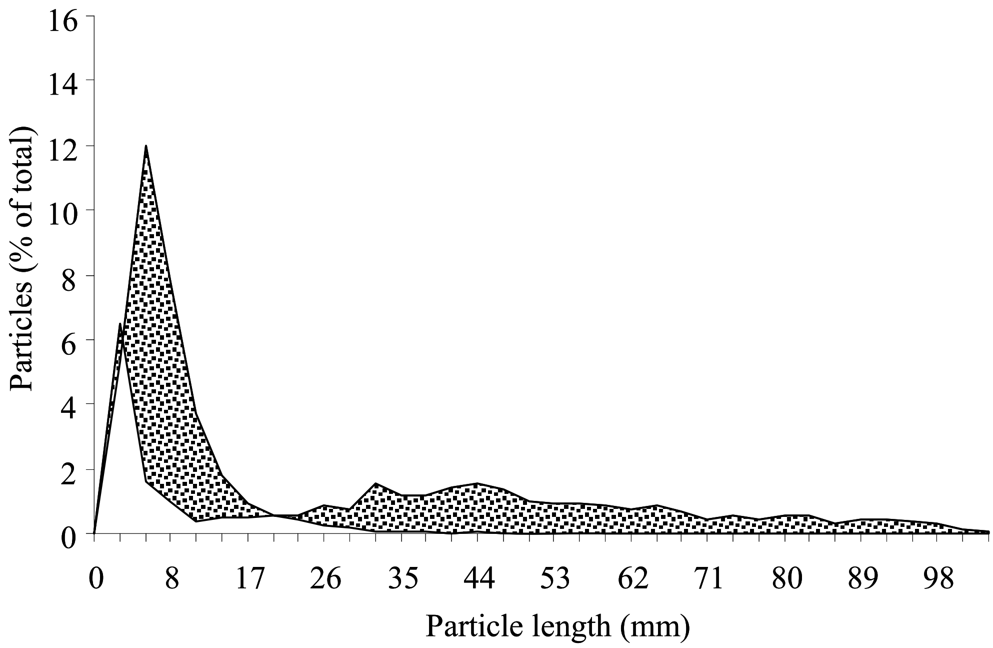


Figure 2.3.c. Difference in particle lengths distribution between rye grass hay particles passing a 19 mm screen but retained on a 8 mm screen and their respective bolus particles.

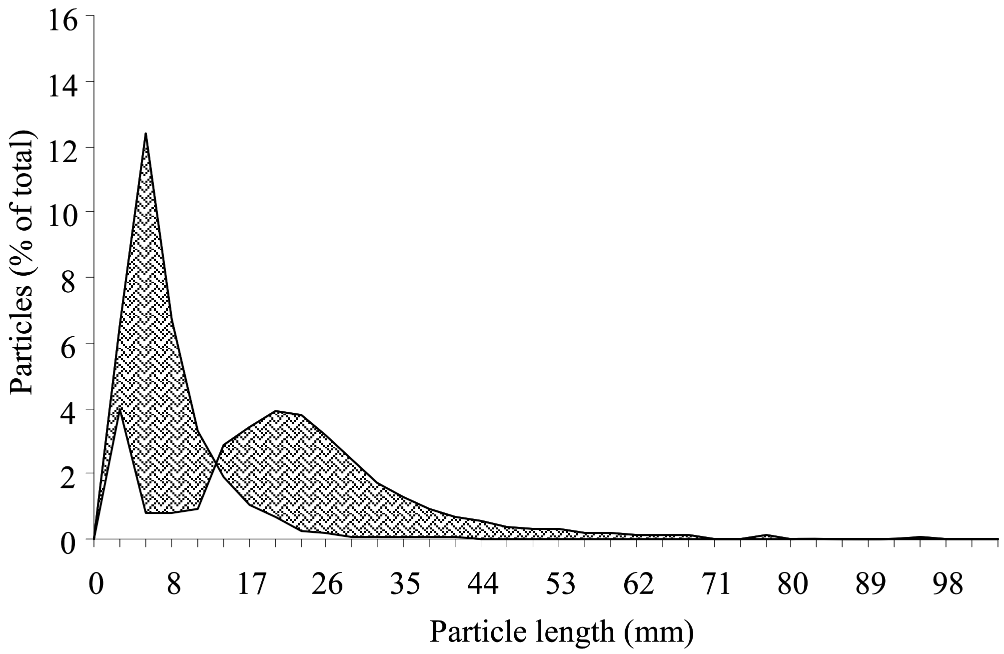


Figure 2.3.d. Difference in particle lengths distribution between rye grass hay particles passing a 8 mm screen but retained on a 1.18 mm screen and their respective bolus particles.

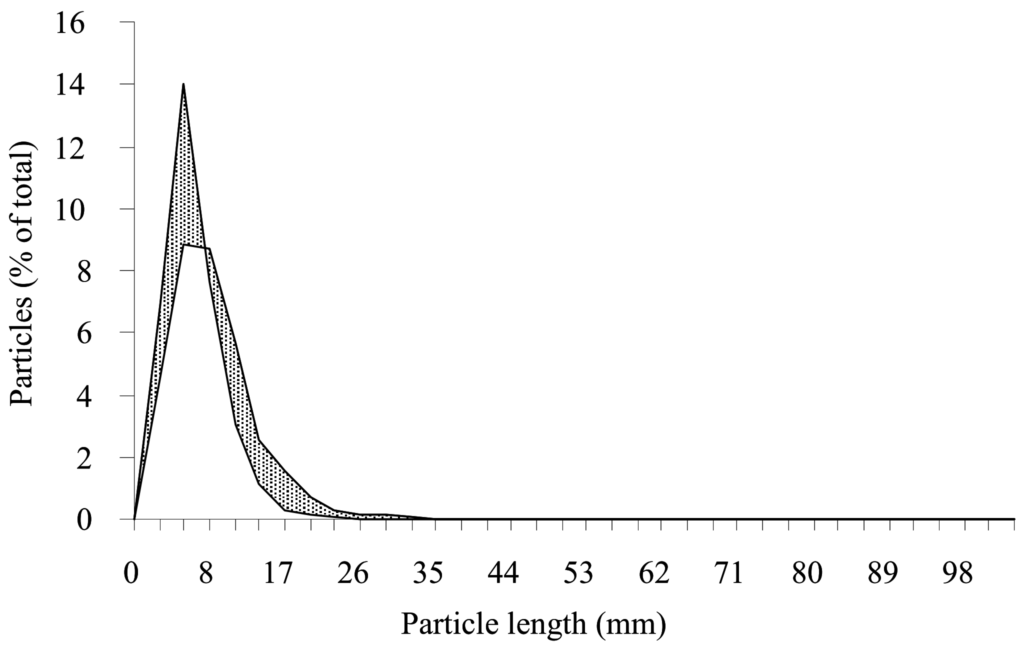


Figure 2.3.e. ▩ Difference in particle lengths distribution between grass silage particles and their respective bolus particles.

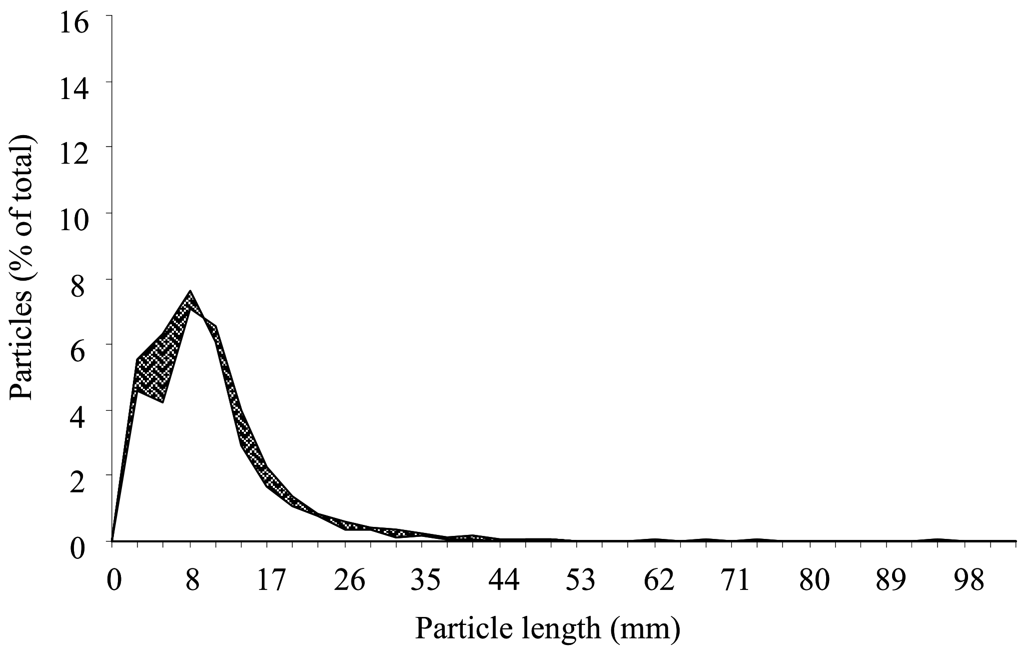


Figure 2.3.f. ▩ Difference in particle lengths distribution between corn silage particles and their respective bolus particles.

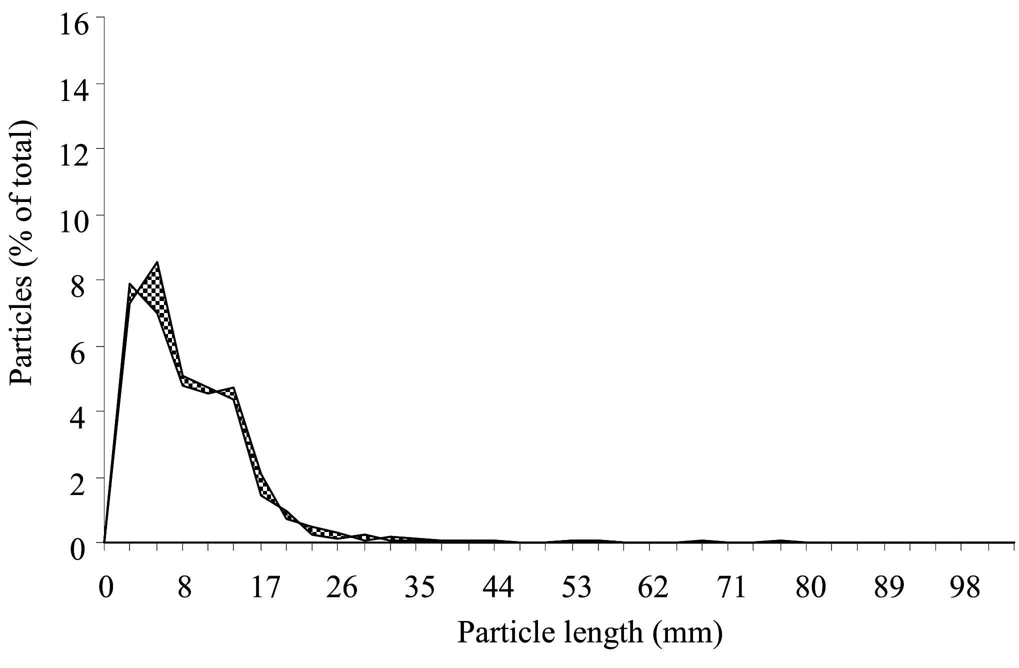
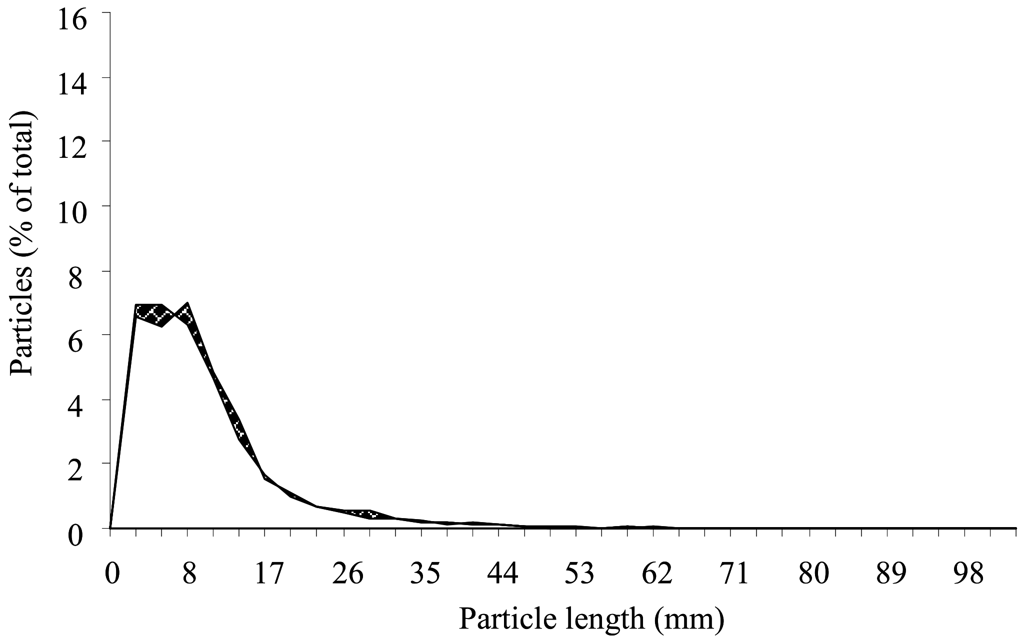


Figure 2.3.g. ▨ Difference in particle lengths distribution between TMR particles and their respective bolus particles.



Figures 2.4. Lengths distribution of rye grass hay particles relative to respective bolus particles (dry and lactating cows averaged, a - g) - *Image analysis* after elimination of small particles by sieving through a 1.6 mm screen.

Figure 2.4.a. Rye grass particles cut at 50 mm length and dried to hay versus respective bolus particles.

○ 1 – 34 mm particles. Less than 1% bolus particles were longer than 34 mm.

▲ 35 – 104 mm particles. Less than 1% hay particles were longer than 104 mm.

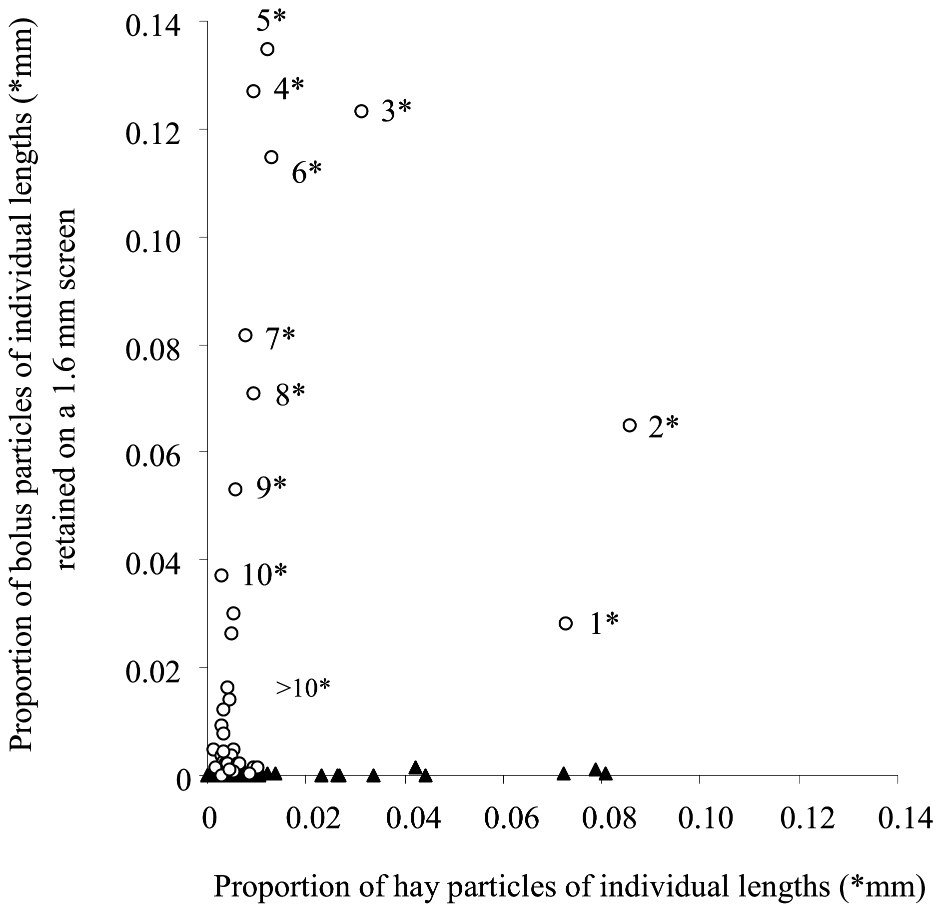


Figure 2.4.b. Rye grass hay particles retained on a 19 mm screen versus respective bolus particles.

○ 1 – 31 mm particles. Less than 1% bolus particles were longer than 31 mm.

▲ 32 – 138 mm particles. Less than 1% hay particles were longer than 138 mm.

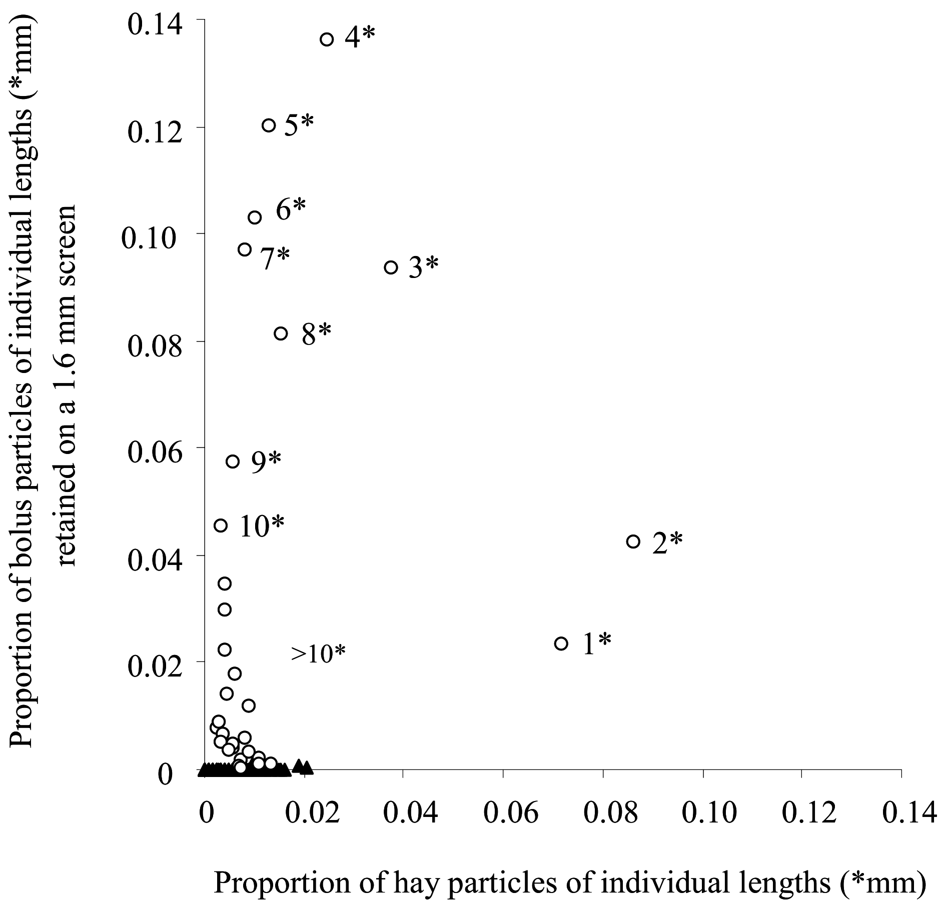


Figure 2.4.c. Rye grass hay particles passing a 19 mm screen but retained on a 8 mm screen versus respective bolus particles.

○ 1 – 27 mm particles. Less than 1% bolus particles were longer than 27 mm.

▲ 28 - 76 mm particles. Less than 1% hay particles were longer than 76 mm.

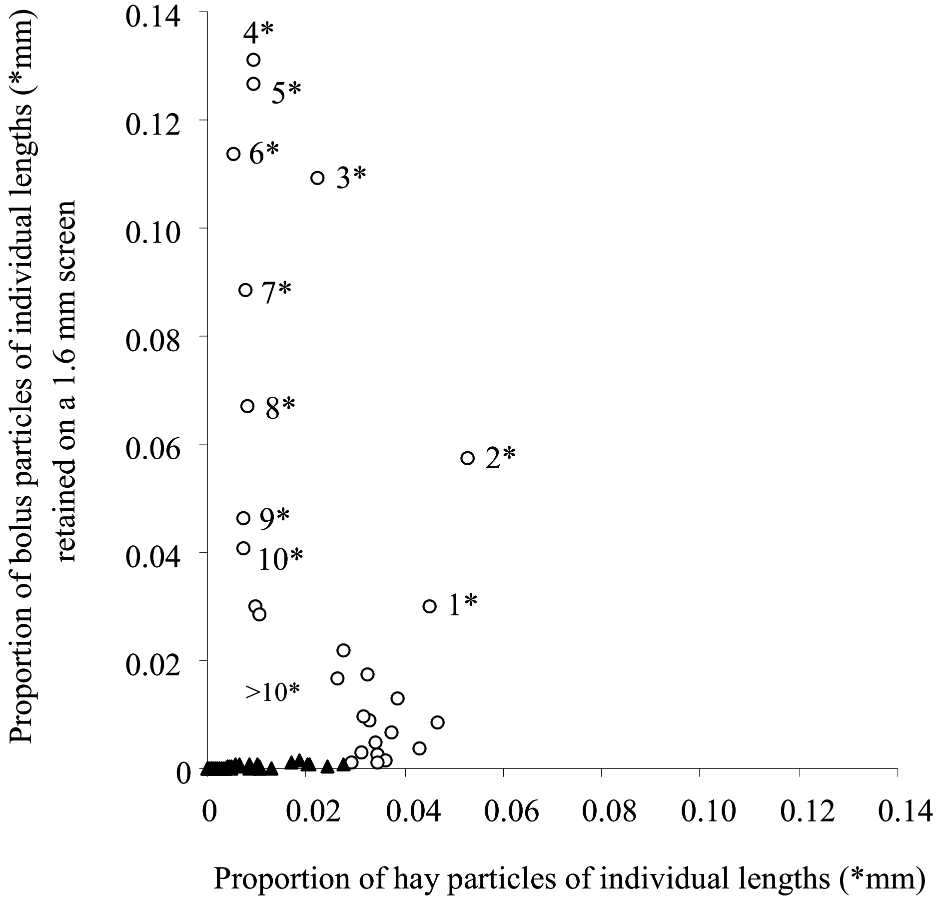


Figure 2.4.d. Rye grass hay particles passing a 8 mm screen but retained on a 1.18 mm screen versus respective bolus particles.

● 1 – 8 mm particles.

○ 9 – 18 mm particles; y = 0.7847x - 0.0064; R^2 = 0.99; n = 10.

Less than 1% bolus particles were longer than 18 mm.

▲ 19 - 23 mm particles. Less than 1% hay particles were longer than 23 mm.

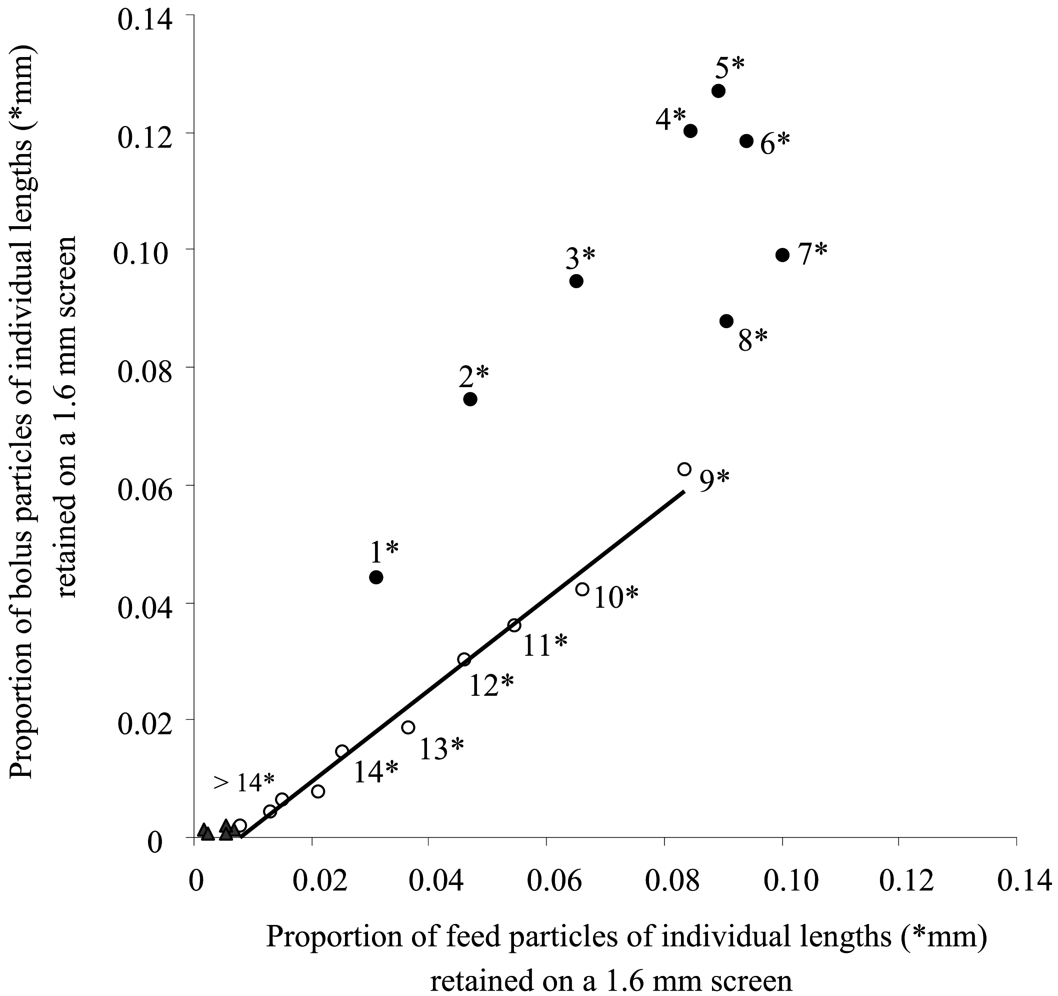


Figure 2.4.e. Grass silage particles versus respective bolus particles.

● 1 – 8 mm particles.

○ 9 – 38 mm particles; y = 0.9055x - 0.0014; R^2 = 0.97; n = 30.

Less than 1% bolus particles were longer than 38 mm.

▲ 39 - 57 mm particles. Less than 1% grass silage particles were longer than 57 mm.

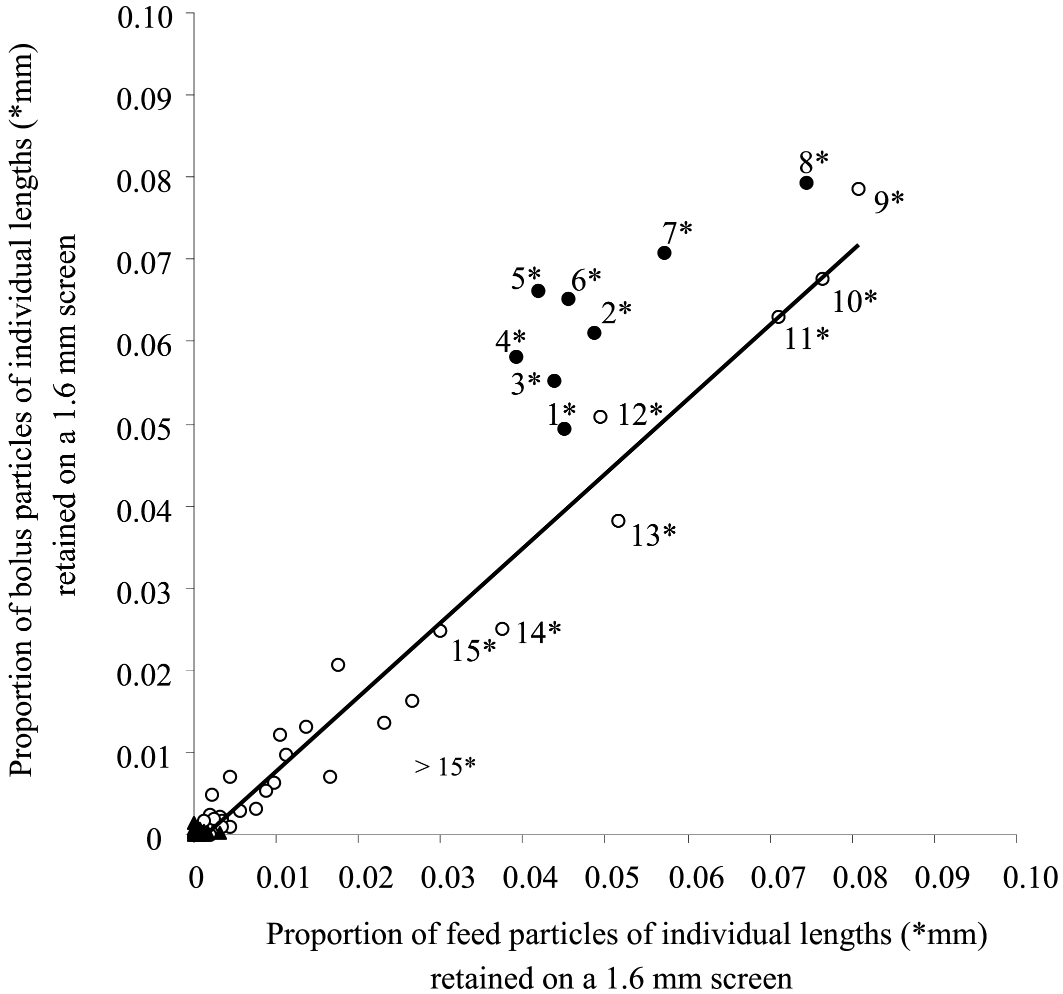


Figure 2.4.f. Corn silage particles versus respective bolus particles..

● 1 mm particle.

○ 2 – 32 mm particles; y = 1.0984x - 0.0019; R^2 = 0.97; n = 31.

Less than 1% bolus particles were longer than 32 mm.

▲ 33 - 45 mm particles. Less than 1% corn silage particles were longer than 45 mm.

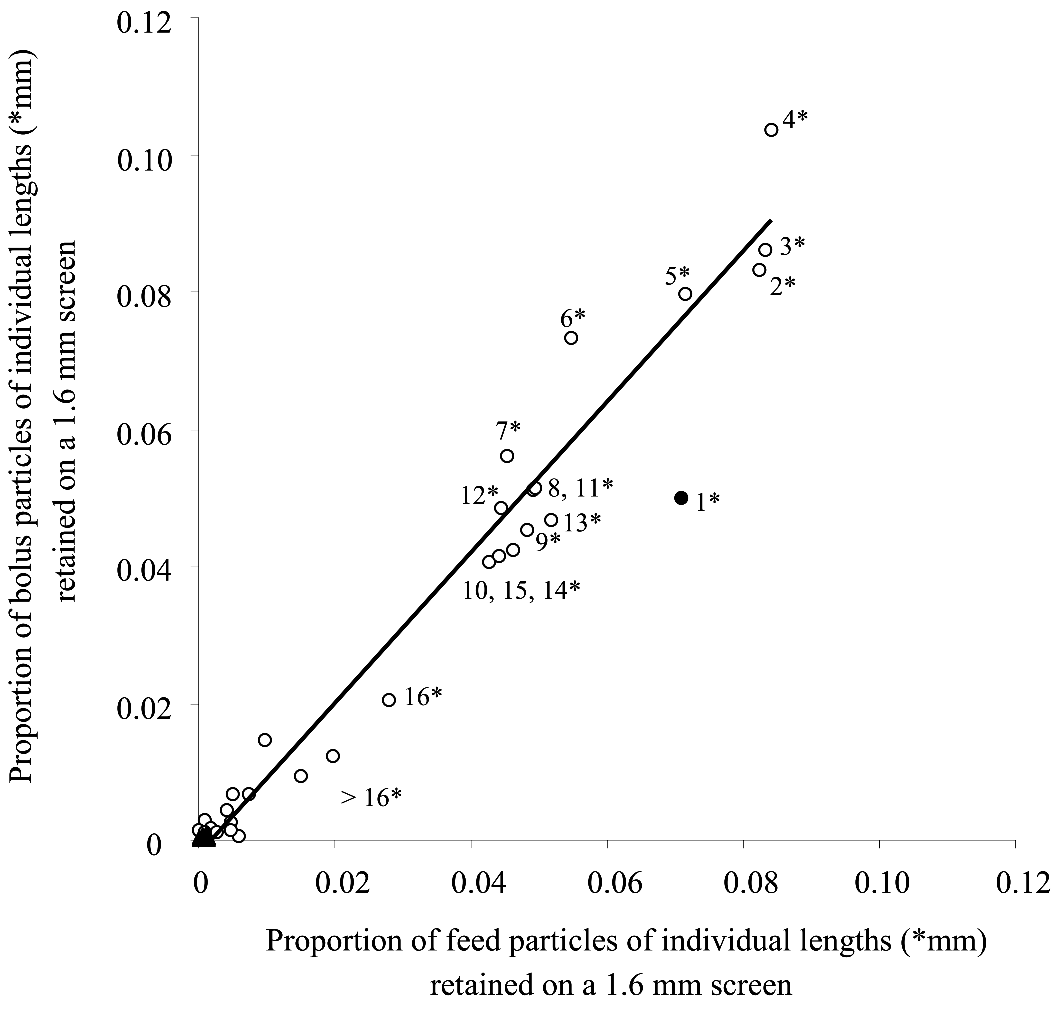


Figure 2.4.g. TMR particles versus respective bolus particles.

● 1 mm particle.

○ 2 – 45 mm particles; y = 0.995x + 0.0004; R^2 = 0.97; n = 44.

Less than 1% bolus particles were longer than 45 mm.

▲ 46 - 49 mm particles. Less than 1% grass silage particles were longer than 49 mm.

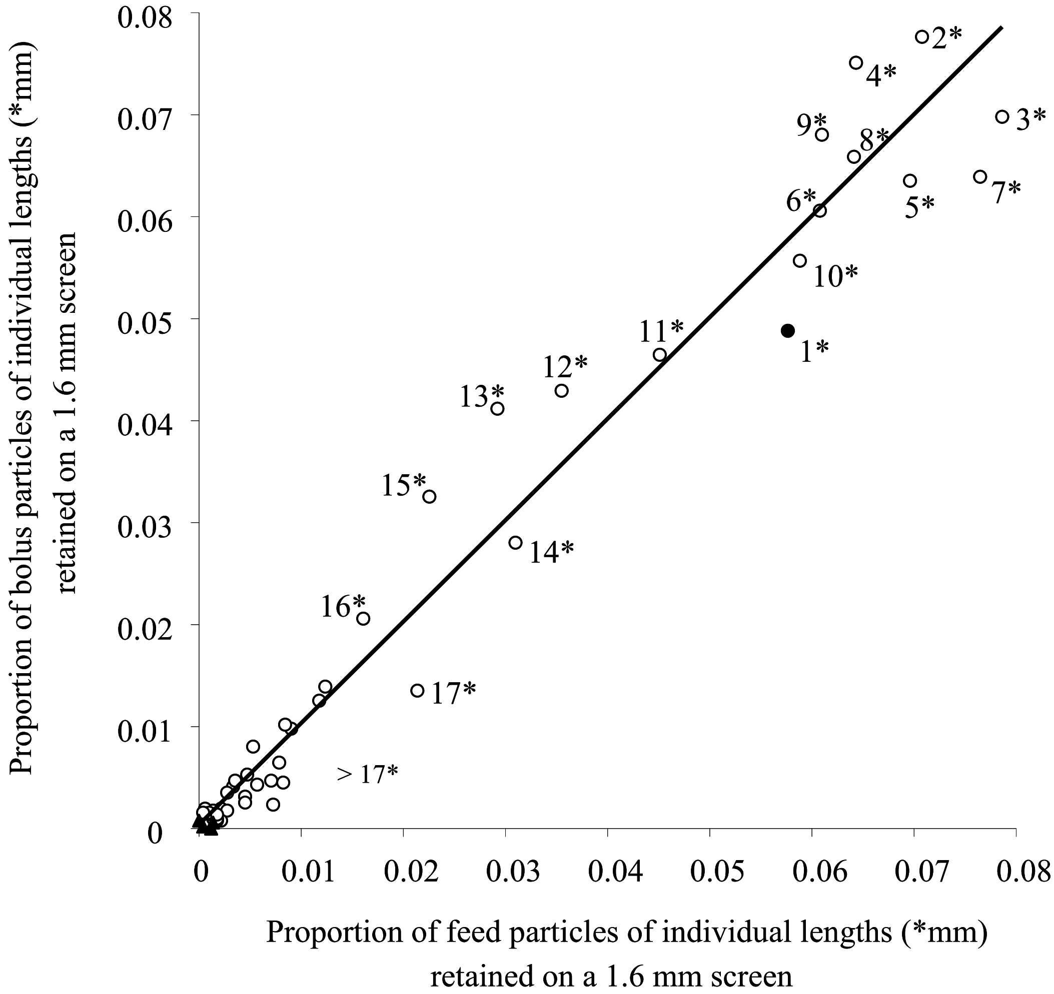


Figure 2.5. 1 - Cumulative lengths distribution of bolus particles retained on a 1.6 mm screen.

- - - Mean TMR bolus particles;

Mean grass silage bolus particles;

Mean corn silage bolus particles distribution;

—◇— Mean bolus particle of rye grass hay passing a 19 mm screen but retained on a 8 mm screen;

—◆— Mean bolus of rye grass hay retained on a 19 mm screen;

—△— Mean bolus of rye grass cut at 50 mm length and dried to hay;

—▲— Mean bolus of long rye grass hay;

—×— Mean bolus of rye grass hay passing a 8 mm screen but retained on a 1.18 mm screen.

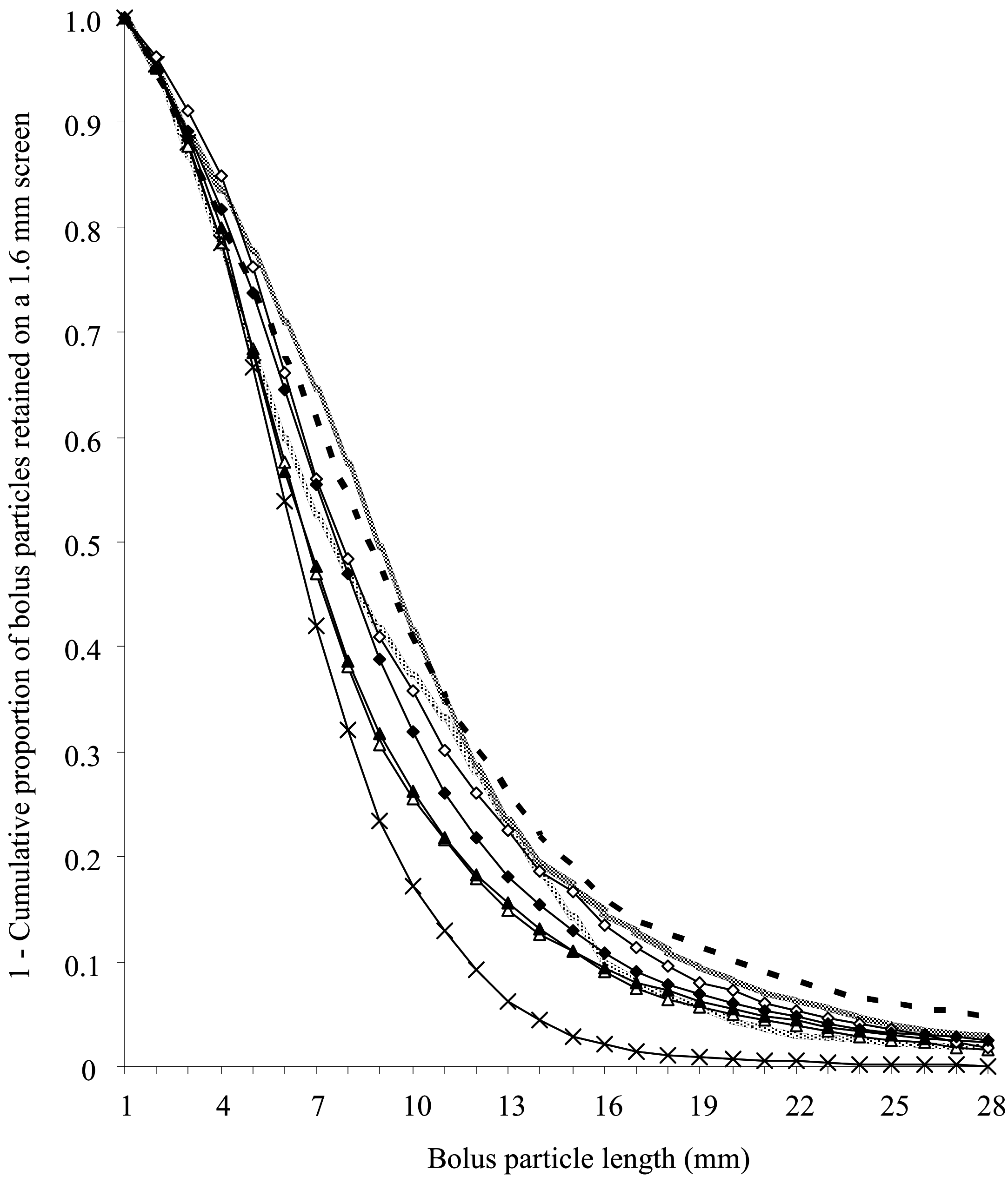
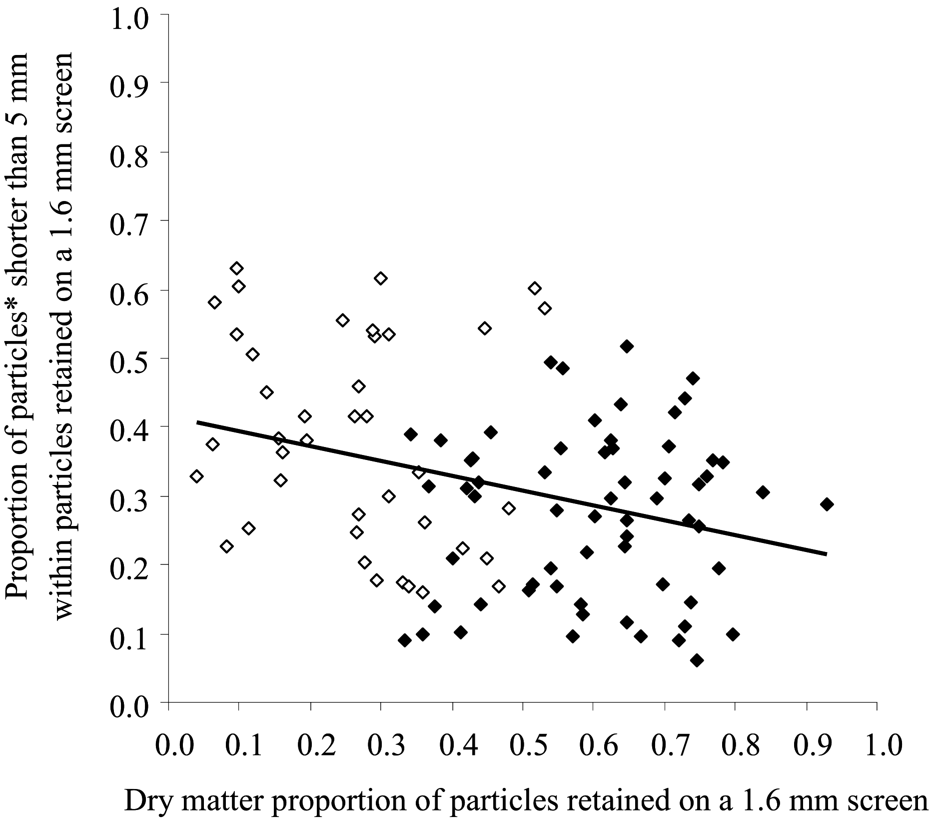


Figure 2.6. How many of the particles retained on a 1.6 mm screen are shorter than 5 mm?

◇ Rumen mat and fecal samples

◆ Bolus and hay samples

y = -0.2136x + 0.4152; R^2 = 0.10; n = 103.



* referred to total numbers of particles

Figures 2.7. Three theoretical examples (a – c) of how feed particle lengths and distributions might influence ingestive chewing, rumination, rumen fill and intake. I assumed that the frequency of chews needed before a certain feed can be swallowed depends on the longest particles in the mouth. I made further the following assumptions in order to simplify the cases and highlight the principles:

- Particle lengths are proportional to volume.
- Particles are broken into half during one chew.

Figure 2.7.a. Example 1. Different proportions of feeds of different lengths are mixed together. Feeds differ in proportion of particles of individual lengths.

- Mean particle length: A > B.
- Ingestive chewing: A = B.
- Rumination: A > B, rumen fill: A > B, intake: A < B.

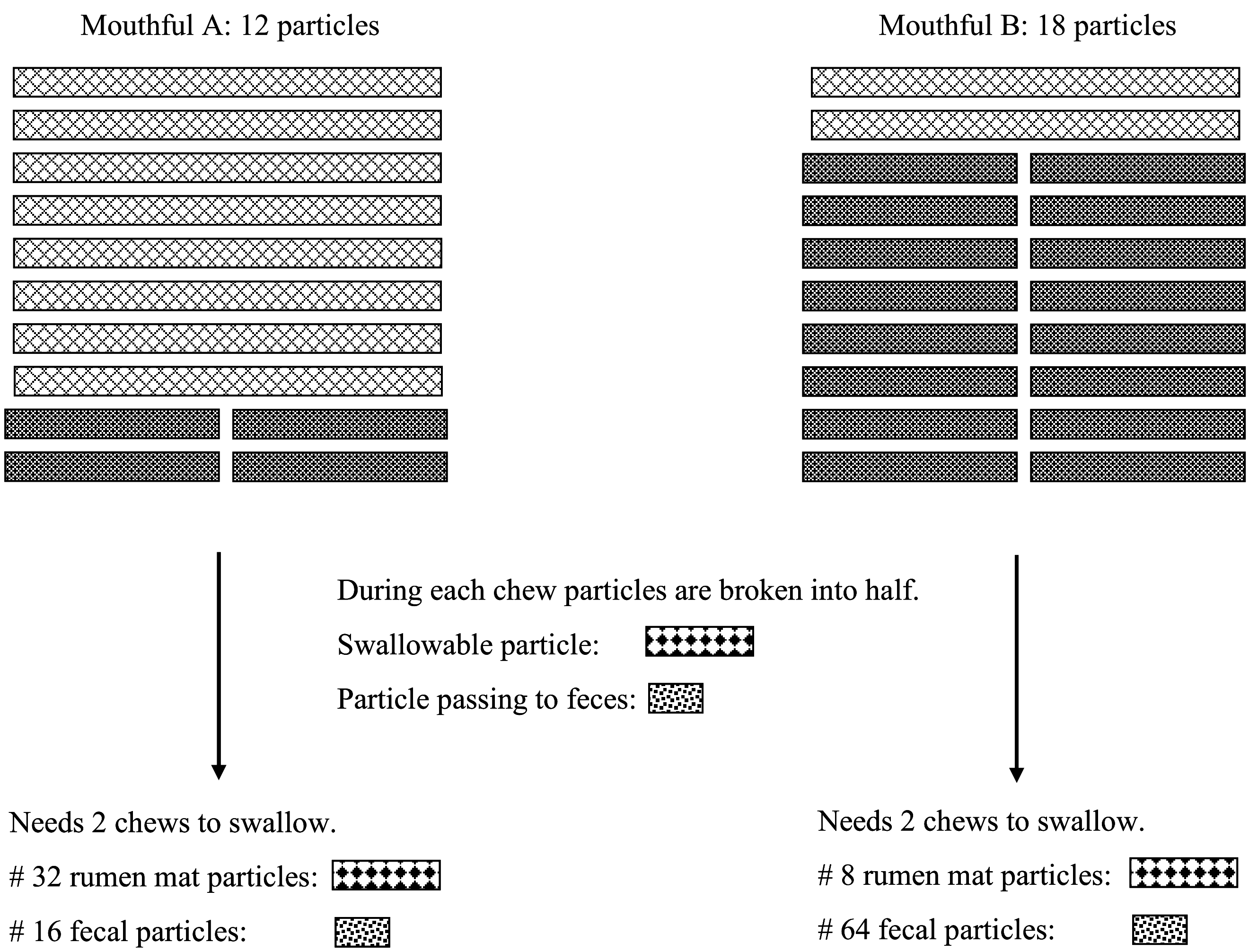


Figure 2.7.b. One feed has longer particles compared to the other. This is the case in our experiment, where treatment feeds were particle residues on screens after a sequential sieving procedure. In reality, this case could occur when one feed is mixed longer in the mixer wagon compared to the other.

- Mean particle length: A > B.
- Ingestive chewing: A > B.
- Rumination: A = B, rumen fill: A = B, intake: A = B.

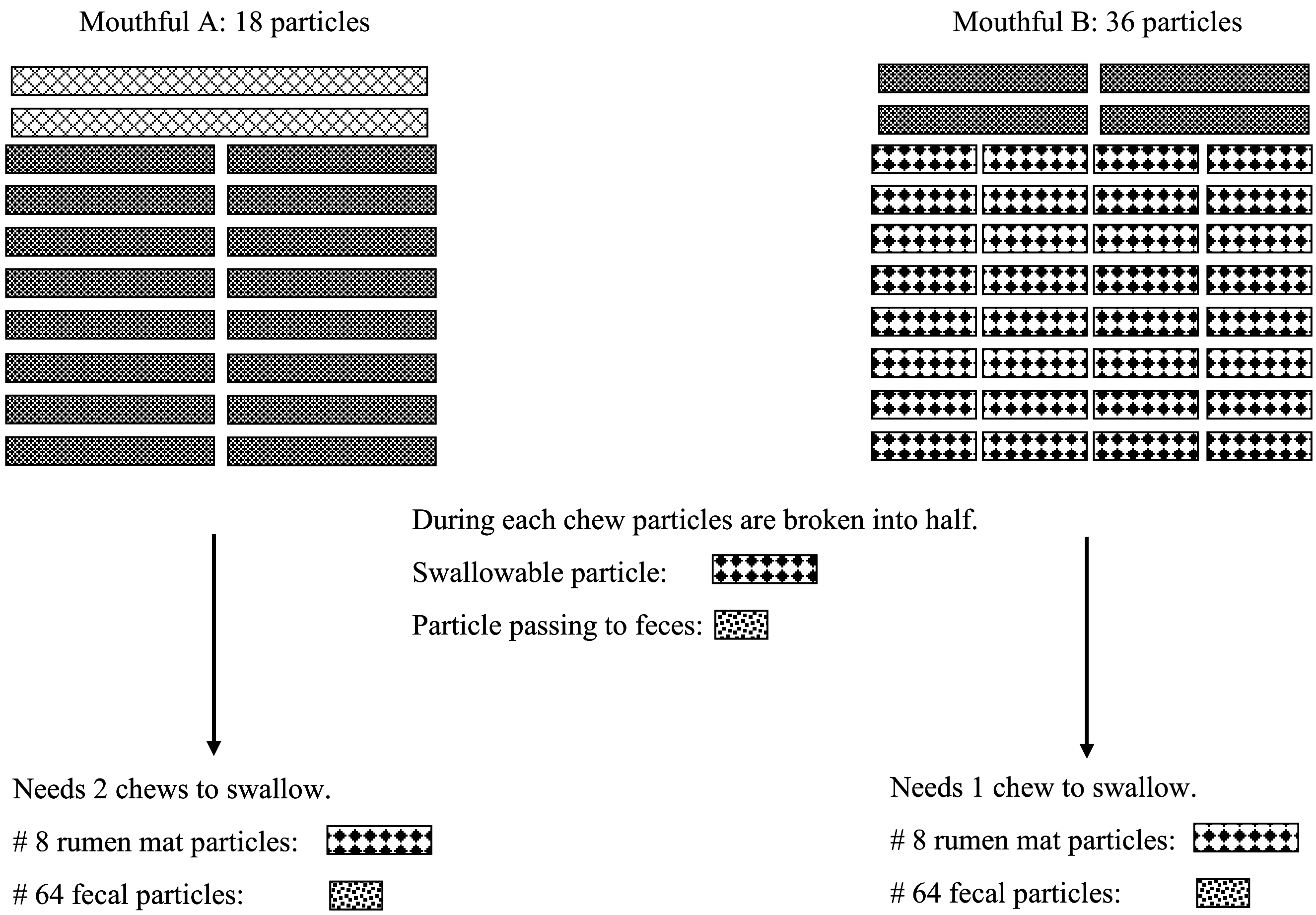


Figure 2.7.c. Both feeds have the same mean length, but differ in distribution. One feed has a wider distribution of particle lengths compared to the other.

- Mean particle length: A = B.
- Ingestive chewing: A > B.
- Rumination: A < B, rumen fill: A < B, intake: A > B.

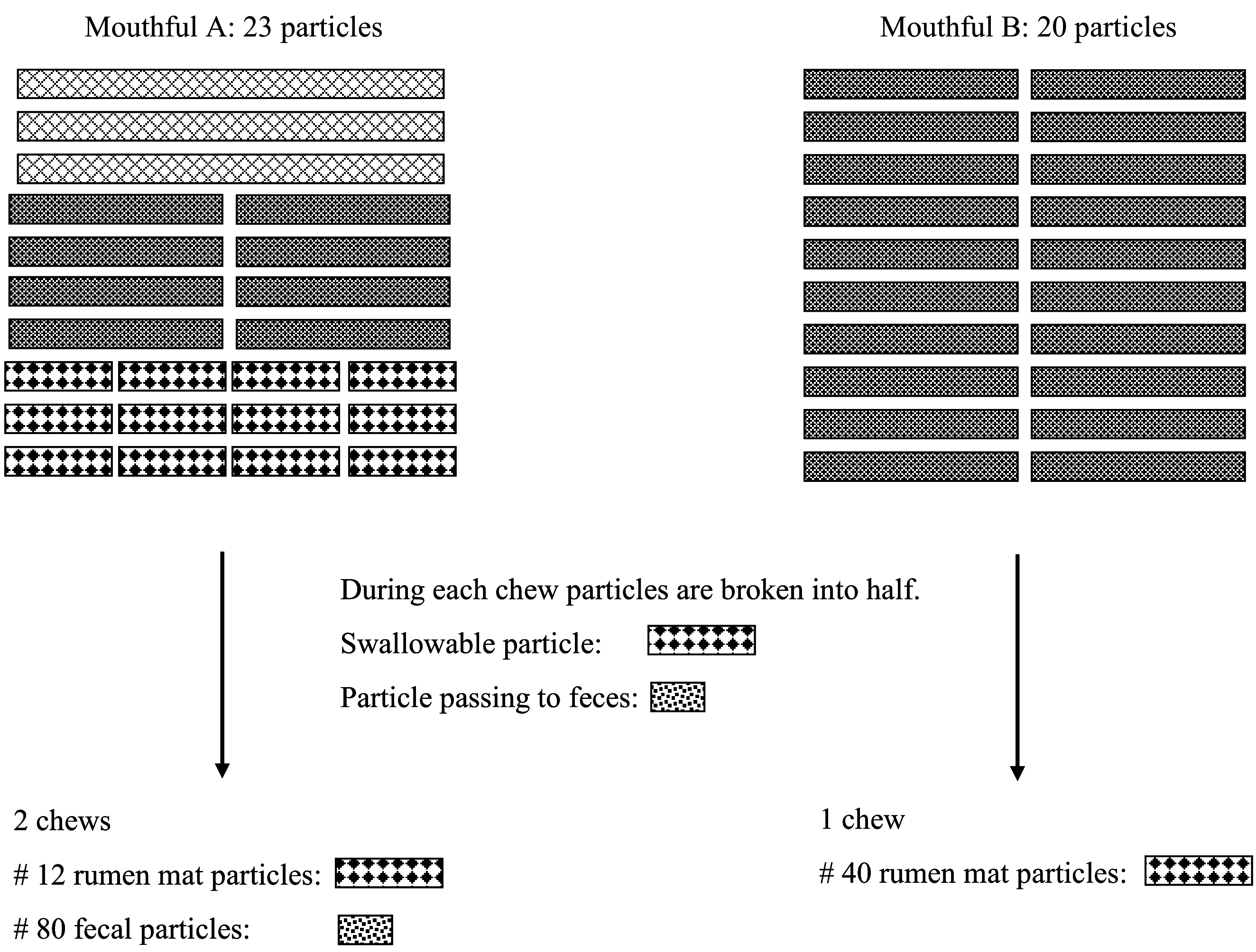


Table 2.1. Ingredient, chemical composition of diets[1], and intake, milk production and body weight of dry and lactating cows.

Item	Dry cows	Lactating cows
Ingredient, % of DM		
Rye grass hay	63.4	12.3
Mixed grass silage[2]		12.9
Corn silage		20.9
Maize grain	20.9	24.8
Barley grain		4.1
Wheat bran	7.2	3.7
Soybean meal (44% CP)	7.5	16.6
Soypass®		1.1
Mix[3]	1.0	3.6
Chemical composition		
DM (%)	86.0	51.0
OM (% DM)	90.5	91.1
CP (% DM)	12.6	16.8
SP, (% CP)	23.0	25.4
aNDF (% DM)	44.8	36.4
ADF (% DM)	26.6	19.5
Lignin (% DM)	4.0	2.5
NFC (% DM)	33.8	36.9
NEL, (Mcal / kg)	1.4	1.7
Intake, (kg / d)		
DM	9.5	21.8
NDS	55.2	63.6
Days in milk		139 ± 73
Milk production		
Yield, (kg / d)		30.9 ± 10.3
Fat (%)		3.2 ± 0.7
Protein (%)		3.2 ± 0.1
Body weight[4], kg	670	640

[1] Rations were formulated using the Cornell-Penn-Miner System (CPM Dairy, Version 3.0) for dry cows and cows in lactation.
DM = dry matter, OM = organic matter, CP = crude protein, SP = soluble protein, aNDF = neutral detergent insoluble fiber, ADF = acid detergent insoluble fiber, NFC = non fiber carbohydrate, NDS = neutral detergent solubles.

[2] Triticale, barley, rye grass.

[3] Vitamin, mineral, urea, amino acid, fat supplement.

[4] assumed.

Table 2.2. Chemical composition of treatment feeds[1].

Treatment hay	DM[1a] %	Ash	CP % DM[1b]	aNDF	pH
Long rye grass hay		10.2[ab]	11.7[c]	57.1[abc]	
Rye grass cut at 50 mm length and dried		10.2[ab]	11.7[c]	58.6[ab]	
Rye grass hay chopped, sieved through PSPS* and retained on:					
Top screen (19 mm)		10.3[ab]	11.8[c]	57.9[abc]	
Second screen (8 mm)		10.6[ab]	12.3[c]	59.1[a]	
Third screen (1.18 mm)		11.3[a]	13.7[b]	54.2[bc]	
Bottom pan		11.7[a]	14.4[b]	54.0[c]	
Grass silage	29.0[b]	9.6[b]	9.3[d]	53.1[c]	3.95[a]
Corn silage	29.1[b]	6.4[c]	9.4[d]	48.1[d]	3.72[b]
TMR	51.3[a]	7.5[c]	16.7[a]	37.7[e]	
SEM	0.90	0.55	0.42	1.56	0.05

[1] DM = dry matter; [1a] dried at ≤ 60°C; [1b] dried at 100°C, CP = crude protein, aNDF = neutral detergent insoluble fiber.

Means within column with different superscript differ by $p<0.05$

*Penn State Particle Separator

Table 2.3. Particle size reduction and number of chews during ingestive mastication - Least square means (LSM) and standard error of mean (SEM) of

- proportional dry residues on a 1.6 mm screen (PROP_1.6),
- mean lengths (ML) of particles retained on a 1.6 mm screen and ≥ 5 mm,
- chews per grams dry matter (DM) ingested.

	PROP_1.6				ML (mm)				Chews (/ g DM)	
	Feed		Bolus		Feed		Bolus			
Treatment hay	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM	LSM	SEM
Long rye grass hay	NE		0.67^{a}	0.041	NE		10.3^{c}	0.43	1.51^{b}	0.14
Rye grass cut at 50 mm length and dried	NE		0.54^{b}	0.044	42.2^{a}	2.78	9.9^{c}	0.47	2.09^{a}	0.13
Rye grass hay chopped, sieved through PSPS* and retained on:										
Top screen (19 mm)	NE		0.67^{a}	0.041	43.5^{a}	1.33	10.7^{bc}	0.43	1.30^{b}	0.08
Second screen (8 mm)	NE		0.69^{a}	0.041	25.1^{b}	0.21	10.8^{bc}	0.43	1.04^{c}	0.04
Third screen (1.18 mm)	0.80^{a}	0.047	0.51^{b}	0.041	9.7^{d}	0.24	8.1^{d}	0.43	1.04^{c}	0.06
Bottom pan	0.33^{c}	0.050	0.13^{d}	0.044	NE		NE		1.22^{b}	0.06
Grass silage	0.66^{a}	0.048	0.59^{ab}	0.042	13.8^{c}	0.32	11.6^{ab}	0.44	0.24^{e}	0.01
Corn silage	0.52^{b}	0.048	0.55^{b}	0.042	12.0^{e}	0.33	11.2^{bc}	0.44	0.33^{d}	0.01
TMR	0.44^{bc}	0.048	0.38^{c}	0.042	13.1^{d}	0.22	12.5^{a}	0.44	0.25^{e}	0.02

Means within column with different superscript differ by $p<0.05$.

*Penn State Particle Separator

NE = not examined

CHAPTER 3

How Do Dairy Cows Chew their Feed?
Part II:
Do lactating cows chew their feed differently from dry cows? How is rumen mat particle size related to bolus and fecal particle size in dry and lactating cows?

INTRODUCTION

There is some evidence in literature showing that optimal nutrition of dairy cows requires, not only a correct diet formulation in regard of chemical composition, but that diet particle size also needs to be attended. Increasing length of forage particles might decrease dry matter intake (Teimouri Yansari and Primohammadi, 2009, Leonardi et al., 2005, Einarson et al., 2004), might alter milk yield (Leonardi et al., 2005; Woodford and Murphy, 1988) or milk composition (Bhandari et al., 2008, Teimouri Yansari et al., 2004, Krause and Combs, 2003). If diet particle size was an important cofactor to influence nutrition of dairy cows, it might be attributed to the size of the masticated particles rather than to the dimensions of the diet particles themselves. In our previous study, we have analyzed lengths distributions of feed and respective bolus particles which are potentially contributing to rumen mat formation and estimated the approximate dry matter proportion of this sample fraction. We have shown that particle size of masticates depends on feed particle size, on a threshold particle size at which feed can be swallowed as well as on chemical parameters of the feed. However, chewing intensity could additionally depend on the physiological stage of the cow. High-producing dairy cows might need to compensate higher nutrient requirement and increased intake, by chewing diets more intensely during ingestion to reduce necessary rumination time and promote passage. Okine et al. (1991) and Le Liboux and Peyraud (1998) observed increased eating time / ingested dry matter (DMI) when feed intake increased in non lactating and milking dairy cows, respectively. In opposite to these studies, other authors observed shorter rumination times in cows with high intakes and / or production even though these cows apparently chewed their feeds less intensely during ingestion. Grant and Albright (1995) reported that high-producing dairy cows are more aggressive eaters, spending less time eating and ruminating per unit of intake. Shaver et al. (1986) reported shorter rumination time / intake of NDF and shorter total chewing / intake of dry matter in cows at early and middle lactation compared to dry cows. These cows were fed the same diet, with decreasing intake from early lactation to the dry period, containing alfalha hay and grain at a ratio of 60:40%. There might be other mechanisms rather than, or in addition to particle size reduction through chewing which might be able to promote passage of longer particles from the rumen. Luginbuhl et al. (1990) found mean rumen mat and fecal particle size increased when steers had higher levels of intake. Deswysen and Ellis (1990) measured longer particles in the dorsal sac and feces of heifers with higher intake. The authors of the latter study observed that higher voluntary intake was positively related with duodenal DM digesta flow per opening of the reticulo-omasal orifice. McBride et al. (1984) observed digesta transfer from the reticulo-rumen to the omasum in cattle by endoscopy. The authors reported that the reticulo-omasal orifice was not a site of discrimination of the size of digesta particles that leave the rumen. Large particles (10 mm in length) could clearly pass through the orifice. The probability for large particles to be in a location which predicates their delivery to the reticulo-omasal orifice might be related to rumen fill and intake.

The objective of the present study was to compare particle size reduction of lactating cows relative to dry cows. We considered reduction of particles from the masticate of the basal diet to rumen mat and to fecal particles as well as reduction if individual feeds during ingestive mastication. Individual feeds included also the basal diets. In particular, we selected 6 rye grass hay samples differing in particle lengths distribution. Additionally, we also tested a corn and a grass silage sample and a sample of a total mixed ration (TMR). Particle size analysis included lengths distributions of particles which are potentially contributing to rumen mat formation and an approximate estimate of the dry matter proportion of that sample fraction.

MATERIALS AND METHODS

The experiment has been conducted at CoRFiLaC, which is a dairy research center located in Ragusa in the Southeast of Sicily, in Italy, and funded by the Sicilian region.

Treatment Feeds and Chemical Analysis

Description in chapter 1, p.27.

Animals and Feeding Protocol

Description in chapter 1, p.28.

Sampling of Feces, Rumen mat and Bolus

Description in chapter 1, p.29. Ingredient, chemical composition of diets, intake and milk production are reported in table 2.1.

Particle Size Analysis

Description in chapter 1, p.29.

Statistical Analysis

Statistical analyses were carried out with SAS (Version 9.1, SAS Inst. Inc., Cary, NC). The GLM procedure of SAS was used to test for differences between the chemical composition of treatment feeds.

Particle frequency by 1 mm lengths was calculated from the Matlab image analysis. Particle mean length (ML) of particles retained on the 1.6 mm sieve and ≥ 5 mm and standard error and statistical tests were determined using PROC LIFETEST, method Kaplan-Meier (KM). The distribution of 1 minus the cumulative frequency proportion as a function of length (l) was calculated. This distribution follows a failure time curve, as the cumulative distribution function (CDF) is related to the survival function as 1-S(t), where S(t) is the survival distribution function evaluated at time t, with length, l, substituted for t. The CDF represented the probability that a length did not exceed length l.

The SAS MIXED procedure was used for testing differences in PROP_1.6, ML and chews / g DM ingested. Differences in PROP_1.6 within feed, bolus and rumen mat samples were tested with cow as repeated subject and the covariance matrix set as VC. Independent variables for feed and bolus samples were treatment, production level and sampling period, for rumen mat samples rumen mat strata (bucket), production level and sampling period. Differences in PROP_1.6 within fecal samples were tested with cow as repeated subject within production level and sampling period and the covariance matrix set as VC. Independent variables for fecal samples were production level and sampling period. Differences in ML within feed, bolus and rumen mat samples were tested with cows set as repeated subject. For feed, bolus and rumen mat tests of ML differences the covariance matrix were set as ARH(1) , AR(1), and AR(1), respectively. Independent variables for feed ML tests were treatment, production level and sampling period, for bolus ML tests treatment, production level, treatment * production level and sampling period, and for rumen mat ML tests rumen mat strata (bucket), production level and sampling period. Differences in feces ML were tested with cow as repeated subject within production level and sampling period and the covariance matrix set as VC. Independent variables for fecal samples ML tests were production level and sampling period. Differences in chews g DM ingested were tested with cows as repeated subject. Covariance matrix was set ARH(1) and independent variables were treatment, production level and treatment*production level.

RESULTS AND DISCUSSION

Mean particle lengths distribution from image analysis of bolus relative to mean rumen mat and fecal samples of the four dry and the four lactating cows are shown in figures 3.1 Image analysis was performed on sample residues after sieving the samples through a 1.6 mm screen.

Figure 3.1.a illustrates bolus from long rye grass hay and rumen mat and fecal particles of dry cows which were fed with long rye grass hay and a concentrate supplement. Figure 3.1.b shows particles of lactating animals which were fed with TMR. Lactating cows had apparently a wider range of particle lengths in bolus particles and as a consequence also in rumen mat and fecal particles, compared to dry cows. Dry cows' bolus samples retained on a 1.6 mm screen, from rye grass hay contained 3.8% of particles > 20 mm, rumen mat samples 5.4% and the fecal samples 0.6%. Lactating cows' bolus samples from TMR feeding contained 8.2% of particles > 20 mm, rumen mat samples 5.4% and the fecal samples 0.6%. The TMR bolus from lactating cows had apparently longer particles compared to the bolus from the long grass hay in dry cows. Higher water content and a lower aNDF content of TMR particles compared to rye grass hay particles might allow bending of particles and the swallowing of longer particles in consequence. However, lengths distributions of rumen mat and especially fecal particles, which were retained on a 1.6 mm screen, from dry and lactating cows were more alike. The forage particle fraction from eaten TMR might need more rumination time relative to the bolus from long rye grass hay.

Lengths distributions of particles retained on a 1.6 mm screen of dry and lactating cows' rumen mat particles relative to bolus and fecal particles are reported in figures 3.2 and 3.3, respectively. Dry cows had very similar bolus and rumen mat particle distributions (figure 3.2.a). Apparently, little reduction in lengths occurred in particles which were retained on a 1.6 mm screen, from eaten hay to rumen mat. Less than 1% bolus particles from long rye grass hay were longer than 30 mm and less than 1% rumen mat particles were longer than 28 mm. Proportions of bolus and rumen mat particles of dry cows were highly correlated (R^2 = 0.97) within a range of lengths of 5 – 28 mm. Proportions of rumen mat particles in dry cows were also highly correlated to fecal particles (R^2 = 0.996) within a range of particles of 1 – 5 mm and (R^2 = 0.98) within a range of particles of 6 – 18 mm (figure 3.2.b). Particle lengths were shorter in fecal particles relative to rumen mat particles. Particles of 7 – 18 mm lengths had higher proportions in the rumen mat relative to feces, and shorter particles were higher in feces. Only 1% of fecal particles were longer than 18 mm. Proportions of rumen mat particles within a range of 19 – 28 mm could not be estimated from proportions of fecal particles. This particle fraction represented only 2.9 % of total numbers of rumen mat particles in dry cows, retained on the 1.6 mm screen. However, these might be the particles which are most important in regards of rumen mat formation and entrapment of small particles. These longer particles supposedly have also higher weights compared to the shorter ones and the low numerical proportion probably did not represent quite well the mass proportion. In lactating cows, proportions of bolus particles at individual lengths were highly correlated to rumen mat particles (R^2 = 0.94), within a range of 7 – 35 mm (figure 3.3.a), $y = 0.8506x – 0.0014$, with y being rumen mat particles' proportion and x bolus particles' proportion. Relative to dry cows, in lactating cows there was a bigger difference in particle lengths between rumen mat and bolus particles. In lactating cows, rumen mat had more particles, compared to bolus particles, in the range of 1 – 6 mm, and fewer particles in the range of 7 – 35 mm. Less than 1% TMR bolus particles were longer than 58 mm and less than 1% rumen mat particles were longer than 35 mm. Proportions of rumen mat particles in lactating cows were also highly correlated to fecal particles ($R^2 = 0.94$) within a range of particles of 1 – 9 mm and ($R^2 = 0.96$) within a range of particles of 10 – 18 mm (figure 3.3.b). Particles of 8 – 18 mm lengths had higher proportions in the rumen mat relative to feces, and shorter particles were higher in feces. In accordance to observations in dry cows, only 1% of fecal particles from lactating cows were longer than 18 mm. Proportions of rumen mat particles within a range of 19 – 35 mm could not be estimated from proportions of fecal particles. This particle fraction represented 6.1 % of total numbers of rumen mat particles retained on the 1.6 mm screen. Figures 3.2 and 3.3 demonstrate that the eaten longer particles from TMR, which were retained on a 1.6 mm screen, might have needed more rumination time to pass from the rumen compared to ingested particles from hay. In both, dry and lactating cows, proportions of rumen mat particles > 4 and > 6 mm, respectively, at individual lengths, retained on a 1.6 mm screen, could be predicted from proportions of bolus particles.

The table 3.1 reassumes PROP_1.6 and ML of feed, bolus, rumen mat and fecal samples from dry and lactating cows. We distinguished basal diet and respective bolus, rumen mat and feces, from treatment feed and bolus. Treatment feed could not be related to rumen mat and fecal samples, because, with exception of long rye grass hay fed to dry cows and the TMR fed to lactating cows, animals were not adapted to these diets. We have highlighted in chapter 1 that PROP_1.6 overestimated mass of particles ≥ 5 mm, especially for the samples with low dry matter residue on the 1.6 mm sieve, because also particles with lengths < 5 mm are retained on that screen. However, overestimation in dry and lactating cows occurs probably to a similar extent. Boli from dry and lactating cows on their basal diet were different from each other ($p < 0.01$), because different feeds were fed. Approximately 67% and 38% dry matter of bolus from dry and lactating cows, respectively, was retained on a 1.6 mm screen. During ingestion, long rye grass hay was apparently chewed more intensely compared to the TMR. Bolus particles retained on a 1.6 mm screen and ≥ 5 mm, had ML of 9.8 mm in dry and 12.6 mm in lactating animals. Dry cows' chewing of regurgitated bolus during rumination, from long rye grass hay reduced the lengths of particles ≥ 5 mm less and increased the particle fraction of small particles passing the 1.6 mm screen more, compared to lactating cows' chewing of regurgitated bolus from TMR, because ingested rye grass hay particles were already shorter compared to ingested TMR particles.

According to Peréz-Barberìa and Gordon (1998) the number of chews has to increase exponentially for small particles to obtain the same rate of comminution as for larger particles. Even though dry and lactating cows received different diets, PROP_1.6 and ML of rumen mat and feces were alike. Approximately 26% and 36% dry matter of rumen mat was retained on a 1.6 mm screen in dry and lactating cows, respectively. Rumen mat particles retained on that screen and ≥ 5 mm, had ML of 8.6 mm in dry and 10.3 mm in lactating cows. We collected the more solid material of rumen digesta in up to six sequential 10 liter buckets and analysed particle size of each bucket. We expected longer particles in the upper strata and smaller material in the lower layers. However, both, PROP_1.6 and ML, did not vary significantly ($p > 0.15$) between layers. There was a trend for higher PROP_1.6 of fecal particles from dry cows compared to lactating cows ($p = 0.105$), but ML of 7.4 and 7.9 in dry and lactating cows, respectively, were not statistically different ($p > 0.15$). Luginbuhl et al. (1990) and Deswysen and Ellis (1990) found longer fecal particles when higher levels of a diet was fed to steers and heifers, respectively. In the present study, we fed two different diets at different levels of intake and measured PROP_1.6 and ML of rumen mat and fecal samples could be the response relative to both, physiological stage and diet characteristics.

There is a close relationship between rumen turnover, rumen contents and feed intake (Van Soest, 1994). As feed intake increases, rumen retention time decreases, and we would expect that the TMR diet is retained less in the rumen compared to the long hay diet, because of the higher intake of lactating compared to dry cows. However, Colucci et al. (1982) showed that rumen particulate retention time, especially forage retention time is not only affected by intake, but also by the forage content of the diet. The authors measured rumen retention time in dry and lactating Holstein cows with low and high intake, respectively, fed high forage and low forage diets. Forage particle retention time was higher with the low forage compared to the high forage diet and with low intake compared to high intake of the low forage diet. Forage particle retention time was equal at either high intakes of low forage diets or low intakes of high forage diets. According to Colucci et al. (1982), forage particles from TMRs, which contain little forage and of which high levels of DM are eaten, could have similar retention times compared to the forage in dry cows rations, which contain basically the forage itself and which is ingested at lower levels. In the current study, rumen retention time was not measured. However, the results of the present trial demonstrated that the lengths of the longer particle fraction from TMR, which was retained on a 1.6 mm screen, were chewed more during rumination compared to the longer fraction of ingested particles from rye grass hay. Only approximately 38% or less of TMR DM were particles potentially retained in the rumen, but these particles might need a longer time for rumination compared to chewed hay particles.

The figure 3.4 illustrates two hypothetical examples, how dimensions of the diet particles could influence rumen fill, intake, bolus, rumen mat and fecal particle lengths. I assumed that the frequency of chews needed before a certain feed can be swallowed depended on the longest particles in the mouth. I also made some further, less realistic, assumptions to illustrate easily possibilities how feeds could be chewed. I assumed particle lengths to be proportional to volume, and that particles are broken into half during one chew. Feed A is more heterogeneous in particle lengths compared to feed B and has the shorter mean particle length. Feed A represents TMR fed to lactating cows and feed B the long hay fed to the dry cows. Feed A needs less chews to be swallowed relative to feed B, because the longest particles are shorter compared to B. Ingestive chewing of feed A produces particles which are small enough to allow passage from the rumen to feces and in consequence cows might be able to eat more feed A relative to B. Feces from feed A might contain smaller particles compared to B, because A contains more heterogeneous particle lengths compared to B. If number of chews for ingestion depended on the longest particles in the mouth, then the small fraction of feed A is reduced more intensely than it would be necessary to allow passage from the rumen. We observed a trend for higher PROP_1.6 in feces from dry cows relative to lactating cows. This difference could be explained, at least in part, by the difference in heterogeneity of feed particle lengths between the diets fed rather than by the different physiological stage of cows.

There is some probability that we didn't determine differences in ML of rumen mat and fecal particles retained on a 1.6 mm screen, between dry cows and cows in lactation, because the big variability of particle lengths distributions between cows might have covered effects of physiological stage. Figures 3.5 and 3.6 show variations in particle lengths between cows, within stage, measured in rumen mat and fecal samples, respectively. We observed up to 16.5% and 18% differences in numbers of particles at individual lengths, which were retained on a 1.6 mm screen, in rumen mat samples in dry and lactating cows, respectively. Differences of particle numbers at individual lengths in fecal samples were up to 17% in dry cows and 16% in lactating cows.

We plotted proportions of rumen mat, fecal and bolus particles of individual lengths, retained on a 1.6 mm screen, from lactating cows against dry cows in figures 3.7, 3.8 and 3.9, respectively. Particle proportions of dry and lactating cows' rumen mat were highly correlated (R^2 = 0.80), within a range of particle lengths between 1 – 9 mm, with y = 0.4793x + 0.0368, and (R^2 = 0.98), within a range of particle lengths between 10 – 35 mm, with y = 1.2235x + 0.0013, and x being dry cows' and y lactating cows' particle proportions referred to total number of imaged particles (figure 3.7). There was no significant difference ($p > 0.15$) between dry and lactating cows particle ML. However, there were apparently more particles of 2 – 8 mm lengths in the rumen mat of dry cows compared to lactating and less particles of 9 – 35 mm lengths. Particle proportions of dry and lactating cows' fecal particles were highly correlated ($R^2 = 0.99$), within a range of particle lengths between 4 – 18 mm, with 0.854x + 0.0016 and x being dry cows' and y lactating cows' particle proportions referred to total number of imaged particles (figure 3.8). Dry cows feces contained apparently more particles of 4 – 18 mm lengths relative to lactating cows and less smaller particles.

The chemical composition of the treatment feeds is reported in table 2.2 of chapter 1. Highest CP was measured in the TMR sample (17% DM), followed by the chopped rye grass hays retained on the third screen and bottom pan (14% DM), by the other rye grass hay treatments (12% DM) and by the silage samples (9% DM). Contents of aNDF was lowest in the TMR (38% DM). The corn silage and the grass silage sample contained 48 and 53 (% DM) aNDF, respectively. The third screen and bottom pan residues of the rye grass hay samples had an average content of 54 (% DM) aNDF. The rye grass hay treatments with the longer particles had aNDF contents ranging from 57 – 59 (% DM). In the hay treatments CP content increased as particle size decreased. Silage and TMR DM was 29% and 51%, respectively.

In the present study, lactating cows had apparently the same chewing behaviour compared to dry cows. Treatment feed and respective bolus PROP_1.6 and ML were not significantly ($p > 0.15$) altered by physiological stage of the tested animals (table 3.1). Chews / g ingested DM which are reported in table 2.3 of chapter 1, were not different either ($p > 0.05$. Proportions of bolus particles at individual lengths of dry and lactating cows were equal (figure 3.9). There was no interaction effect of treatment feed by physiological stage on bolus ML ($p > 0.15$). We plotted the 1-cumulative mean proportions of dry and lactating cows' bolus particles retained on a 1.6 mm screen for the individual treatments (figures 3.10). Curves of dry and lactating cows from many treatments were overlapping over the entire range of particle lengths tested. However, lactating cows eating rye grass cut at 50 mm length and dried had apparently equal numbers of bolus particles of ≥ 13 mm, but some less particles in the range of 3 – 12 mm compared to dry cows. Lactating cows eating chopped rye grass hay which had passed the upper PSPS screen, but was retained on the middle screen with 8 mm openings, had apparently equal numbers of bolus particles ≥ 20 mm, but some less particles in the range of 9 – 19 mm compared to dry cows. Lactating cows eating chopped rye grass hay which had passed the middle PSPS screen, but was retained on the lower PSPS screen with 1.18 mm openings, had apparently equal numbers of bolus particles ≥ 13 mm, but some more particles in the range of 7 – 12 mm compared to dry cows. Lactating cows eating grass silage had apparently equal numbers of bolus particles ≥ 24 mm, but some less particles in the range of 10 – 23 mm compared to dry cows. Lactating cows eating corn silage had apparently equal numbers of bolus particles ≥ 15 mm, but some less particles in the range of 10 – 14 mm compared to dry cows.

This experiment might have been performed under conditions which were not ideal for a detection of differences in chewing behaviour of dry and lactating cows. Besides the basal diets, cows were not adapted to tested feeds. Animals were presumably hungry, because feed was with held for 4 hours. We needed hungry animals in order to avoid eventual treatment refusals. We could only sample masticates over a very short time period, too, which might not necessarily have represented eating behaviour of cows during the entire day. DeVries et al. (2003a) reported that feeding activity of Holstein cows was highest after delivery of fresh feed and after return from milking. Chewing intensity might be driven by hunger and competition at the feed alley especially when fresh feed is delivered. However, the results of our study are in accordance with Gibb et al. (1999) and Shaver et al. (1988). The authors of the former study reported bite mass and bites / minute not affected by physiological stage (dry versus lactation) of grazing Holstein cows. Shaver et al. (1988) reported equal particle size of alfalfa hay masticates from dry cows and cows in early lactation. If cows changed their chewing behaviour relative to physiological stages, the non-response of eating behaviour observed in our study, might be explained, at least in part, by variability of chewing behaviour among cows. Acosta et al. (2007) and Nelson (1988) reported animal differences in particle size reduction during ingestive mastication. Acosta et al. (2007) and Nelson (1988) measured mastication of cows in late lactation and in steers, respectively. De Boever et al. (1990) listed productive potential, ingestive capacity, chewing efficiency and / or body weight to be possible causes for individual variations of chewing activity. The authors reported that animals with higher intake capacity needed less time to eat and ruminate and that rate of eating or rumination was positively correlated to body weight. Peréz-Barberìa and Gordon (1998) explained that chewing effectiveness or particle size reduction was related to bite force and tooth morphological features such as occlusal surface area, occlusal contact area cutting enamel edges and enamel features. These authors reported that body mass and age of the animal might be related to bite force and tooth wear. DeVries et al. (2003b) suggested that the large amount of between-cow variability for all measures necessitates the use of within-cow tests when testing for changes in feeding behaviour. We had two cows in our study which were passing from 200 days in milk to the dry stage. However, when we tested only these two cows, responses of PROP_1.6 or ML of bolus from treatment feeds, of rumen mat or fecal particles, to physiological stage were the same as when all cows were considered.

CONCLUSIONS

In the present study, chewing behaviour of dairy cows was not altered by physiological stage or an interaction of treatment feed by physiological stage. Bolus PROP_1.6 and ML from dry cows were not different from lactating cows ($p > 0.15$). Even though dry and lactating cows received different diets, PROP_1.6 and ML of rumen mat and feces were alike. Approximately 26% and 36% dry matter of rumen mat was retained on a 1.6 mm screen in dry and lactating cows, respectively. Rumen mat particles retained on that screen and ≥ 5 mm, had ML of 8.6 mm in dry and 10.3 mm lactating cows. There was a trend for higher PROP_1.6 of fecal particles from dry cows compared to lactating cows ($p = 0.105$), but ML of 7.4 and 7.9 in dry and lactating cows, respectively, were not statistically different ($p > 0.15$).

The lengths of the longer particle fraction from TMR, which was retained on a 1.6 mm screen, were reduced to a higher extent during rumination compared to the longer fraction of ingested particles from rye grass hay. Only approximately 38% or less of TMR DM were particles potentially retained in the rumen. However, these particles might need a longer time for rumination compared to chewed hay particles.

In both, dry and lactating cows, proportions of rumen mat particles > 4 and > 6 mm, respectively, at individual lengths, retained on a 1.6 mm screen, were highly correlated to proportions of bolus particles. Rumen mat ML can be estimated from lengths distribution of bolus particles retained on a 1.6 mm screen.

Figures 3.1. Mean particle distribution of bolus relative to mean rumen mat and fecal samples of four dry cows and four lactating cows. - *Image analysis* after elimination of small particles by sieving through a 1.6 mm screen.

Figure 3.1.a. Dry cows which were fed basically with long rye grass hay.

—— Bolus;

—○— Rumen mat;

—◆— Feces.

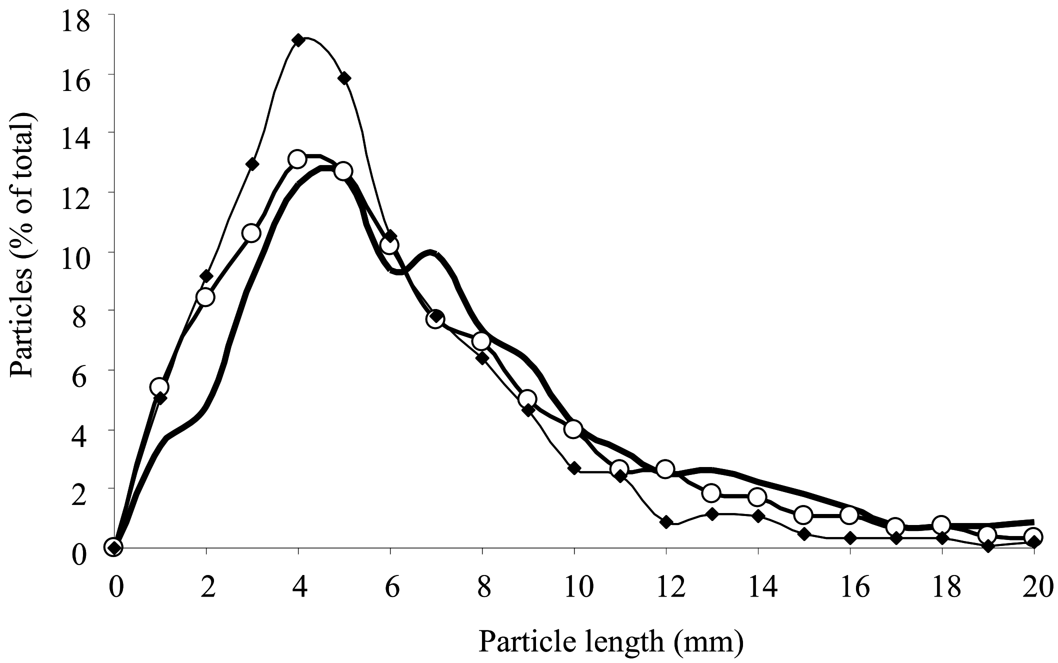
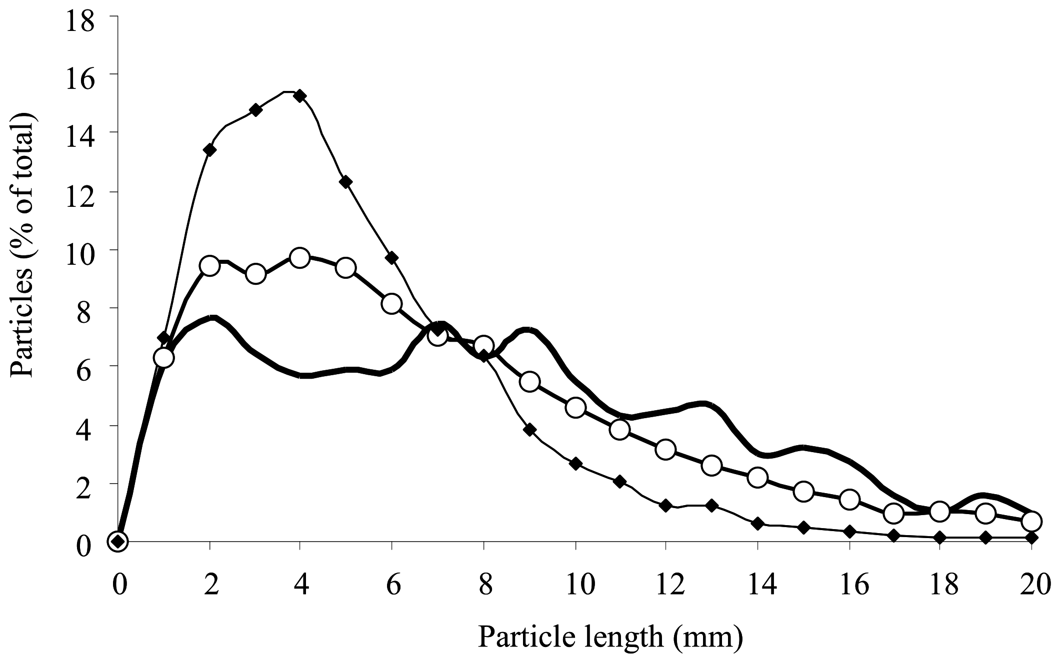


Figure 3.1.b. Lactating cows which were fed with TMR.

— Bolus;

—○— Rumen mat;

—◆— Feces.



Figures 3.2. Lengths distribution of dry cows rumen mat particles relative to long rye grass hay bolus and fecal particles- *Image analysis* after elimination of small particles by sieving through a 1.6 mm screen.

Figure 3.2.a. Rumen mat particles versus bolus particles.

◆ 1 – 4 mm particles.

○ 5 – 28 mm particles; y = 0.9588x - 0.002; R^2 = 0.97; n = 24.

Less than 1% long rye grass hay bolus particles were longer than 30 mm.

Less than 1% rumen mat particles were longer than 28 mm.

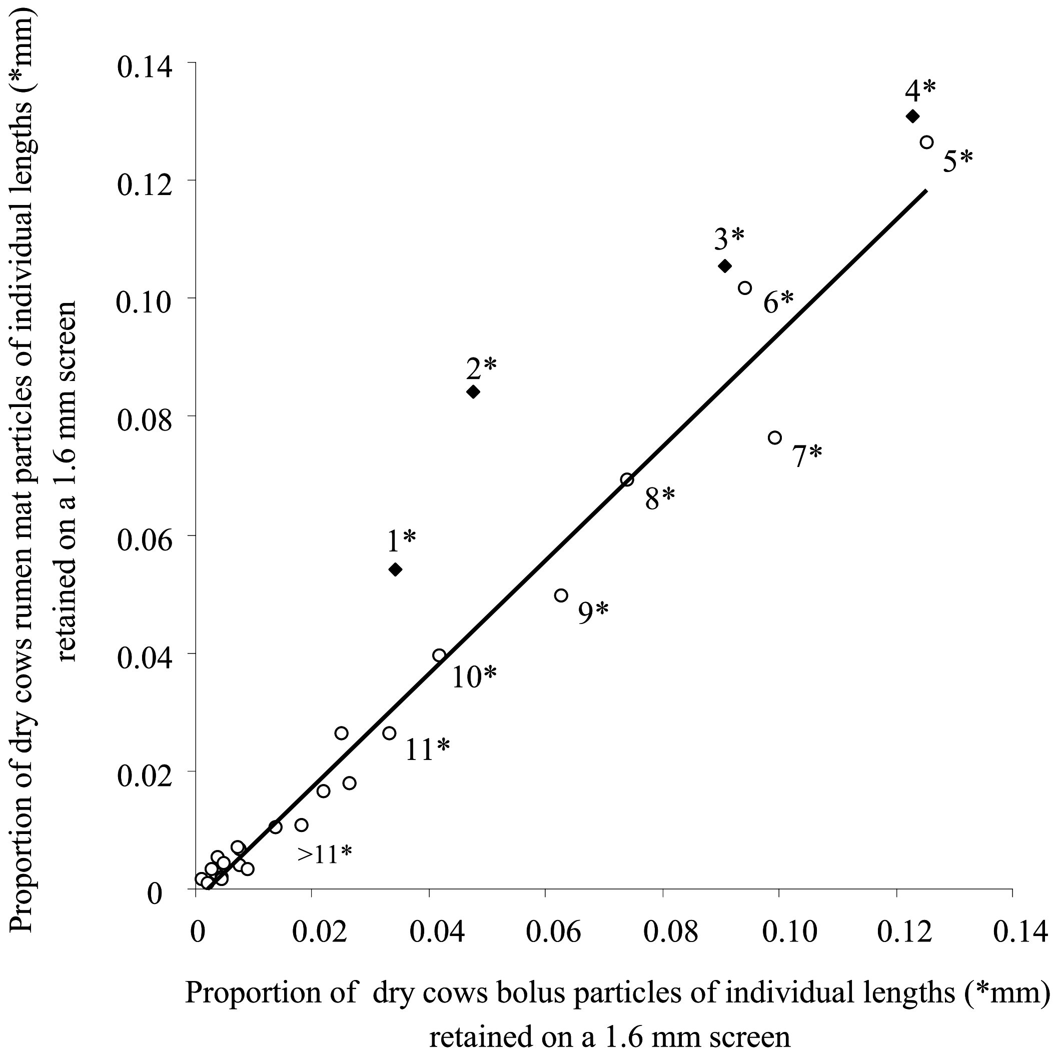


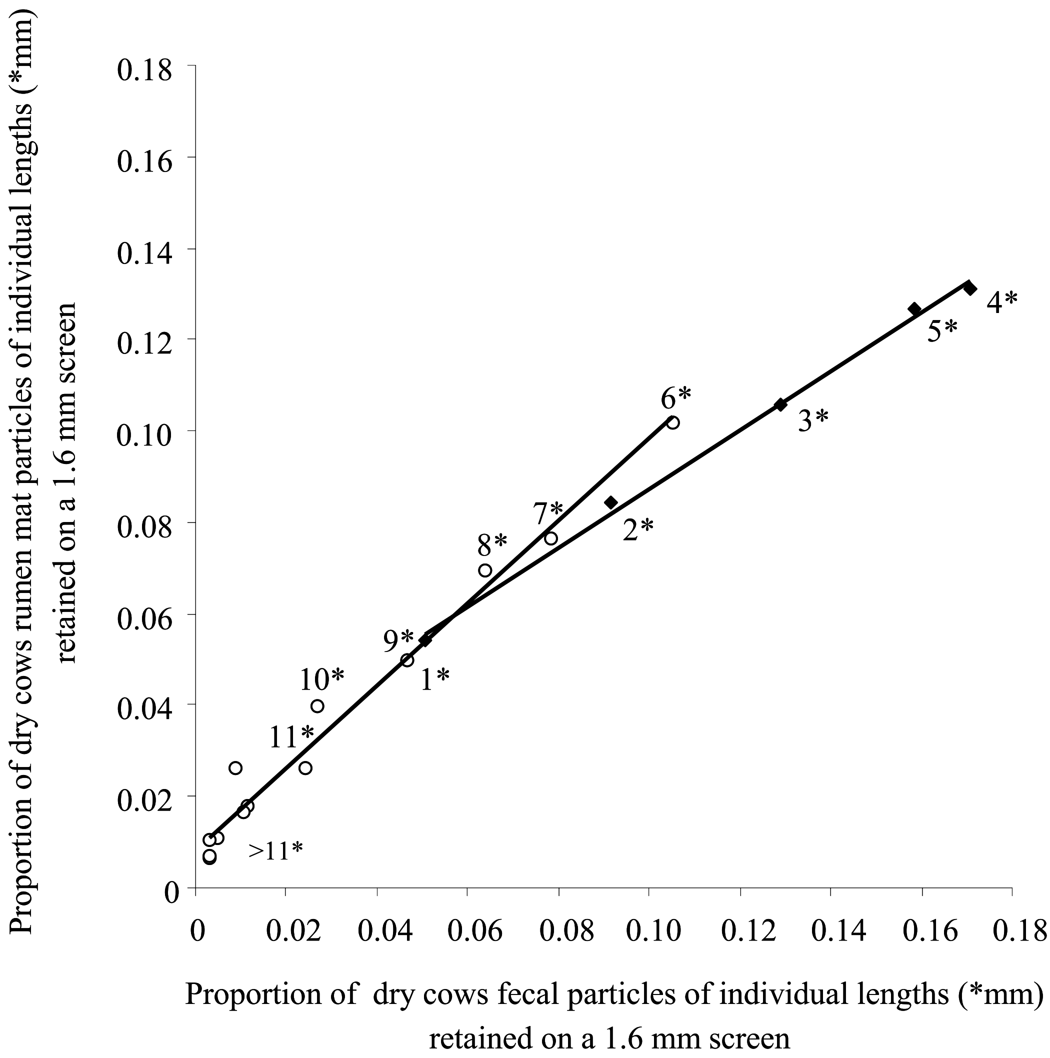
Figure 3.2.b. Rumen mat particles versus fecal particles.

◆ 1 – 5 mm particles; y = 0.6437x + 0.023; R^2 = 0.996; n = 5.

○ 6 – 18 mm particles; y = 0.9011x + 0.0083; R^2 = 0.98; n = 13.

Less than 1% rumen mat particles were longer than 28 mm. (19 – 28 mm particles represent 2.9 % of total rumen mat particles).

Less than 1% fecal particles were longer than 18 mm.



Figures 3.3. Lengths distribution of lactating cows rumen mat particles relative to TMR bolus and fecal particles- *Image analysis* after elimination of small particles by sieving through a 1.6 mm screen.

Figure 3.3.a. Rumen mat particles versus bolus particles.

◆ 1 – 6 mm particles.

○ 7 – 35 mm particles; y = 0.8506x - 0.0014; R^2 = 0.94; n = 29.

Less than 1% TMR bolus particles were longer than 58 mm.

Less than 1% rumen mat particles were longer than 35 mm.

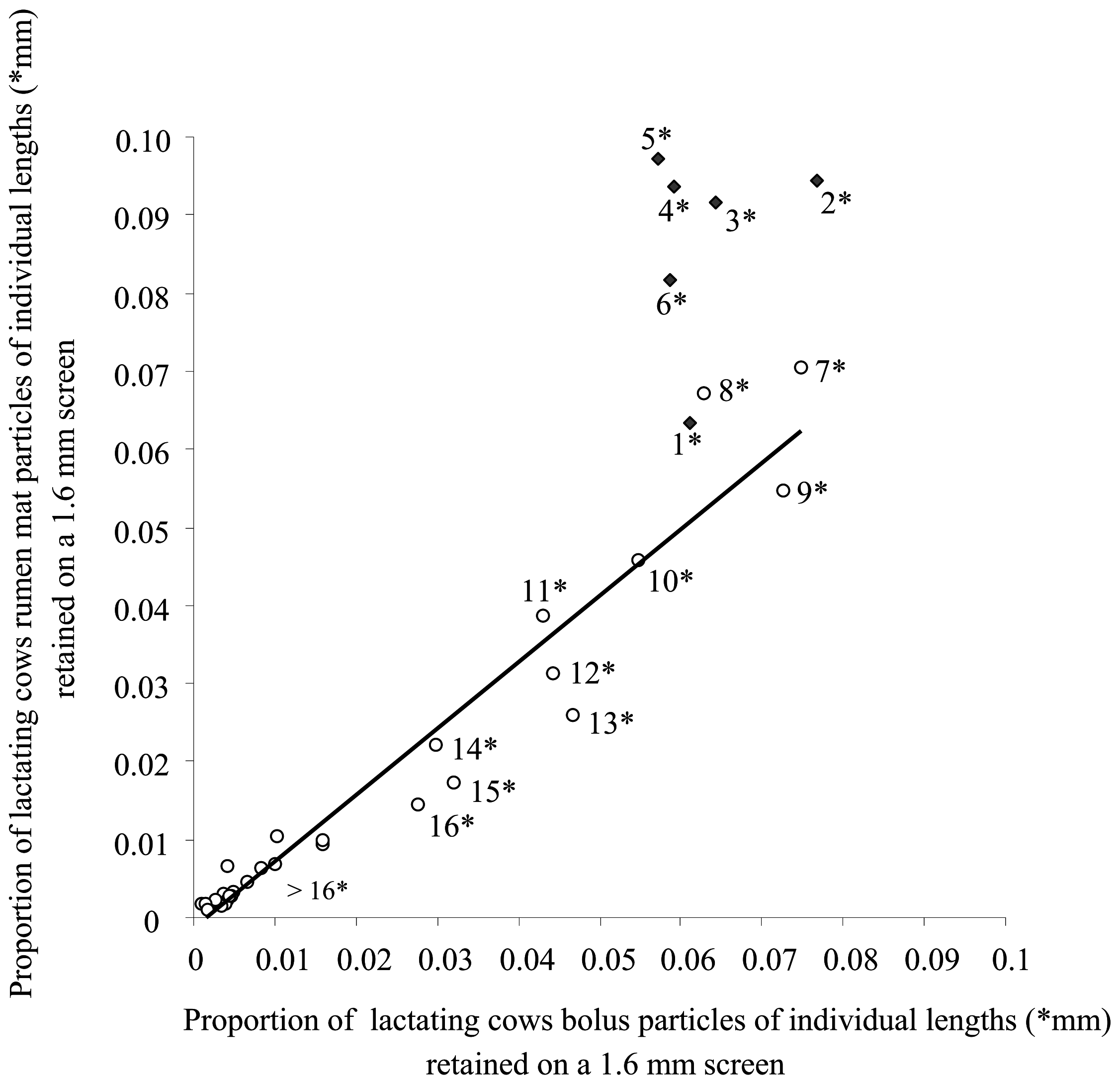


Figure 3.3.b. Rumen mat particles versus fecal particles.

◆ 1 – 9 mm particles; y = 0.3719x + 0.0422; R^2 = 0.94; n = 9.

○ 10 – 18 mm particles; y = 1.4244x + 0.0095; R^2 = 0.96; n = 9.

Less than 1% rumen mat particles were longer than 35 mm. (19 – 35 mm particles represent 6.1 % of total rumen mat particles).

Less than 1% fecal particles were longer than 18 mm.

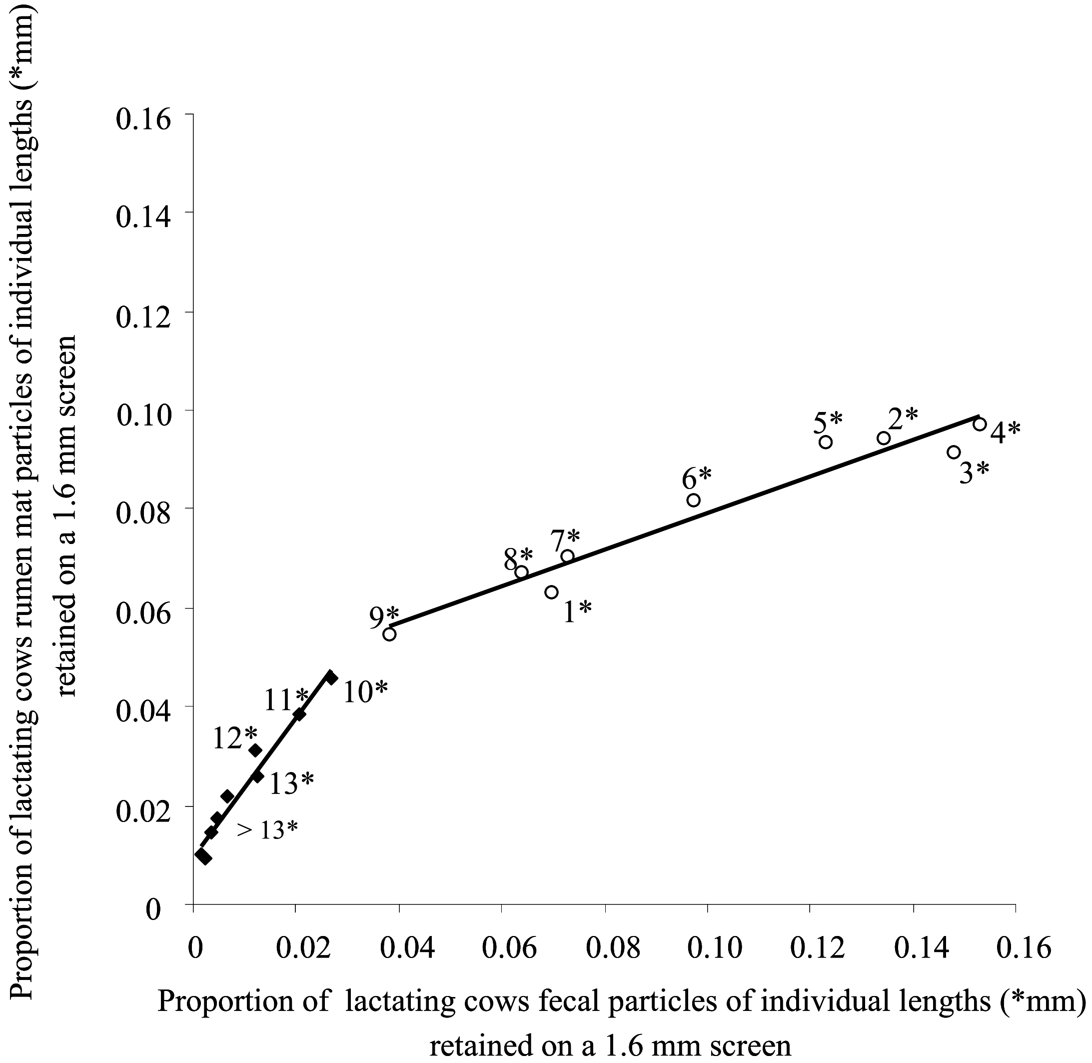


Figure 3.4. Example of how feed particle lengths and distributions might influence rumen fill, intake, bolus, rumen mat and fecal particle length and distribution.

I assumed that the frequency of chews needed before a certain feed can be swallowed depends on the longest particles in the mouth.

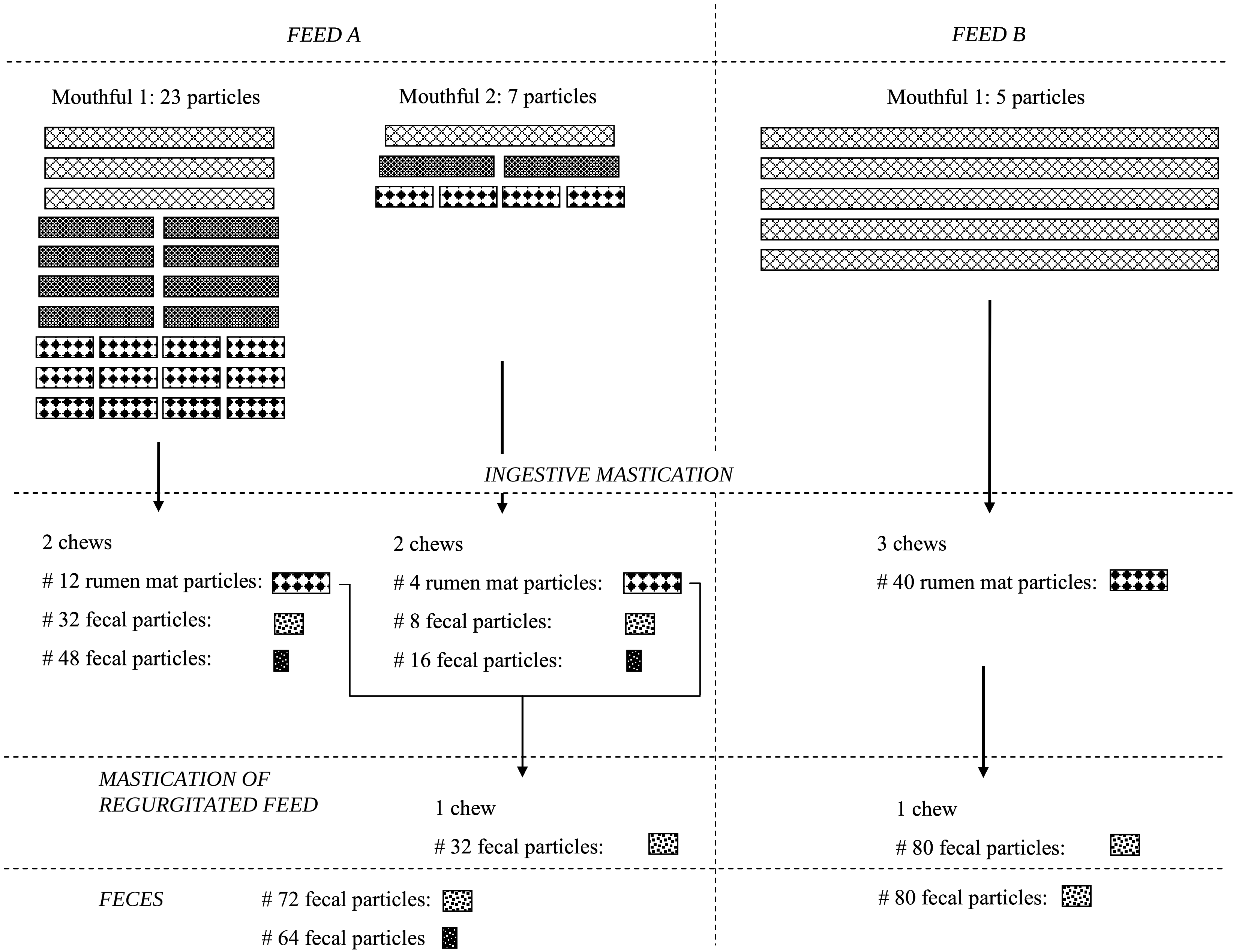
I made further the following assumptions in order to simplify the cases and highlight the principles:

- Particle lengths are proportional to volume.
- Particles are broken into half during one chew, maximum length of particles to be swallowed, maximum length of particles passing to feces.

Feed A: TMR, different proportions of feeds of different lengths are mixed together.

Feed B: Long hay.

- Mean feed particle length: A < B.
- Bolus mean particle length: A < B.
- Intake: A > B.
- Rumen mat particle length: A = B.
- Fecal mean particle length: A < B.


FEED A
FEED B
Mouthful 1: 23 particles
Mouthful 2: 7 particles
Mouthful 1: 5 particles
INGESTIVE MASTICATION
2 chews
12 rumen mat particles:
32 fecal particles:
48 fecal particles:
2 chews
4 rumen mat particles:
8 fecal particles:
16 fecal particles:
3 chews
40 rumen mat particles:
MASTICATION OF
REGURGITATED FEED
1 chew
32 fecal particles:
1 chew
80 fecal particles:
FECES
72 fecal particles:
64 fecal particles
80 fecal particles:

Figures 3.5. Variability of particle lengths distribution within rumen mat samples of four dry cows and four lactating – *Image analysis* after elimination of small particles by sieving through a 1.6 mm screen.

Figure 3.5.a. Dry cows which were fed basically with long rye grass hay.

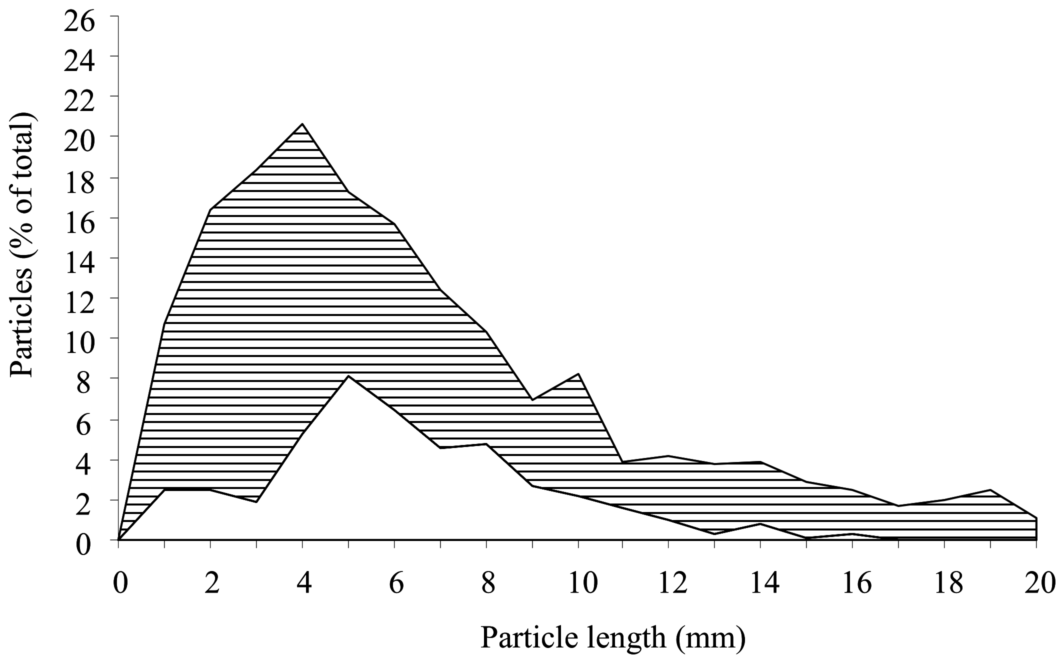
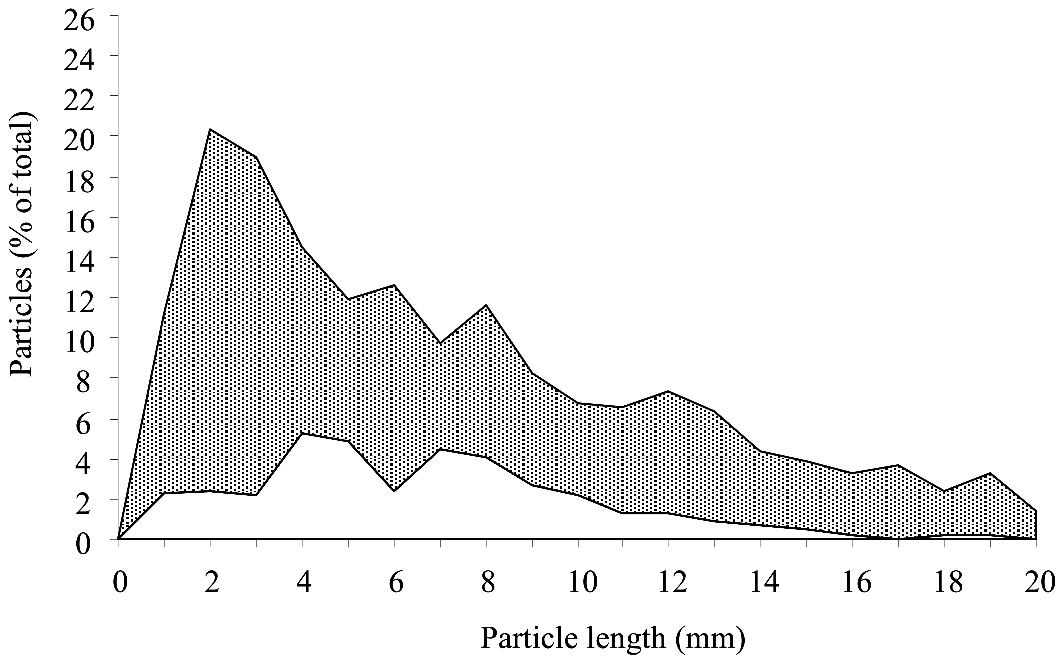


Figure 3.5.b. Lactating cows which were fed with TMR.



Figures 3.6. Variability of particle lengths distribution within fecal samples of four dry cows and four lactating – *Image analysis* after elimination of small particles by sieving through a 1.6 mm screen.

Figure 3.6.a. Dry cows which were fed basically with long rye grass hay.

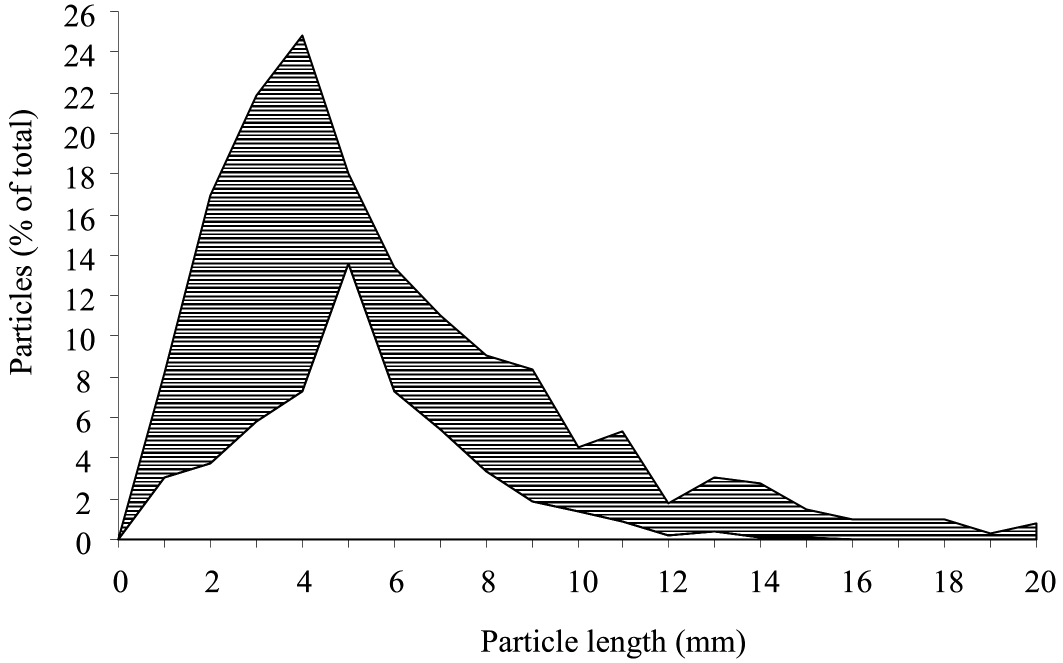


Figure 3.6.b. Lactating cows which were fed with TMR.

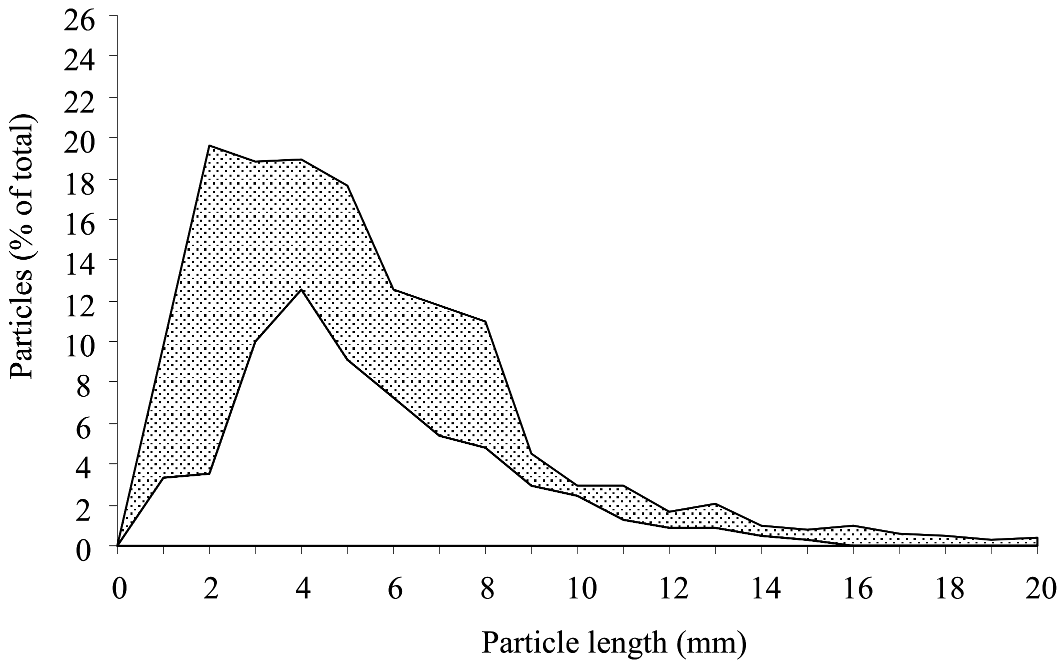


Figure 3.7. Comparison between dry and lactating cows rumen mat particle lengths distribution – *Image analysis* after elimination of small particles by sieving through a 1.6 mm screen.

Dry cows: long rye grass hay plus concentrate supplement.

Lactating cows: TMR.

◆ 1 – 9 mm particles; y = 0.4793x + 0.0368; R^2 = 0.80; n = 9.

○ 10 – 35 mm particles; y = 1.2235x + 0.0013; R^2 = 0.98; n = 26.

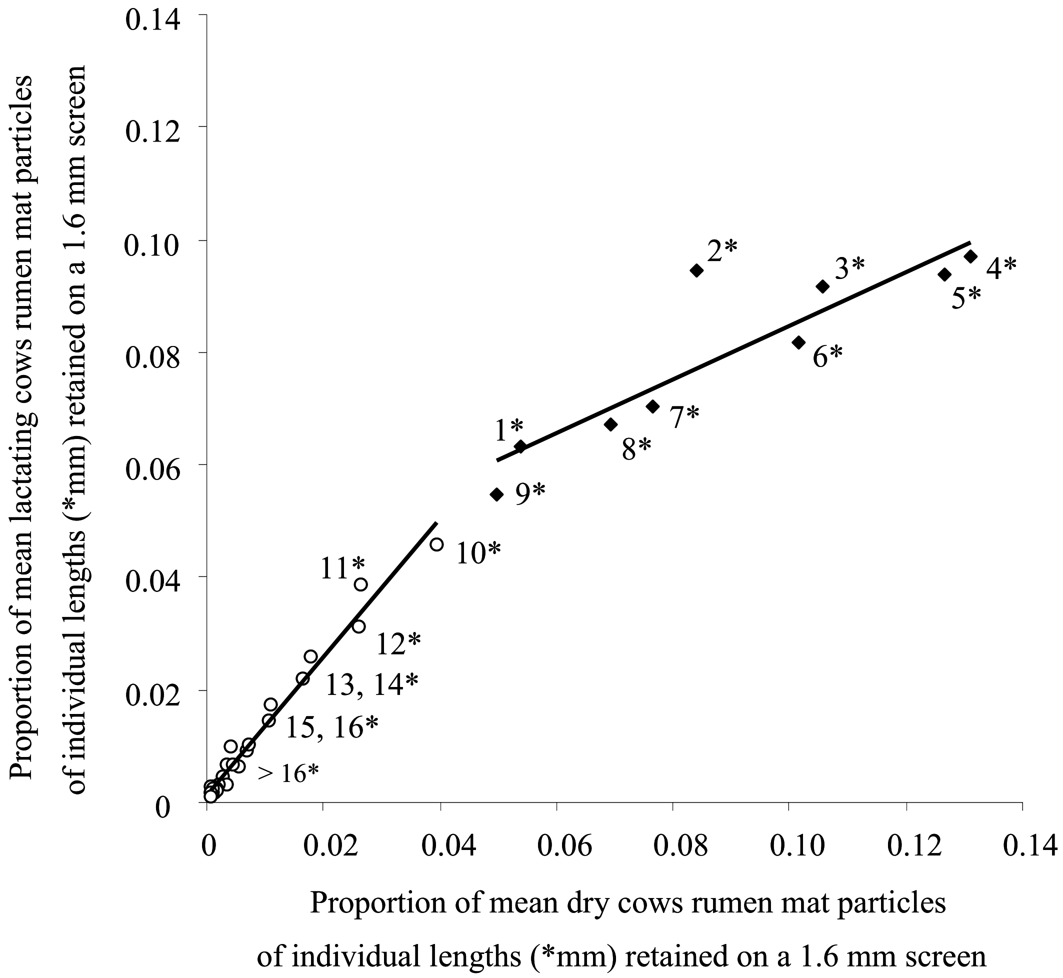


Figure 3.8. Comparison between dry and lactating cows fecal particle lengths distribution – *Image analysis* after elimination of small particles by sieving through a 1.6 mm screen.

Dry cows: long rye grass hay plus concentrate supplement.

Lactating cows: TMR.

◆ 1 – 3 mm particles.

◯ 4 – 18 mm particles; y = 0.854x + 0.0016; R^2 = 0.99; n = 15.

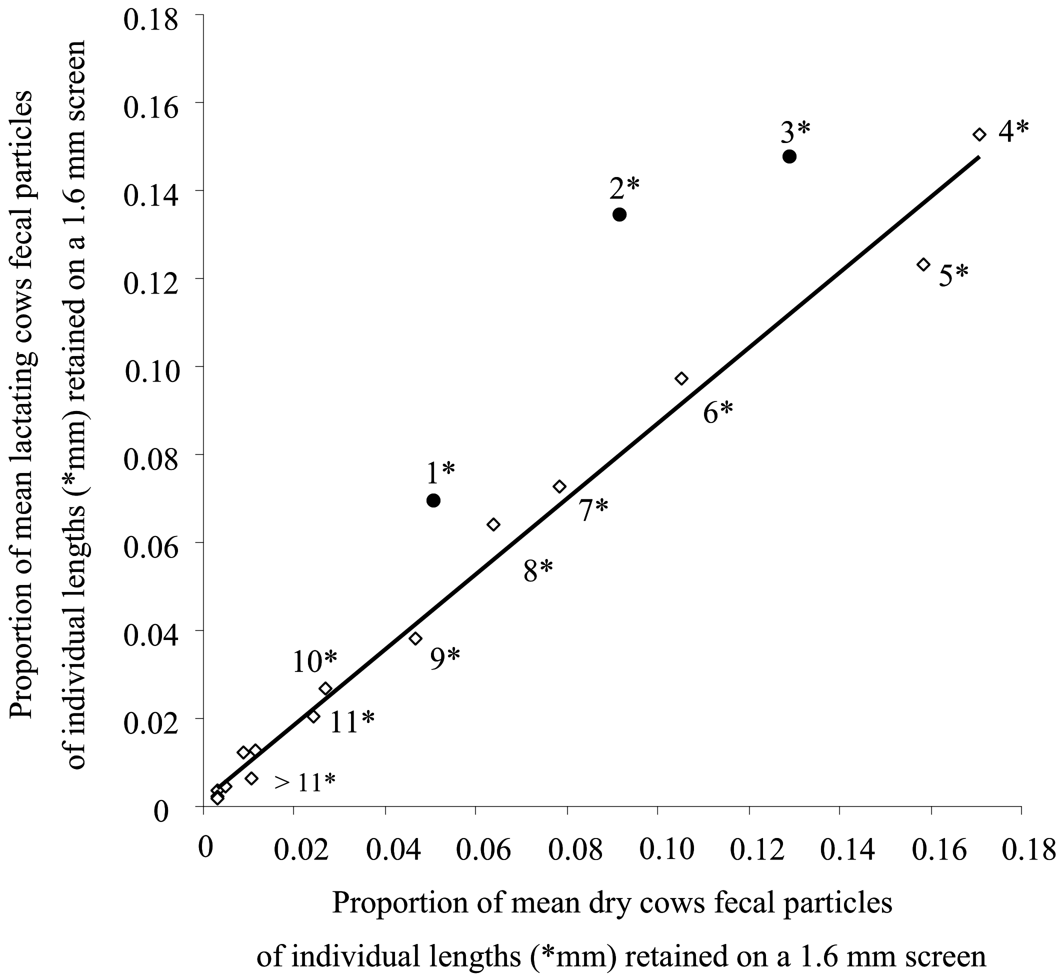
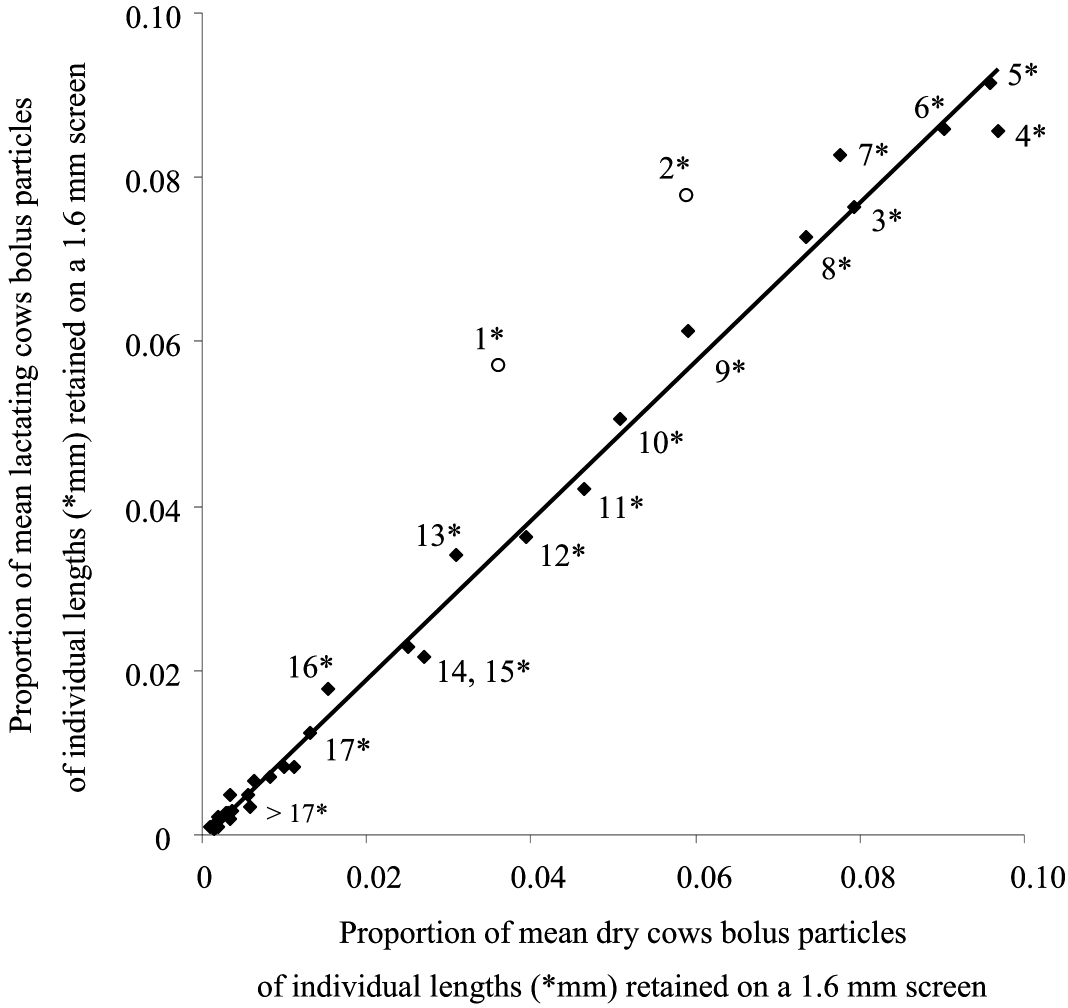


Figure 3.9. Comparison between mean* dry and lactating cows bolus particle lengths distribution – *Image analysis* after elimination of small particles by sieving through a 1.6 mm screen.

* Mean particle proportions of all treatment feeds.

○ 1- 2 mm particles.

◆ 3 – 35 mm particles; y = 0.9657x - 0.0003; R^2 = 0.99; n = 33.



Figures 3.10. Comparison between individual dry and lactating cows bolus particle lengths distribution (a – h). 1 - Cumulative lengths distribution of bolus particles retained on a 1.6 mm screen from *image analysis*.

Figure 3.10.a. Long rye grass hay.

—◆— Dry cows bolus particles.

—○— Lactating cows bolus particles.

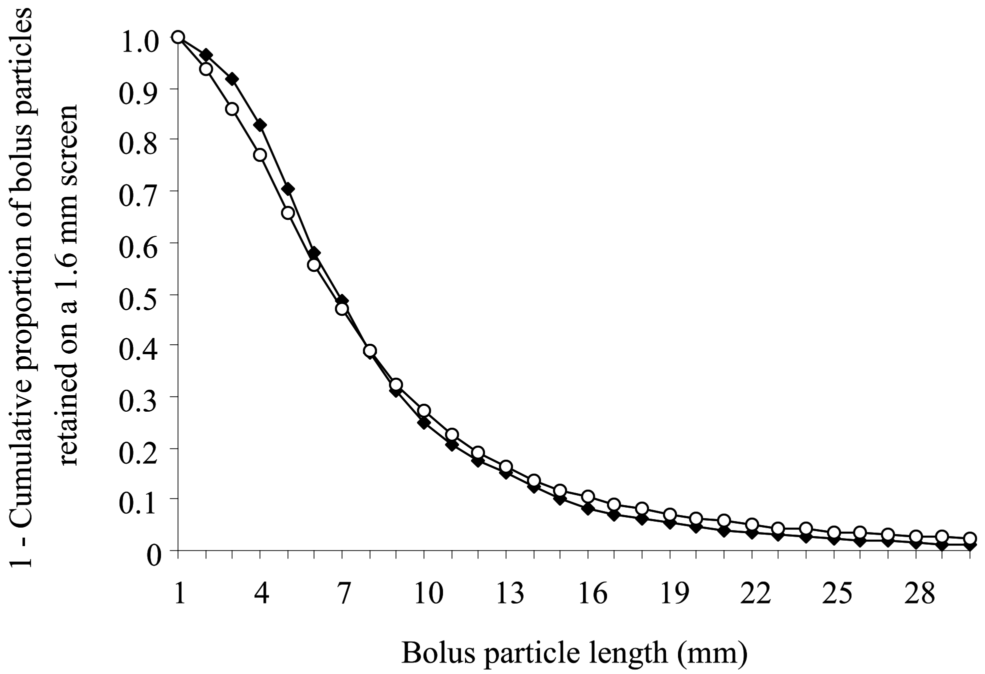


Figure 3.10.b. Rye grass particles cut at 50 mm length and dried to hay.

—◆— Dry cows bolus particles.

—○— Lactating cows bolus particles.

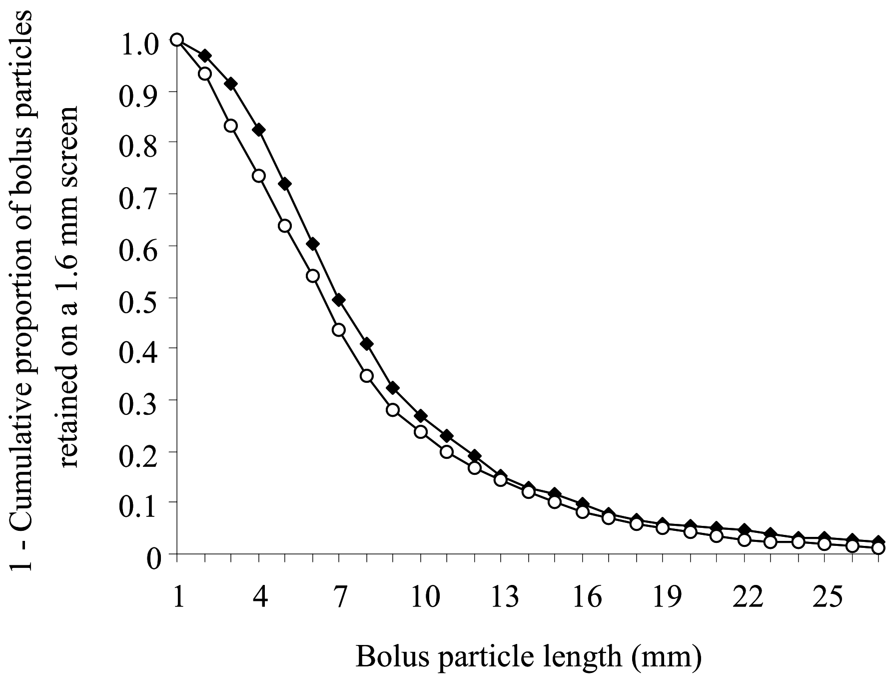


Figure 3.10.c. Rye grass hay particles retained on a 19 mm screen.

Dry cows bolus particles.

Lactating cows bolus particles.

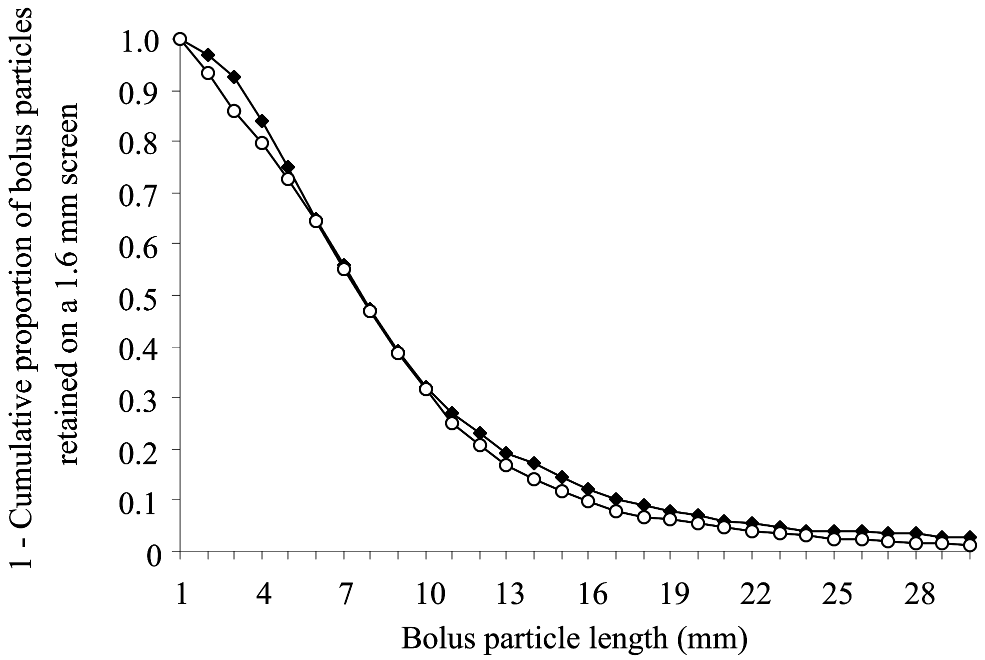


Figure 3.10.d. Rye grass hay particles passing a 19 mm screen but retained on a 8 mm screen.

Dry cows bolus particles.

Lactating cows bolus particles.

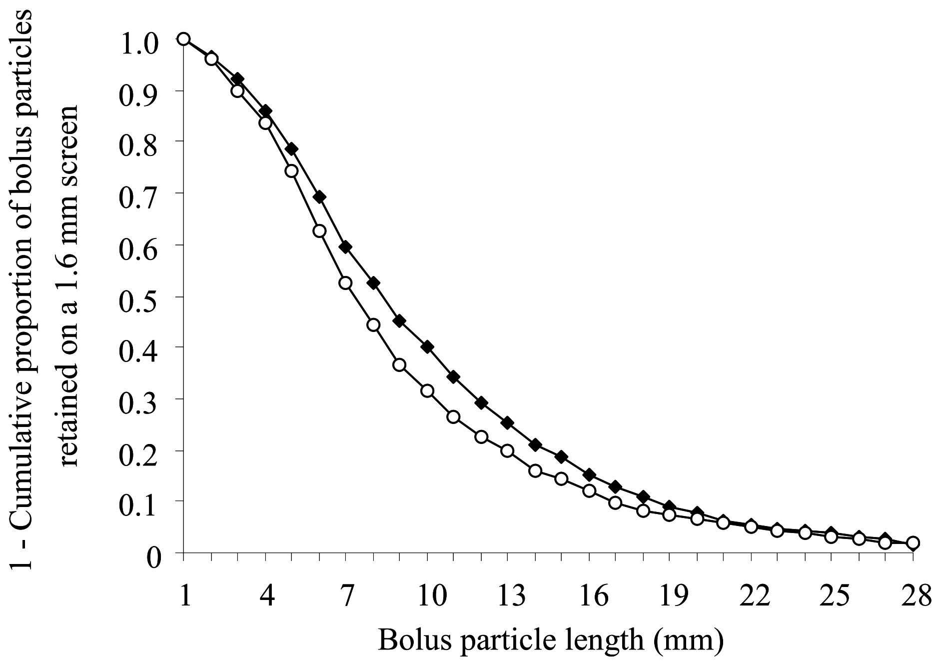


Figure 3.10.e. Rye grass hay particles passing a 8 mm screen but retained on a 1.18 mm screen.

—◆— Dry cows bolus particles.

—○— Lactating cows bolus particles.

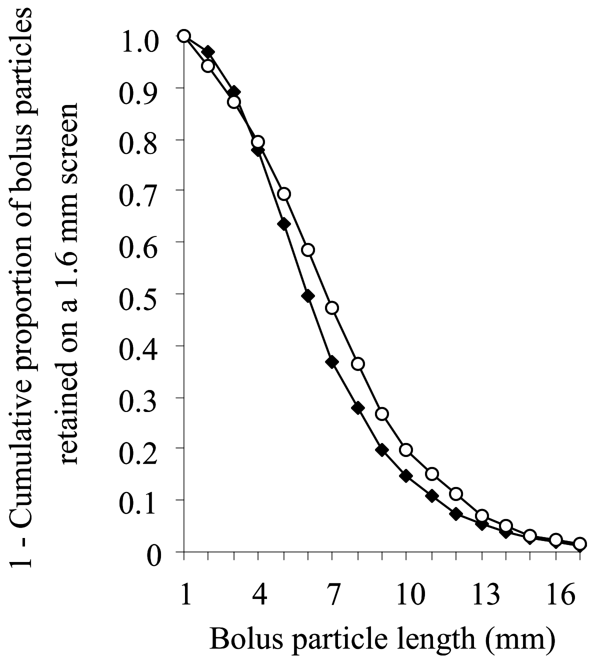


Figure 3.10.f. Grass silage

—◆— Dry cows bolus particles.

—○— Lactating cows bolus particles.

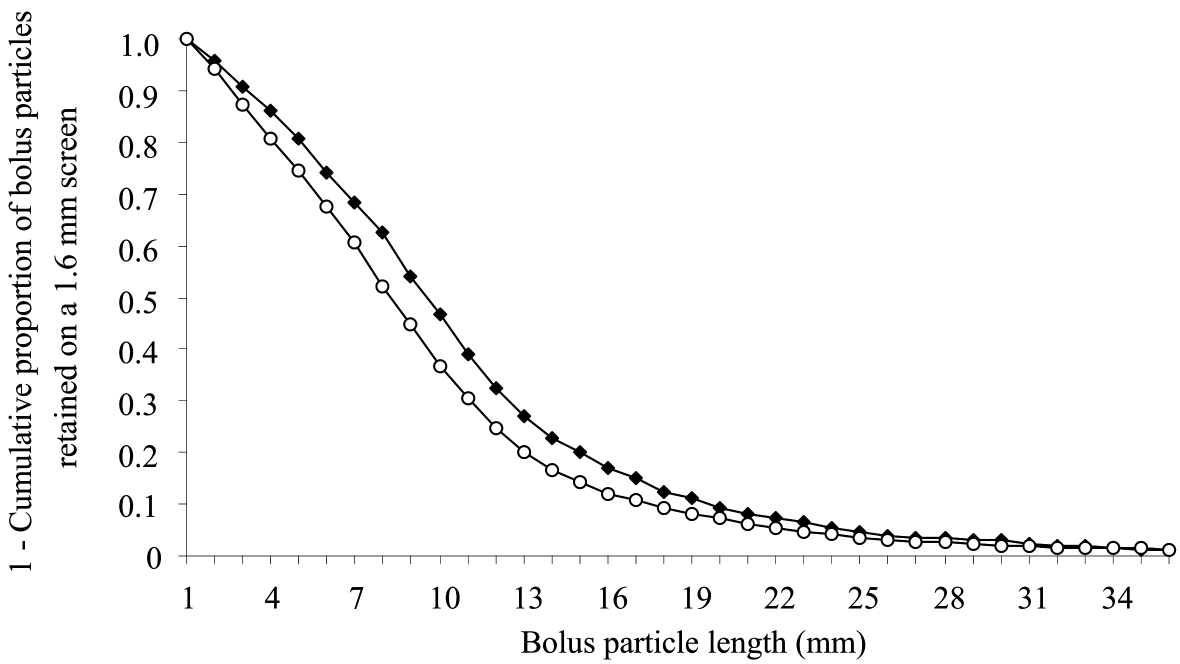


Figure 3.10.g. Corn silage

—◆— Dry cows bolus particles.

—○— Lactating cows bolus particles.

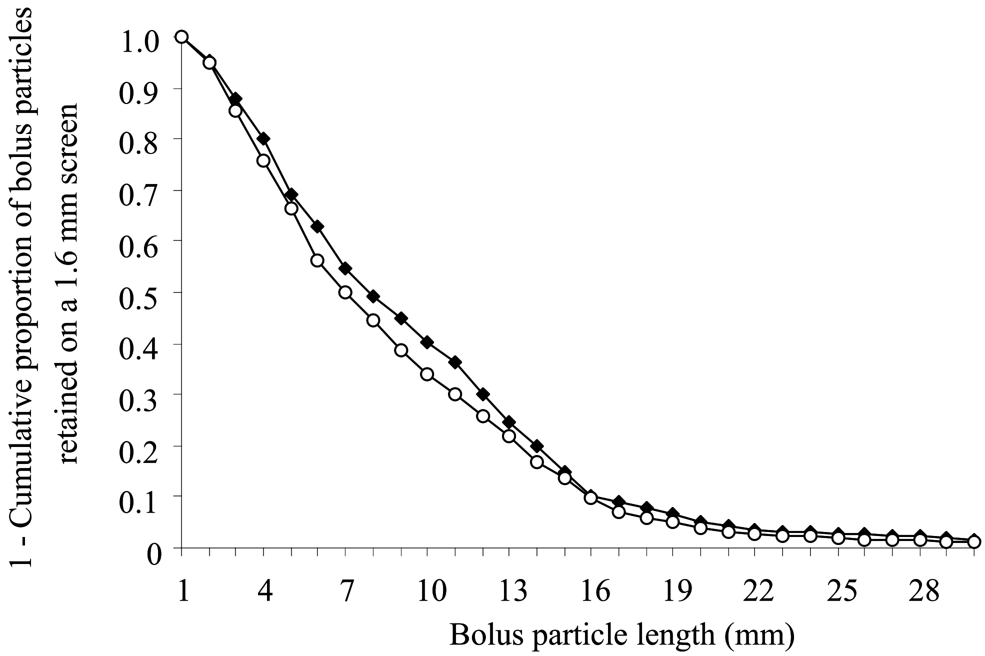


Figure 3.10.h. TMR

—◆— Dry cows bolus particles.

—○— Lactating cows bolus particles.

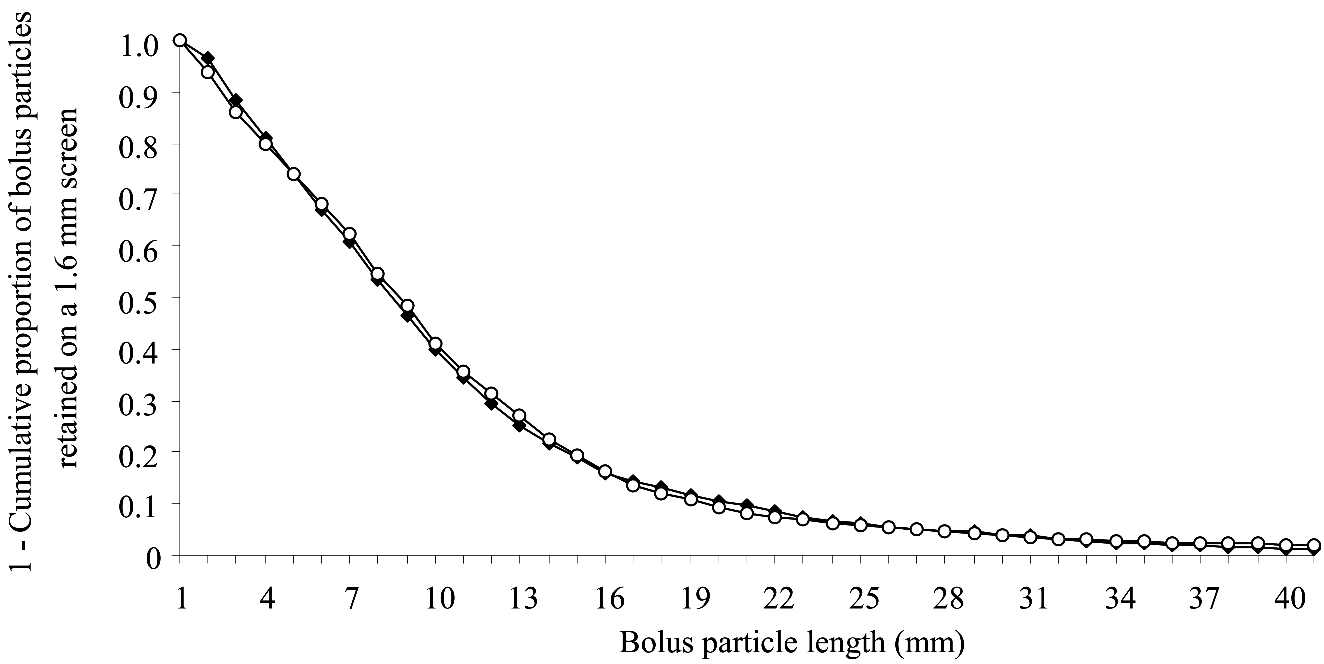


Table 3.1. Effect of physiological stage (dry versus lactating) of dairy cows on particle size of bolus, rumen mat and fecal samples - Least square means (LSM) and standard error of mean (SEM) of:

- proportional dry residues on a 1.6 mm screen (PROP_1.6),
- mean lengths (ML) of particles retained on a 1.6 mm screen and ≥ 5 mm.

Item	PROP_1.6					ML (mm)				
	Dry cows		Lactating cows			Dry cows		Lactating cows		
Treatment	LSM	SEM	LSM	SEM	p^a	LSM	SEM	LSM	SEM	p^a
Feed	0.531	0.035	0.569	0.029	ns	23.03	0.52	22.49	0.50	ns
Bolus	0.510	0.020	0.540	0.020	ns	10.75	0.22	10.50	0.23	ns
Basal										
Diet*	NE		0.434	0.052		NE		13.07	0.22	
Bolus	0.670	0.043	0.381	0.044	***	9.77	0.61	12.58	0.63	***
Rumen mat	0.257	0.026	0.359	0.027	ns	8.55	0.56	10.30	0.55	ns
Feces	0.200	0.042	0.081	0.042	0.105	7.44	0.46	7.89	0.46	ns

[a] Effect of physiological stage, ns = not significant $p > 0.15$; *** = $p < 0.01$.

* Dry cows: long rye grass hay with supplement; lactating cows: TMR.

CHAPTER 4

How Do Dairy Cows Chew their Feed?
Part III:
Particle size analysis of feed and ingested bolus particles from fractions of total mixed rations which are differing in particle length and composition. How is chemical composition of feed related to particle size reduction during chewing?

INTRODUCTION

The understanding of how cows chew their feed might be one fundamental previous step necessary to study if, and how feed particle size could alter efficient feeding, milk production and composition and animal health. In our previous studies we fed rye grass hays cut into various lengths to dairy cows and measured particle size of both, the forages and the respective boli. In our first experiment he have chosen to analyse reduction of grass hay particles, because besides straw, which is not commonly used for nutrition of dairy cows, grass hay ought to be chewed most intensely due to its high content of neutral detergent fiber (NDF). Mertens (1997) showed, summarizing chewing data from other research studies, that, although the variation in chewing among long forages is related primarily to differences in NDF concentration, chewing per kg of NDF increased as the NDF in long forages increased. According to the review of Peréz-Barberìa and Gordon (1998) physical structure as well as chemical composition might effect chewing and particle size reduction. The authors cited studies where ingestive chewing and rumination varied with feed type and with plant parts and which reported increased chewing activity when more mature forages were fed. Asadi Alamouti et al. (2009) evaluated effects of partial replacement of neutral detergent soluble fiber from pelleted beet pulp, for starch from ground barley or maize grain, in diets on chewing activities. Eating time not rumination time / kg dry matter ingested (DMI) was higher the more beet pulp was fed. Kowsar et al. (2008) measured changes in chewing behaviour of dairy cows when chopped alfalfa hay in the ration was gradually replaced by corn silage. Diet dry matter (DM) and acid detergent fiber decreased the higher the corn silage content in the diets was. Eating and rumination time / kg DMI was not affected by corn silage content in the diet even though the diet which contained only alfalfa hay without any corn silage had apparently the shortest particles. The alfalfa hay diet without any corn silage had the lowest percentages of particles retained on the top and the middle screen of the Penn State Particle Separator (PSPS) with openings of 19 mm and

8 mm, respectively, and highest residues on the lower screen with openings of 1.18 mm. Vincent (1983) reported that drying of leaves of Lolium perenne and Phleum pratense reduced their water content and increased the tensile strength of intervening cells, although the force required to fracture across the veins was almost independent of water content. However, there is some evidence that water content in the diet might alter chewing behaviour as well. Beauchemin et al. (2003) evaluated ratio of alfalfa silage to alfalfa hay of diets on chewing activity. The diet which contained more alfalfa silage had the longer particles, but required less eating time / kg DMI. Teimouri Yansari and Primohammadi (2009) evaluated the effects of two methods of alfalfa feeding, dry and reconstituted, on chewing. Hays were reconstituted 24 h before feeding by placing the required amount of dry hay into an industrial container and adding slowly water at ambient temperature to the hay during mixing to achieve a theoretical DM content of 350 g / kg. Diets containing the reconstituted hay had shorter eating and rumination times / kg DMI.

In our first study, one corn silage sample, one grass silage sample and one total mixed ration (TMR) sample have been analysed additionally to the grass hays, in order to get an initial sense of how chemical composition of feeds might influence chewing behaviour. We observed that apparently longer particles can be swallowed from TMR compared to rye grass hay particles which were long enough to be retained on the second PSPS screen with 8 mm openings. Following detailed particle size studies of masticates from various individual forages at individual lengths would have been the logical consequence to our first study. However, when it came to the choice, which feeds to analyze, we decided to focus on TMRs, because this is actually the most commonly feed used in North America and large parts of Europe as well. It is defined as the practice of weighing and blending all feedstuffs into a complete ration which provides adequate nourishment to meet the needs of dairy cows. It can contain several different forages as well as numerous by-products and concentrates. We have selected 10 TMRs from Sicilian dairy farms, which were differing in chemical and ingredient composition as well as in distribution of particle lengths. We fractionated these TMRs by sequential sieving through 4 screens. The fractions differed not only in particle size but also in chemical composition, as especially the shorter particle fractions contained more grain.

The objective of our study was to measure lengths distributions of unprocessed TMRs and TMR fractions and respective bolus particles which are potentially contributing to rumen mat formation and estimate the dry matter proportion of this sample fraction.

MATERIALS AND METHODS

The experiment has been conducted at CoRFiLaC, which is a dairy research center located in Ragusa in the Southeast of Sicily, in Italy and funded by the Sicilian region.

Treatment Feeds and Chemical Analysis

We have selected 10 total mixed rations (TMR) differing in composition and particle size. Diets were formulated for herds at different production levels. Table 4.1 shows ingredient, chemical and physical composition of selected TMRs with respective milk production levels. Forage content ranged from 39.7% to 53.1% (dry matter DM), DM from 48.5 to 63.8%, crude protein (CP) from 14.8 to 25.0%, neutral detergent insoluble fiber (aNDF) from 26.2 to 35.0%, milk yield /d /cow from 19.7 to 38.8 kg, wet residue on the top layer of the Penn State Particle Separator (PSPS) with 19 mm openings from 3 – 22%, on the mid layer with 8 mm openings from 25 to 45%, on the lower layer with 1.18 mm openings from 32 to 46% and on the bottom pan from 16 to 25%.

Our first treatment was the unprocessed TMRs. We provided further treatments by processing the TMRs, as fed, through the PSPS. Approximately 400g of TMR was placed on the top screen, shaken 5 times on each side for two full turns. We added an additional sieve to further subdivide the particle fraction which had passed the 8 mm screen but was retained on the 1.18 mm screen. We have shown in our previous study that rye grass hay particles retained on the 8 mm screen of the PSPS were all chewed to a constant particle size which can be swallowed by the cow independently from their original length. Only the fraction of rye grass hay particles passing the 8 mm screen but retained on the 1.18 mm screen had smaller bolus particles. Chewing of this fraction produced bolus particles, which were, at least in part, shorter than the maximum length of particles which can be swallowed by the cows. The threshold of feed particles to produce maximum length bolus particles might be described by a sieve in between 8 and 1.18 mm. This was the reason for us to test an additional treatment of feed particles which had passed the 8 mm screen but were retained on a screen with openings in between 8 and 1.18 mm. We first tried to process the TMR fraction which had passed the 8 mm screen but was retained on the 1.18 mm screen, through a screen with 4 mm openings. There was not enough residue on that screen to provide an additional treatment. Therefore we chose the next smaller sieve size, for us available, which was a screen with 2.5 mm square openings to provide the additional treatment feed. Summarising, our treatments were the unprocessed TMRs and TMR fractions retained on a 19 mm, 8 mm, 2.5 mm, 1.18 mm screen and on the bottom pan, after using a sequential horizontal sieving technique on the fresh material. We continued the whole sieving procedure until sample size was sufficient for our trial.

Chemical analysis consisted in the determination of dry matter (DM), organic matter (OM), neutral detergent fiber (aNDF), and crude protein (CP). Feeds were dried overnight at 105°C to obtain DM and ashed in a muffle furnace at 550°C for 4 hours to obtain ash content and OM. We analysed aNDF content according to Mertens (2002) using sodium sulfite and heat stable α-amylase (Sigma-Aldrich, Steinheim, Germany). Nitrogen content was determined by a standard Kjeldahl procedure with Cu^{2+} as a catalyst, and multiplied by 6.25 to obtain CP.

Sampling of Faeces

We selected 5 cows from each farm to which the tested TMRs were fed. Animals were 70 – 200 days in milk. We collected approximately 500 g faeces from the rectum of each cow into a polyethylene bag, sealed the bag and placed it on ice.

Animals, Feeding Protocol and Sampling of Bolus

Three ruminally fistulated, dry, mature Holstein cows were used in the study. The animals were fed a ration composed of ad libitum rye grass hay and 3 kg of concentrate (18% CP, 22% NDF, 8.5% ash, containing corn meal, soy bean meal, wheat bran, minerals and vitamins). Animals were housed in a communal pen with free access to hay and water. Grain was offered once a day from a common feed manger. Prior to experimental sampling, animals were moved to individual tie stalls, feed was with held for 4 hours. We removed rumen cannulas and emptied rumen digesta into buckets. Following rumen evacuation, approximately 1000 g treatment feed was offered. The sequence of treatments was random for each individual cow. The cows were allowed to swallow two boli within a treatment prior to sampling three boli for particle size analysis. Boli were obtained by manual collection through the rumen cannula at the rumen-reticular oesophageal orifice when cows were observed to swallow. The three boli were composited in one polyethylene bag, sealed, labelled with cow ID and treatment number and placed on ice. Residual feed was removed from the feed manager and the next treatment offered. Boli collection procedures were followed for each treatment until all treatments had been offered to each cow. Excess boli for each treatment were removed from the rumen prior to the delivery of the next feed treatment.

Particle Size Analysis

The analysis is explained and described in the previous section (chapter 1, p. 29). The main objective was to analyse in detail particle lengths and distribution of particles ≥ 5 mm. Particles < 5 mm have been eliminated by sieving the samples through a 1.6 mm screen. We have determined proportional dry matter retained on the 1.6 mm (PROP_1.6) screen. We separated approximately 3 – 3.5 g of wet particles retained on the screen, by hand and analysed lengths of each particle with an image analysis technique. We calculated mean length (ML) of these particles considering only particles ≥ 5 mm.

Statistical Analysis

Statistical analyses were carried out with SAS (Version 9.1, SAS Inst. Inc., Cary, NC). The GLM procedure of SAS was used to test for differences between the chemical composition of treatment feeds.

Particle frequency by 1 mm lengths was calculated from the Matlab image analysis. Particle mean length (ML) of particles retained on the 1.6 mm sieve and ≥ 5 mm and standard error and statistical tests were determined using PROC LIFETEST, method Kaplan-Meier (KM). The distribution of 1 minus the cumulative frequency proportion as a function of length (l) was calculated. This distribution follows a failure time curve, as the cumulative distribution function (CDF) is related to the survival function as 1-S(t), where S(t) is the survival distribution function evaluated at time t, with length, l, substituted for t. The CDF represented the probability that a length did not exceed length l.

The GLM procedures of SAS was used to test for differences in PROP_1.6 and ML between treatment feed and respective bolus. Mean values of the three cows were considered for the testing. The model was set with PROP_1.6 and ML = provenance treatment source treatment*source. Source was either feed or bolus.

In order to evaluate effects of chemical parameters (DM, CP, aNDF) on particle size reduction during ingestive mastication, also samples tested in our previous studies were considered. In particular, chopped rye grass hay which was retained on a 19 mm screen, which had passed a 19 mm screen and was retained on a 8 mm screen, which had passed a 8 mm screen and was retained on a 1.18 mm screen, rye grass cut at 50 mm length and dried to hay, one grass silage, one corn silage and one TMR, and their respective boli from 4 dry and 4 lactating dairy cows were considered together with the results of the above described experiment. A test using the GLM procedures of SAS was performed on selected feeds, which had most similar particle size but different chemical characteristics (rye grass hay versus TMR fractions). In order to test the effect on bolus ML, feeds were assigned to two blocks, “long particles” and “mid size particles”. “Long particles” were from samples retained on a 19 mm screen and from samples which had passed a 19 mm screen and were retained on a 8 mm screen, with 13 observations from each feed source. “Mid size particles” were from samples which had passed a 8 mm screen and were retained on a 1.18 mm screen (rye grass hay) and from samples passing a 8 mm screen but retained on a 2.5 mm screen (TMR), with 8 observations from each feed source. The model was set with ML (feed and bolus), DM, CP, aNDF = feed source block feed source*block. In order to test the effect on bolus PROP_1.6, feeds were assigned to two blocks, “mid size particles” and “short particles”. “Short particles” were from samples passing a 1.18 mm screen but retained on the bottom pan (rye grass hay) and from samples passing a 2.5 mm screen but retained on a 1.18 mm screen (TMR), with 7 observations for each feed. The model was set with PROP_1.6 (feed and bolus), DM, CP, aNDF = feed source block feed source*block.

RESULTS AND DISCUSSION

Chemical composition of treatment feeds are reported in table 4.2. The unprocessed TMRs had in average 55.1 % DM and contained 7.7 (% DM) ash, 18.6 (%DM) CP and 31.7 (%DM) aNDF. DM increased from the longest particles retained on the 19 mm screen (51.8%) to the particles which had passed the 2.5 mm screen but were retained on the 1.18 mm screen (63.6%). The smallest particles which had passed all the screens had an intermediate DM of 56.7%. There was the same trend for CP, which increased from the longest particles retained on the 19 mm screen with 13.5 (% DM) to the particles which had passed the 2.5 mm screen but which were retained on the 1.18 mm screen with 24.0 (% DM). The smallest particles which had passed all the screens had an intermediate content of 21.6 (% DM) CP. The content of aNDF decreased from the longest particles with 48.4 (% DM) to the smallest retained on the bottom pan with 14.8 (% DM). Higher DM, CP and lower aNDF were measured generally in the smaller TMR particle fractions, because they contained more grain compared to the longer particles retained on the upper screens.

The figures 4.1 present the variability of particle lengths distribution within and between TMR treatments from image analysis. Image analysis was performed after elimination of small particles by sieving samples through a 1.6 mm screen. The treatment particle fraction which had passed all the screens of 19, 8, 2.5 and 1.18 mm and was retained on the bottom pan was not imaged. On this treatment we determined only the dry proportion of particles on a 1.6 mm screen. The 10 unprocessed TMRs (figure 4.1.a) contained in average 12.4% of particles < 5 mm. The majority of particles, which appeared at least at 1% on the 1.6 mm screen, were of lengths up to 27 mm. The imaged TMR fractions which were retained on the upper PSPS screen had 19.2% of particles < 5 mm and particle lengths which were represented at ≥ 1% were in a range between 9 and 49 mm (figure 4.1.b). The imaged TMR particles which had passed the 19 mm screen but were retained on a 8 mm screen had 9.6% of particles < 5 mm (figure 4.1.c). The majority of particles (≥ 1%) were of lengths up to 26 mm. The imaged TMR fractions which had passed the 8 mm screen but which were retained on a 2.5 mm screen, had 12.3% of particles < 5 mm and particle lengths which were represented at ≥ 1% were up to 16 mm (figure 4.1.d). The imaged TMR fractions which had passed the 2.5 mm screen and were retained on a 1.18 mm screen had 29.9% of particles < 5 mm (figure 4.1.e). The majority of particles which appeared at least at 1%, were of lengths up to 13 mm. The 10 unprocessed TMRs and the longer TMR fractions which were retained on the top and the second PSPS screen with 19 and 8 mm openings, respectively, had apparently a larger variation of particle lengths distributions within treatment compared to the shorter TMR fractions which were retained on the 2.5 and the 1.18 mm screens.

Figures 4.2 show the mean reduction of particle lengths during ingestive mastication. Particle % relative to total imaged particles, at individual lengths was plotted for both, treatment feed and respective bolus. Mean distribution within 3 mm intervals and means of three animals were plotted. Lengths distributions of imaged bolus particles, which were retained on a 1.6 mm screen, were very similar to respective distributions of imaged feed particles, when unprocessed TMRs were fed and when the smaller TMR fractions were fed, which were retained on a 2.5 and 1.18 mm screen (figures 4.2.a, 4.2.d, 4.2.e). The TMR fraction retained on a 19 mm screen had more particles in the imaged feed compared to the imaged bolus when particles were ≥ 23 mm (figure 4.2.b). The TMR fraction which had passed the 19 mm screen but was retained on a 8 mm screen had more particles in the imaged feed relative to the imaged bolus when particles were ≥ 11 mm (figure 4.2.e).

In figures 4.3 proportions of imaged bolus particles are plotted upon respective proportions of imaged TMR particles at equal length. These figures show both, reduction in particle lengths and correlation between feed and bolus particles. Average lengths distributions of imaged particles from the unprocessed TMRs and their respective boli are apparently not different from each other (figure 4.3.a). Less than 1% of imaged particles from unprocessed TMR were > 70 mm and less than 1% of imaged bolus particles were > 57 mm. Figure 4.3.b presents lengths distributions of the imaged TMR particles which were retained on a 19 mm screen and of the respective bolus. There was less correlation between feed and bolus particle proportions at individual lengths compared to the other TMR treatments. Proportions of imaged feed and bolus particles of lengths in a range between 3 and 22 mm were correlated with $R^2 = 0.16$ and particles of lengths from 23 to 66 mm were correlated with $R^2 = 0.56$. Also figure 4.3.b, like previously figure 4.2.b, shows, that there were more imaged feed particles from this longest TMR fraction relative to bolus particles when lengths were ≥ 23 mm. Less than 1% TMR particles from this longest fraction were longer than 108 mm and less than 1% of respective bolus particles were longer than 66 mm. Lengths distributions of imaged TMR particles which had passed the 19 mm screen and were retained on a 8 mm screen and the respective bolus particles are highly correlated with $R^2 = 0.87$ and $R^2 = 0.96$, within ranges of particle lengths between 1 – 13 mm and 14 – 37 mm, respectively (figure 4.3.c). At particle lengths of 1 – 11 mm, there were more bolus relative to TMR particles and at lengths of 12 – 37 mm particle proportions were higher in the feed compared to the bolus. Less than 1% of bolus particles were longer than 37 mm and less than 1% of respective TMR particles were longer than 44 mm. There was apparently very little reduction in particle size during ingestive mastication of TMR fractions which were retained on the 2.5 and 1.18 mm sieves (figures 4.3.d, 4.3.e). Less than 1% of both, feed and bolus particles were longer than 22 and 15 mm in the treatments retained on the 2.5 and 1.18 mm screens, respectively.

The table 4.3 reports PROP_1.6 and ML of treatment feeds and respective boli and also of fecal samples which had been collected from 5 cows which ate the unprocessed TMRs. The PROP_1.6 decreased from 0.83 in the longest TMR fraction which was retained on the 19 mm PSPS screen to 0.01 in the smallest TMR particles of the bottom pan. Unprocessed TMRs had an intermediate PROP_1.6 of 0.50. Bolus PROP_1.6 decreased from 0.69 to 0.02 with feeding TMR fractions decreasing in particle size. The chewed unprocessed TMR had a PROP_1.6 of 0.44. Particle ML decreased from 34.3 mm in the TMR fractions which were retained on the 19 mm screen to 7.2 mm in the fractions which had passed the 2.5 mm screen and were retained on the 1.18 mm screen. Respective bolus ML decreased from 18.3 to 7.2 mm. TMR particles retained on the bottom pan and respective bolus were not imaged. The longest ML of a TMR particle the cows were able to swallow was about 18 mm. This was nearly twice the longest ML of rye grass hay bolus particles, which could be swallowed at ML ≤ 10 – 11 mm (table 2.3). Unprocessed TMRs had a ML of 16.9 mm and a ML of 15.7 mm after chewing and ingestion. Ingestive chewing reduced PROP_1.6 of the unprocessed TMRs. Eating reduced also PROP_1.6 of TMR fractions retained on screens with openings of at least 2.5 mm, but not the TMR fractions passing that screen. Eating reduced ML of TMR fractions retained on screens with openings of at least 8 mm, but neither particles which had passed the 8 mm screen, nor ML of the unprocessed TMRs were reduced significantly ($p > 0.05$). TMR particles retained on a 2.5 mm screen were reduced only in PROP_1.6 but not in ML. During eating, the particles that short might be cut only at their edges. Lengths of these particles might be reduced only at a very small extent, but many small particles might be produced at the same time, which are able decrease PROP_1.6. Particles passing the 2.5 mm screen might not be cut at all and in consequence also PROP_1.6 might not decrease. According to Peréz-Barberìa and Gordon (1998) the number of chews has to increase exponentially for small particles to obtain the same rate of comminution as for larger particles. The authors explained that chewing effectiveness or particle size reduction was related to bite force and tooth morphological features such as occlusal surface area, occlusal contact area cutting enamel edges and enamel features.

In the present study, there was no reduction of particle size during eating when particles were retained on a 1.18 mm screen or were shorter to pass that screen. The PSPS according to Kononoff et al. (2003a) consist in three sieves without the 2.5 mm screen. The fraction of particles on the 1.18 mm screen reported in literature might have contained therefore also a fraction of longer particles which could be retained on a 2.5 mm screen. Particles retained on a 1.18 mm screen without an additional sieve might be less likely to stimulate chewing activity if the proportion of longer particles which could be captured with an additional sieve of 2.5 mm, is small. Kononoff and Heinrichs (2003) and Kononoff et al. (2003b) suggested using the sum of proportions of feed particles retained on a 1.18 mm, a 8 mm and a 19 mm screen to calculate parameters such as physical effective fiber (pef1.18) or NDF (peNDF1.18). However, the results of the present study show, that this proportion might overestimate physical effectiveness, as it might contain particles which are probably not stimulating chewing activity. Zebeli et al. (2006) reported considerable variation in chewing / DMI based on peNDF1.18. However, others didn't observe correlation between pef1.18 and chewing activity. Yang and Beauchemin (2007a) fed alfalfa silages differing in chop lengths. Treatment pef1.18 was not affected by chop length, but total chewing time / kg dry matter ingested (DMI) was higher when the diets with the longer silage particles were fed. Zebeli et al. (2008) didn't measure differences in peNDF1.18 either, when chop lengths of corn silage in the diet varied. We suggest the use of a sequential sieve set containing a 19 mm, a 8 mm sieve and a screen with 2.5 mm openings for diet evaluation on the farm, rather than a screen with 1.18 mm openings. This size is similar to the size Cotanch et al. (2010) suggested. The authors reported that the traditional PSPS method with two screens (Lammers et al., 1996) and an additional sieve with 3.18 mm openings was able to predict dry TMR proportions retained on a 1.18 mm screen using a vertical sieving technique.

The PSPS has been used in several research studies (table 1.2) to describe the physical effective particle fraction from the sum of sample proportions on two or three screens, independently from the feed source. Individual conserved forages as hay, haylage or silage, as well as TMRs were analysed with the same equipment and method. However, the results of the present and the previous study suggest, that the same PSPS fractions of either TMR particles or grass hay particles, might stimulate chewing to a different extent. This difference might be attributed, at least in part, to the fact that in our study longer hay particles were generally retained on each screen compared to TMR particles. However, as a difference to TMR particles, all rye grass hay fractions probably stimulated chewing to some extent. Even the smallest rye grass hay particles retained on the PSPS bottom pan were apparently reduced in PROP_1.6 (table 2.3) although this fraction is not defined physical effective (Kononoff and Heinrichs, 2003), whereas TMR particles stimulated chewing only when they were long enough to be retained on a 2.5 mm screen. The interpretation of feed particle size using only the mass proportions from one single sieving procedure might be not accurate. Individual forage at individual conservation procedures, as well as TMRs might need either individual sieving procedures differing in screen sizes, or one single procedure with probably more additional sieves and additional consideration of chemical parameters of the feeds to analyze. This suggestion is in agreement with Cotanch et al. (2010) who recommended the use of an additional sieve of either 3.18 mm or 4.76 mm to the traditional PSPS with 19 mm and 8 mm screens (Lammers et al., 1996), to measure pef1.18 of either corn silage or haycrop silage and TMR.

In our previous study we have observed that PROP_1.6 overestimated mass of particles ≥ 5 mm, especially for the samples with low dry matter residue on the 1.6 mm sieve, because also particles with lengths < 5 mm are retained on that screen. Table 4.4 reports the percentage of 1 – 4 mm feed and bolus particles retained on the 1.6 mm screen. We counted 12.4 and 13.5% of the imaged particles with lengths of 1 – 4 mm in the unprocessed TMR and respective bolus. We imaged in average 19.2%, 9.6% and 12.3% of 1 – 4 mm particles in the TMR fractions retained on the 19 mm, the 8 mm and the 2.5 mm screens, respectively, and 12.6%, 10.6% and 12.8% in the respective bolus samples. Highest percentages of small particles were observed in the TMR fraction retained on the 1.18 mm screen, with 29.9% in the TMR fraction and 32.7% in the respective bolus. Percentage of 1 – 4 mm particles (x) was related to dry matter proportion of particles retained on a 1.6 mm screen (y), with $R^2 = 0.52$ for 106 observations and $y = -27.09x + 31.253$. The relative mass proportions of these small particles are different from the numeric proportions assessed from image analysis. However, it is impossible to estimate the mass proportions from the counted particles and lengths distributions, also because of the variety of different feed ingredients with different densities in the TMRs used. The longer TMR particles might be composed mainly by forage, silage and hay, stover and whole corn kernels, by some pellets and by-product flakes. The shorter particles might be composed less by forage fragments but more by grain, concentrates and by-product fragments. Comparing grain particles and forages of a same size, the grain particles might be heavier compared to forages. On the other hand, this difference might be less important for silages particles which contain more water relative to grain. We eliminated probably more grain and less small forage particles with our wet sieving procedure. Grain fragments are heavier and were more likely to sink through the 1.6 mm sieve openings, whereas the forage particles floated on top of the water. In our study, the smaller fragments retained on the 1.6 mm screen were probably lighter compared to the longer ones. Table 4.4 reports a weighted percentage of imaged feed and bolus particles of 1 – 4 mm lengths. Individual particles were weighted by their lengths and the calculated percentage of 1 – 4 mm particles was referred to the weighted total. The weighted percentage of 1 – 4 mm particles (x) was also related to the dry matter proportion of particles retained on a 1.6 mm screen (y), with $R^2 = 0.64$ for 106 observations and $y = -20.395x + 16.257$. With exception of the treatment with particles retained on the 1.18 mm screen, the weighted percentage of particles of 1 – 4 mm lengths, of all other treatments and respective boli was less than 5 %. However, the particle fraction passing the 2.5 mm screen was not reduced in particle size anyway, and a description of particle size in that fraction might not be necessary. Mass of feed and bolus particles ≥ 5 mm were overestimated by PROP_1.6, but to a similar extent. The percentages, as well as the weighted percentages of small particles, were similar for both feed and respective bolus samples.

Figures 4.4 summarize all data, including the observations from our previous study, relative to particle size reduction, PROP_1.6 (figure 4.4.a) and ML (figure 4.4.b) of feed and respective bolus particles. The black diamonds represent unprocessed TMRs and TMR fractions. The empty triangles represent silages and crosses and stars were rye grass hay particles. Feed and bolus PROP_1.6 were highly correlated ($R^2 = 0.94$, 65 observations), when unprocessed TMRs and TMR fractions were fed, with $y = 0.79x + 0.03$ and y being bolus and x feed PROP_1.6. Rye grass hay particles were apparently chewed more intensely. Bolus PROP_1.6 of rye grass hays were apparently lower compared to TMR bolus PROP_1.6. Particles with ML > 20 mm were apparently chewed to constant lengths. Chewing intensity of these particles might depend on the size at which particles can be swallowed and this critical size might depend also on physical and chemical properties of the feed (figure 4.4.b). Rye grass hay particles were dry, whereas TMR treatments contained 36 – 48% water (table 4.2). Rye grass hay particles contained 12 – 14 (% DM) CP and 54 – 59 (% DM) aNDF, whereas CP and aNDF of TMRs were 14 – 24 and 20 – 48 (% DM), respectively (table 2.2., table 4.2). Longest average bolus ML from TMR feeding was approximately 18 - 19 mm, whereas longest average ML from rye grass hay feeding was approximately 10 – 11 mm. Higher water content and a lower aNDF content of TMR particles compared to rye grass hay particles might allow bending of particles and the swallowing of longer particles in consequence. Importance of chemical and physical properties seemed to decrease with feed particle size when particle ML ≤ 20 mm. Feed and bolus ML were highly correlated ($R^2 = 0.86$, 47 observations), when unprocessed TMRs and TMR fractions were fed and feed ML ≤ 20 mm, with $y = 0.76x + 2.08$ and y being bolus and x feed ML. When rye grass hay was fed and feed ML ≤ 20 mm, feed and bolus ML were correlated with $R^2 = 0.43$ at 21 observations, with $y = 0.24x + 6.13$ and y being bolus and x feed ML.

In order to statistically test chemical effects (DM, CP and aNDF) on bolus ML, feeds were assigned to two blocks, “long particles” and “mid size particles”. “Long particles” were from samples retained on a 19 mm screen and from samples passing a 19 mm screen but retained on a 8 mm screen, with 13 observations from each feed source (rye grass hay or TMR). “Mid size particles” were from samples passing a 8 mm screen but retained on a 1.18 mm screen (rye grass hay) and from samples passing a 8 mm screen but retained on a 2.5 mm screen (TMR), with 8 observations from each feed source. Subsamples were selected in order to provide most similar particle size and different chemical parameters (rye grass hay versus TMR) within each block. Rye grass hay ML was 19.7 mm and TMR ML 19.6 mm. Rye grass hay DM was approximately 33% higher, CP 5.7 (% DM) lower and aNDF 16.8 (% DM) higher compared to TMR. Rye grass hay and TMR ML were not different ($p > 0.05$), but respective rye grass hay bolus ML was 4.5 mm shorter than TMR bolus ML ($p < 0.01$) (table 4.5.a). The same attempt was made in order to select subsamples to evaluate effects of chemical parameters on bolus PROP_1.6. Feeds were assigned to two blocks, “mid size particles” and “short particles”. “Short particles” were from samples passing a 1.18 mm screen but retained on the bottom pan (rye grass hay) and from samples passing a 2.5 mm screen but retained on a 1.18 mm screen (TMR), with 7 observations for each feed. Chemical parameters differed between TMR and rye grass hay samples. Rye grass hay DM was approximately 27.8% higher, CP 10.9 (% DM) lower and aNDF 28.8 (% DM) higher compared to TMR. The selected subsamples provided also different PROP_1.6 of rye grass hays and TMRs ($p < 0.01$). Rye grass hay had a PROP_1.6 of 0.57 and TMR of 0.48. However, rye grass hay bolus PROP_1.6 did not differ significantly from TMR bolus PROP_1.6 ($p > 0.05$). During eating, cows reduced rye grass hay PROP_1.6 by 12% more compared to TMR PROP_1.6 (table 4.5.b). Figures 4.5 illustrate the effects of DM (figure 4.5.a) and aNDF (figure 4.5.b) on bolus ML and figures 4.6 on bolus PROP_1.6. The same subsamples which were selected for the statistical test of chemical effects on particle size reduction, were plotted. In figures 4.5, empty triangles were “long TMR particles”, full triangles were “long rye grass hay particles”. Triangles had an average ML of approximately 30 mm. Empty circles were “mid size TMR particles” and full circles “mid size rye grass hay particles” Circles had an average ML of 9.5 mm. Both parameters, DM and aNDF, were negatively correlated to bolus ML of “long particles” as well as to “mid size particles”. However, chemical parameters had more impact on the reduction of “long particles”. In figures 4.6, empty triangles were “mid size TMR particles” with average PROP_1.6 of 0.71, full triangles were “mid size rye grass particles” with average PROP_1.6 of 0.80. Empty circles were “short TMR particles” with an average PROP_1.6 of 0.23 and full circles were “short rye grass hay particles” with an average PROP_1.6 of 0.34. Chemical parameters had apparently higher impact on bolus PROP_1.6 in the “short particles” compared to the “mid size particles”. Only in the “short particles” there was a trend ($p < 0.10$) for reduced PROP_1.6 when DM or NDF increased. Feed particles higher in NDF and DM were chewed more intensely. The longer the particles the higher the impact on lengths of particles which are retained on a 1.6 mm screen. During eating, smaller particles might be cut more likely at their edges. Those particles won’t be reduced much in their lengths. However, more particles small enough to pass the 1.6 mm screen might be produced.

It was difficult to distinguish individual effects of chemical parameters on particle size reduction during eating, because all parameters were correlated to each other. In figure 4.7, we tried to subsample feeds which were similar in feed ML, CP and NDF, but which were different in DM. The figure 4.7 shows two plots of bolus ML upon feed ML. Empty diamonds were TMR samples with an average DM of 53%, an average aNDF of 47.5 (% DM) and an average CP of 13.0 (% DM). Full diamonds were rye grass hay samples with an average DM of 89.9%, an average aNDF of 51.0 (% DM) and an average CP of 12.8 (% DM). For both sample sets, bolus ML increased significantly ($p < 0.05$) when feed ML increased. TMR samples had always the longer bolus particles. Using the regression formulas, we calculated differences between the two sample sets, in bolus ML, of 5.1 mm and 8.9 mm inserting the extreme TMR particle lengths of the sample set of 14.7 and 43.7 mm. In this range of particle lengths, differences in dry matter between rye grass hays and TMRs, increased also from 25.2% at 14.7 mm particle lengths to 43.0% at 43.7 mm particle lengths. For each % decrease in sample DM bolus ML increased approximately 0.2 mm.

CONCLUSIONS

The longest ML of a TMR particle the cows were able to swallow was about 18 mm. This was nearly twice the longest ML of rye grass hay bolus particles, which could be swallowed at ML ≤ 10 – 11 mm.

Ingestive chewing reduced PROP_1.6 of the unprocessed TMRs. Eating reduced also PROP_1.6 of TMR fractions retained on screens with openings of at least 2.5 mm, but not the PROP_1.6 of TMR fractions passing that screen. Eating reduced ML of TMR fractions retained on screens with openings of at least 8 mm, but neither ML of particles which had passed the 8 mm screen, nor ML of the unprocessed TMRs were reduced significantly ($p > 0.05$). The sum of TMR residues on the two upper PSPS screens with 19 and 8 mm openings might underestimate TMR pef, whereas the sum of residues from all three PSPS screens might overestimate TMR pef. We suggest the use of a sequential sieve set containing a 19 mm, a 8 mm sieve and a screen with 2.5 mm openings for diet evaluation on the farm, rather than a screen with 1.18 mm openings.

Feed and bolus PROP_1.6 were highly correlated ($R^2 = 0.94$, 65 observations), when unprocessed TMRs and TMR fractions were fed, with $0.79x + 0.03$ and y being bolus and x feed PROP_1.6. Rye grass hay particles were apparently chewed more intensely and having lower PROP_1.6. Feed and bolus ML were highly correlated ($R^2 = 0.86$, 47 observations), when unprocessed TMRs and TMR fractions were fed and feed ML ≤ 20 mm, with $y = 0.76x + 2.08$ and y being bolus and x feed ML. When rye grass hay was fed and feed ML ≤ 20 mm, feed and bolus ML were correlated with $R^2 = 0.43$ at 21 observations, with $y = 0.24x + 6.13$ and y being bolus and x feed ML. Rye grass hay and TMR particles with ML > 20 mm were apparently chewed to constant lengths. Chopped rye grass hay particles with ML > 20 mm were particles retained on the 19 and the 8 mm PSPS screen, whereas TMR with ML > 20 mm were retained only on the 19 mm screen. Only particle size of feeds with ML under this threshold might be able to influence parameters such as rumen retention time, intake and rumen degradation of feed, if these parameters were related to bolus particle size.

Rye grass hay was chewed more intensely compared to TMR particles. Rye grass hay particles were dry, whereas TMR treatments contained 36 – 48% water. Rye grass hay particles contained 12 – 14 (% DM) CP and 54 – 59 (% DM) aNDF, whereas CP and aNDF of TMRs were 14 – 24 and 20 – 48 (% DM), respectively. During eating, chemical parameters influenced more reduction of ML of longer compared to shorter particles, but reduction of PROP_1.6 was more affected in the shorter particles. For each % decrease in sample DM bolus ML increased approximately 0.2 mm, under the particular condition where feed particles ML ranged between 14.7 and 43.7 mm, CP content ranged between 12.8 and 13 (% DM) and aNDF content ranged between 47.5 and 51 (% DM).

Figures 4.1. Variability of particle lengths distribution within TMR treatments – *Image analysis* of (a – e) after elimination of small particles by sieving through a 1.6 mm screen.

Figure 4.1.a. ▩ Unprocessed TMR.

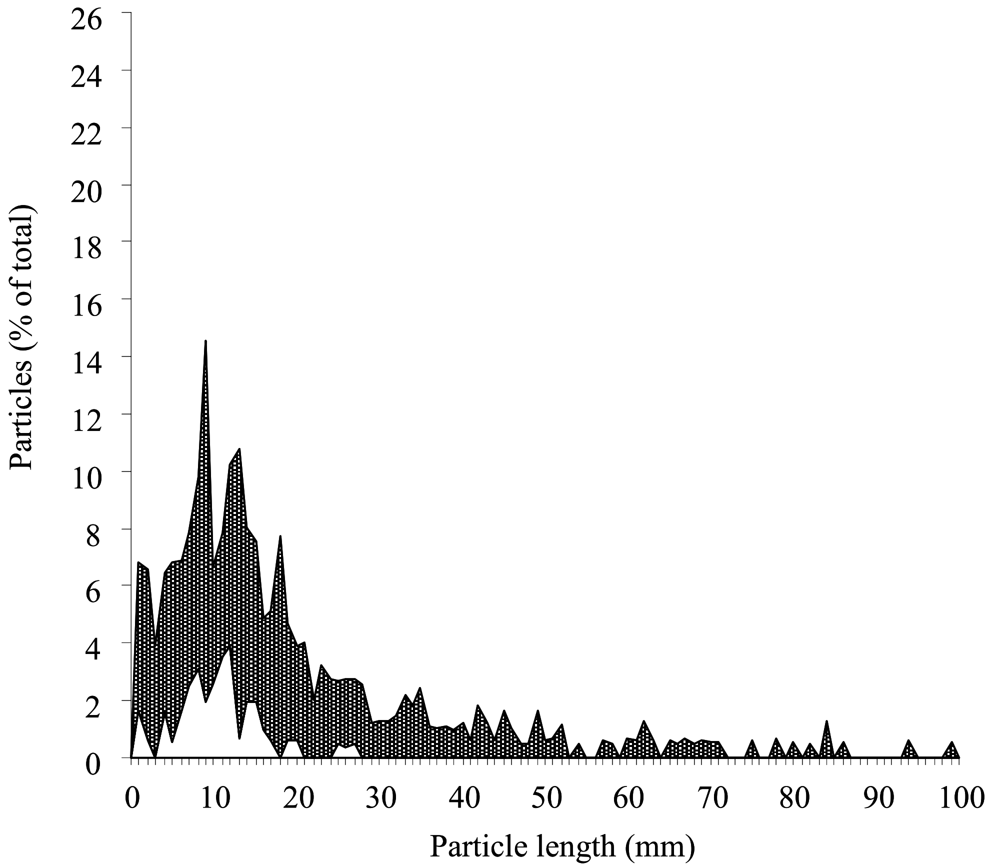


Figure 4.1.b. TMR particles retained on a 19 mm screen.

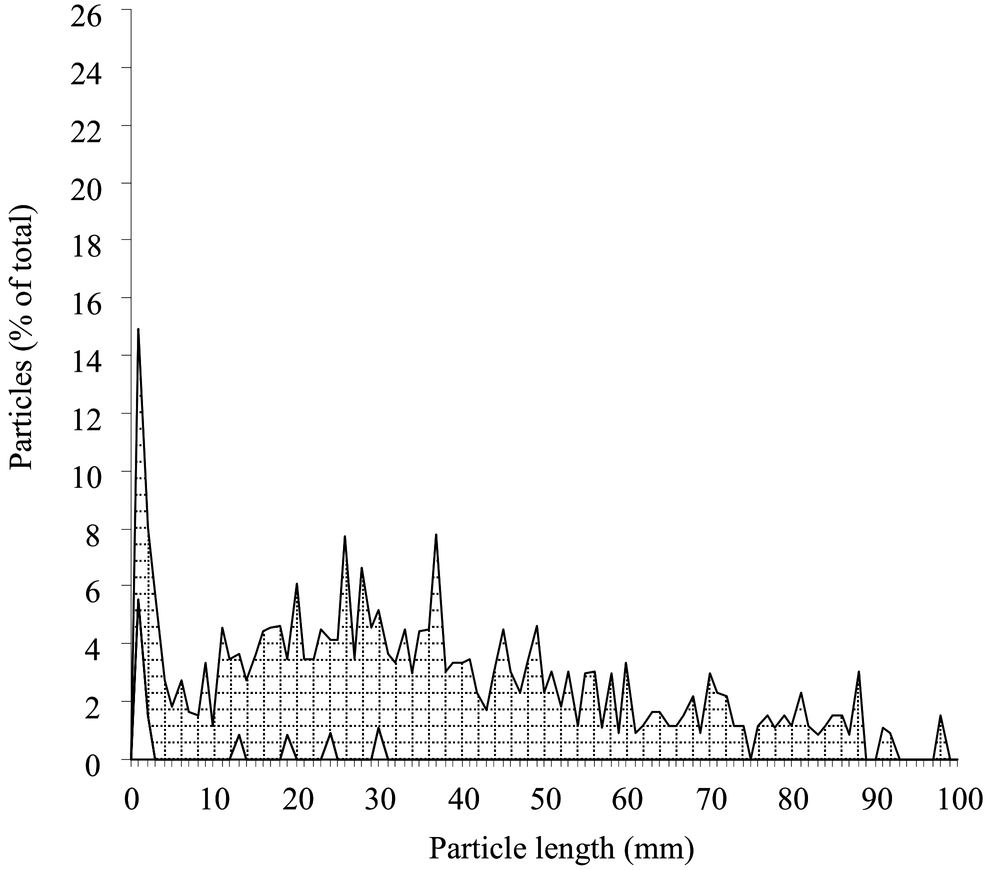


Figure 4.1.c. TMR particles passing a 19 mm screen but retained on a 8 mm screen.

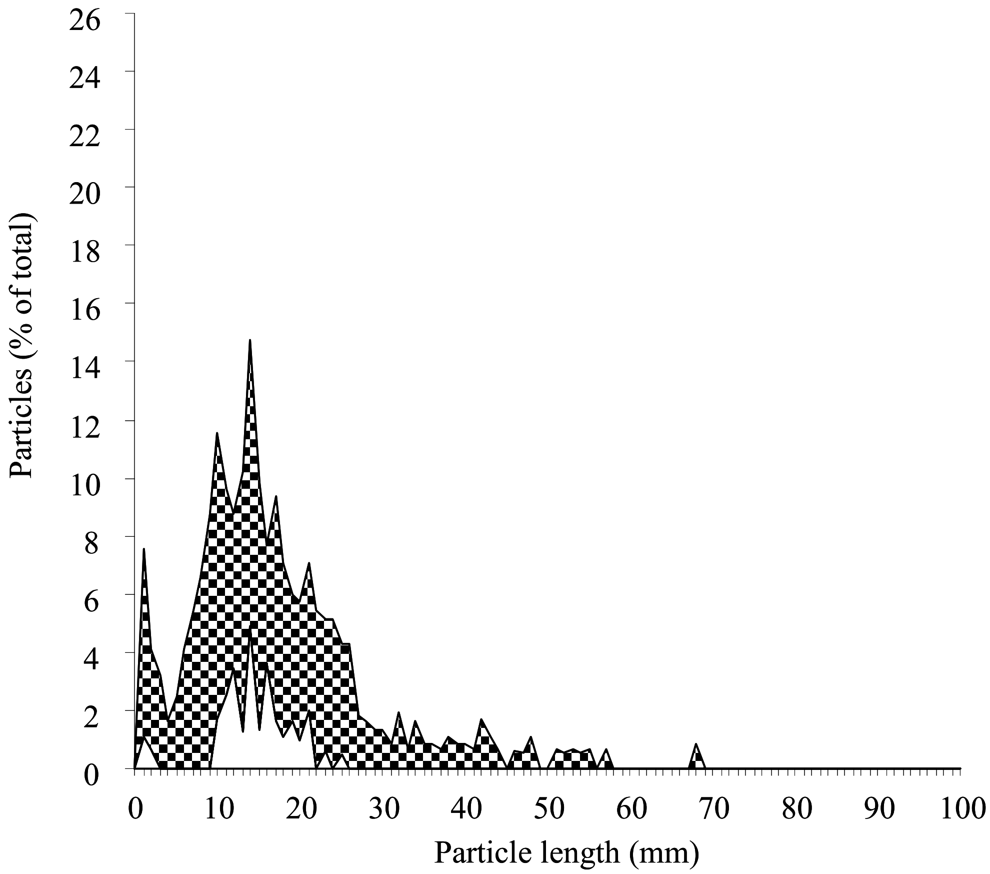


Figure 4.1.d. TMR particles passing a 8 mm screen but retained on a 2.5 mm screen.

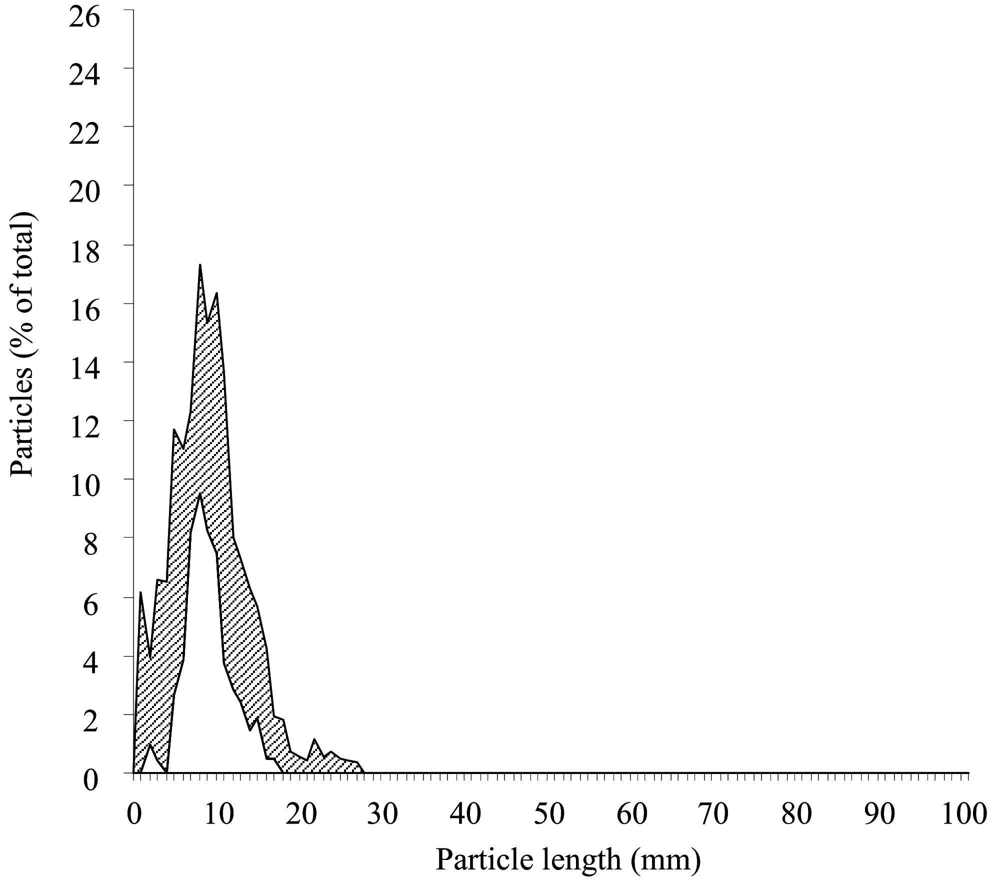
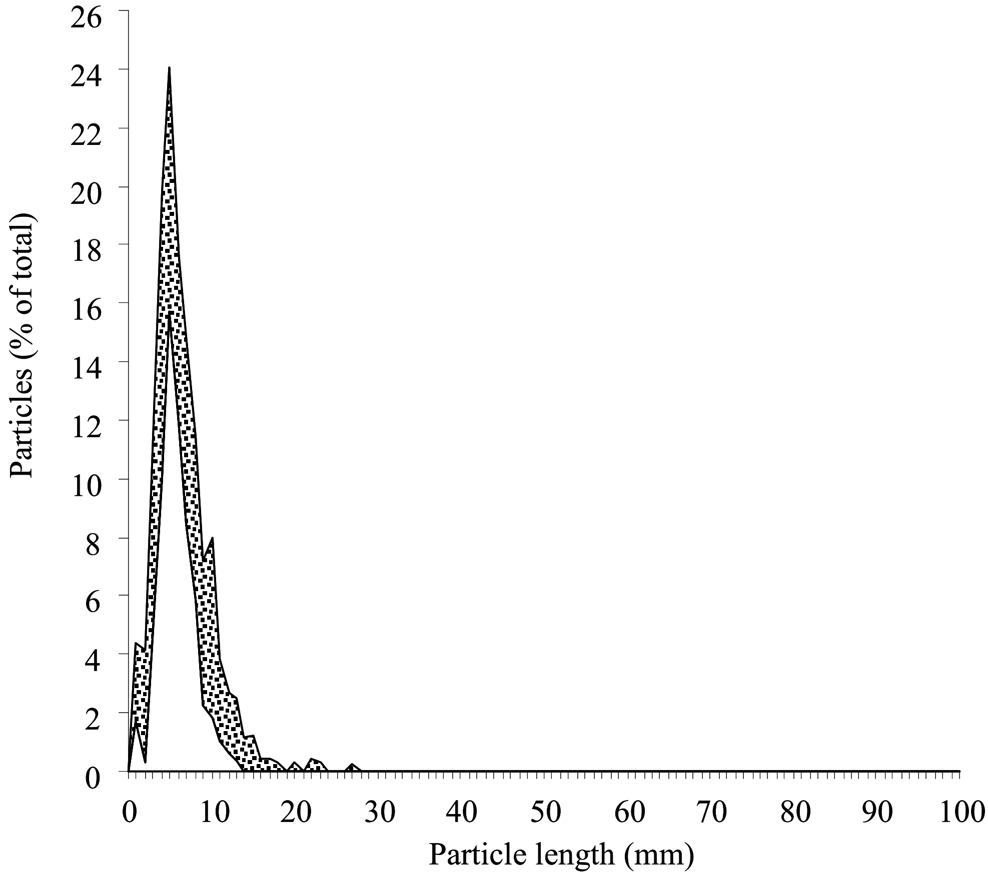


Figure 4.1.e. TMR particles passing a 2.5 mm screen but retained on a 1.18 mm screen.



Figures 4.2. Mean* reduction of particle lengths during ingestive mastication – *Image analysis* of individual treatment TMR particles and respective boli (a – d) after elimination of small particles by sieving through a 1.6 mm screen.

* Mean distribution within 3 mm intervals and, regarding the boli, means of three animals were considered.

Figure 4.2.a. ▥ Unprocessed TMR.

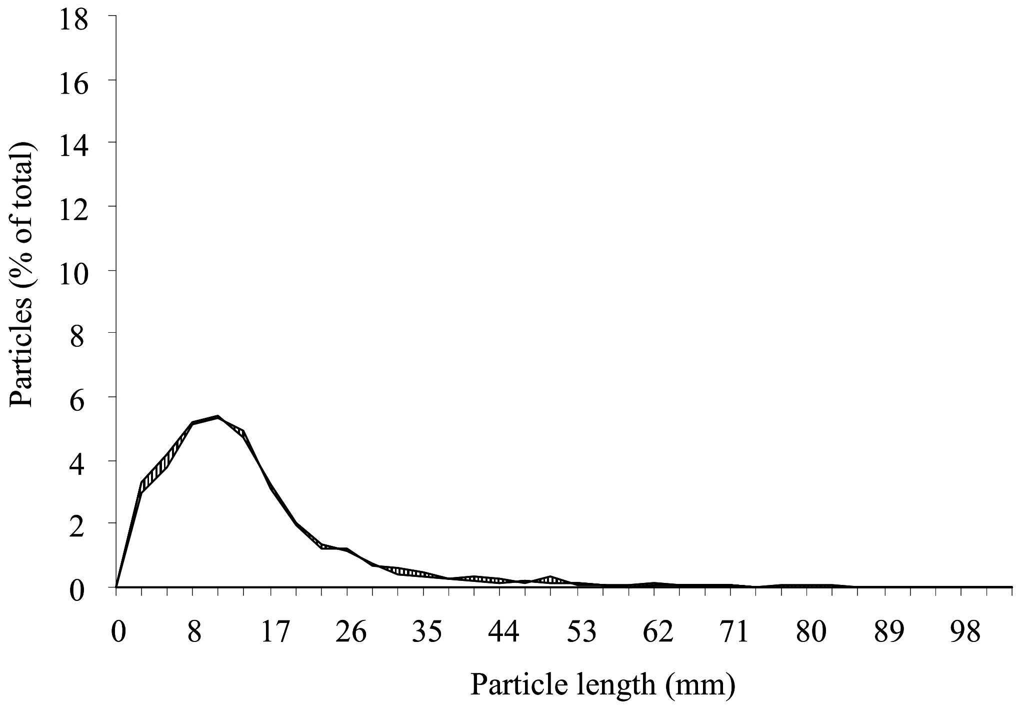


Figure 4.2.b. TMR particles retained on a 19 mm screen.

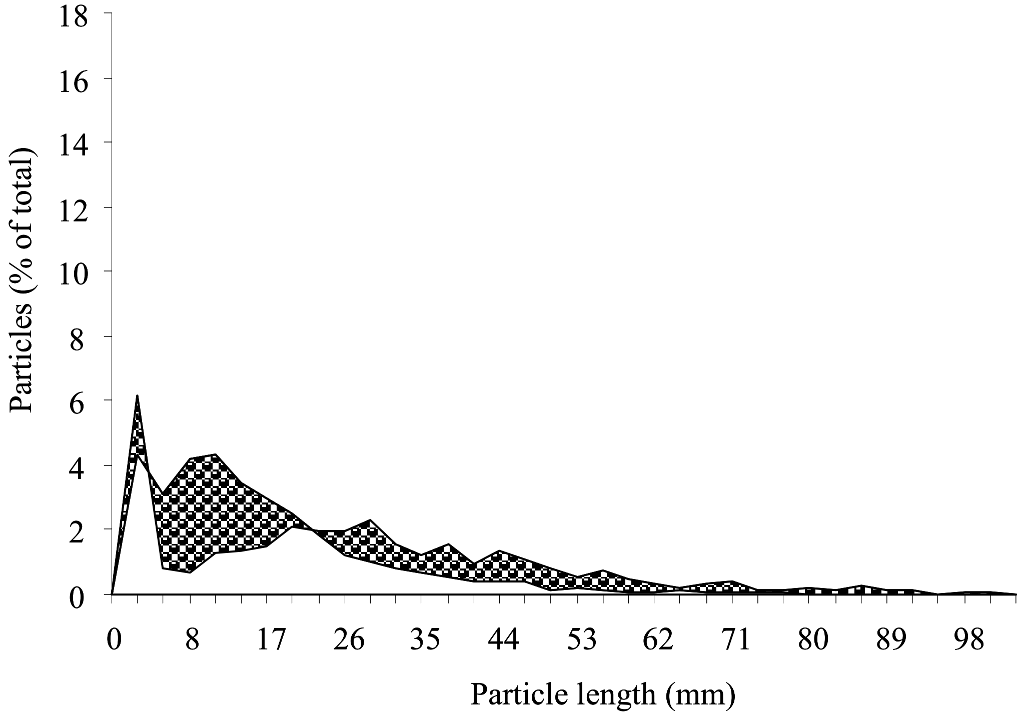


Figure 4.2.c. TMR particles passing a 19 mm screen but retained on a 8 mm screen.

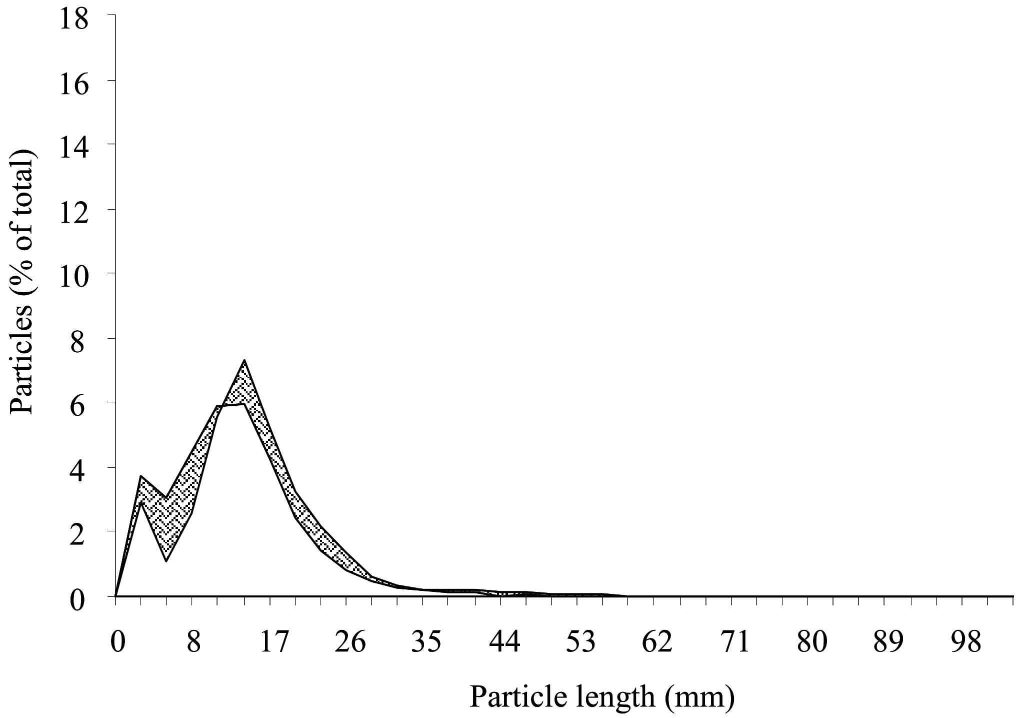


Figure 4.2.d. TMR particles passing a 8 mm screen but retained on a 2.5 mm screen.

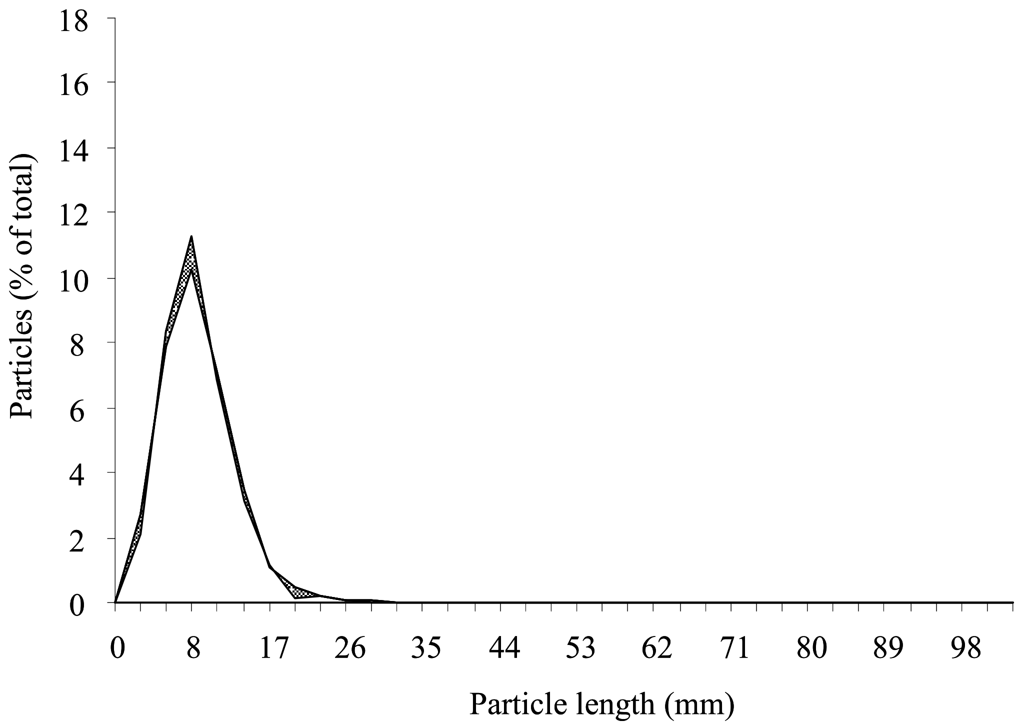
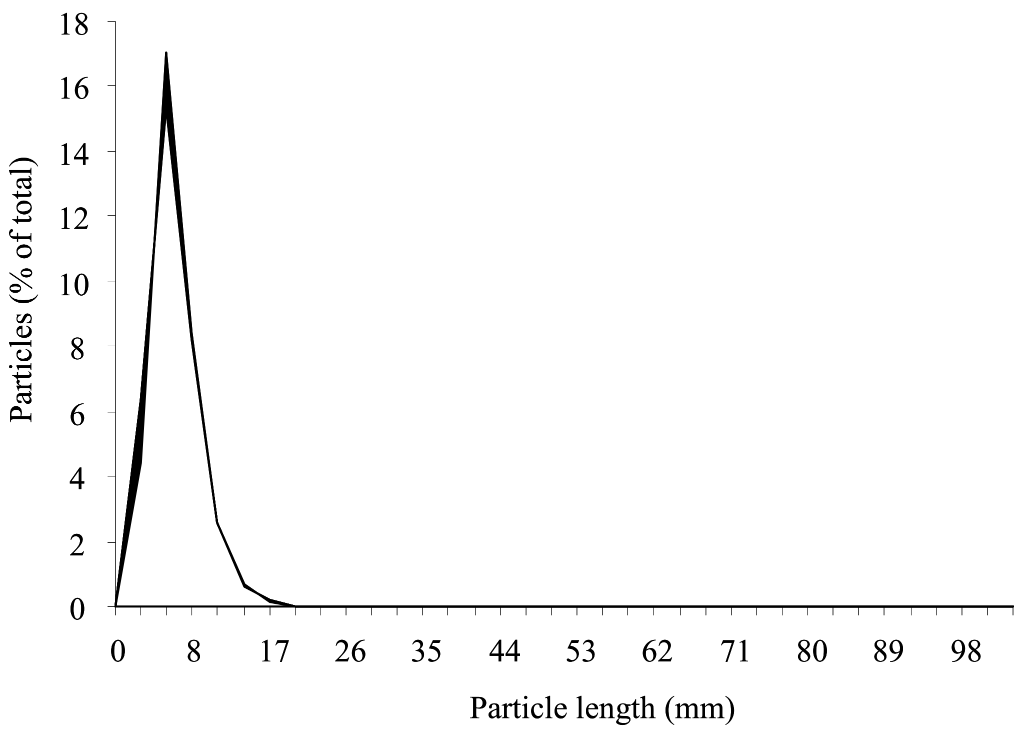


Figure 4.2.e. TMR particles passing a 2.5 mm screen but retained on a 1.18 mm screen.



Figures 4.3. Lengths distribution of individual TMR treatment particles relative to respective bolus particles - *Image analysis* after elimination of small particles by sieving through a 1.6 mm screen.

Figure 4.3.a. Unprocessed TMR.

○ 1 – 57 mm particles; y = 0.9968x + 0.0002; R^2 = 0.96; n = 57.

Less than 1% bolus particles were longer than 57 mm.

▲ 58 - 70 mm particles. Less than 1% feed particles were longer than 70 mm.

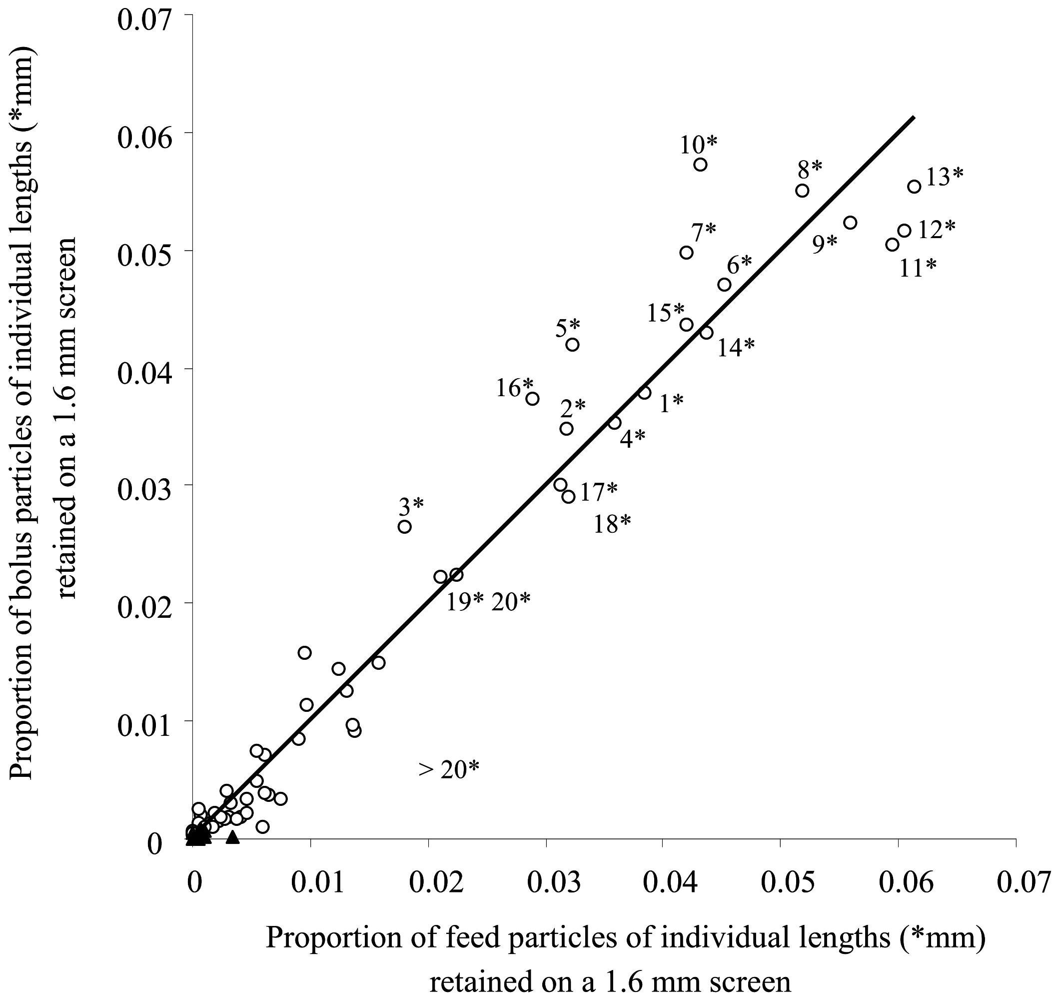


Figure 4.3.b. TMR particles retained on a 19 mm screen.

□ 1 – 2 mm particles.

◆ 3 – 22 mm particles; y = -0.532x + 0.0401; R^2 = 0.16; n = 20.

○ 23 – 66 mm particles; y = 0.4675x - 0.0002; R^2 = 0.56; n = 44.

Less than 1% bolus particles were longer than 66 mm.

▲ 67 - 108 mm particles. Less than 1% feed particles were longer than 108 mm.

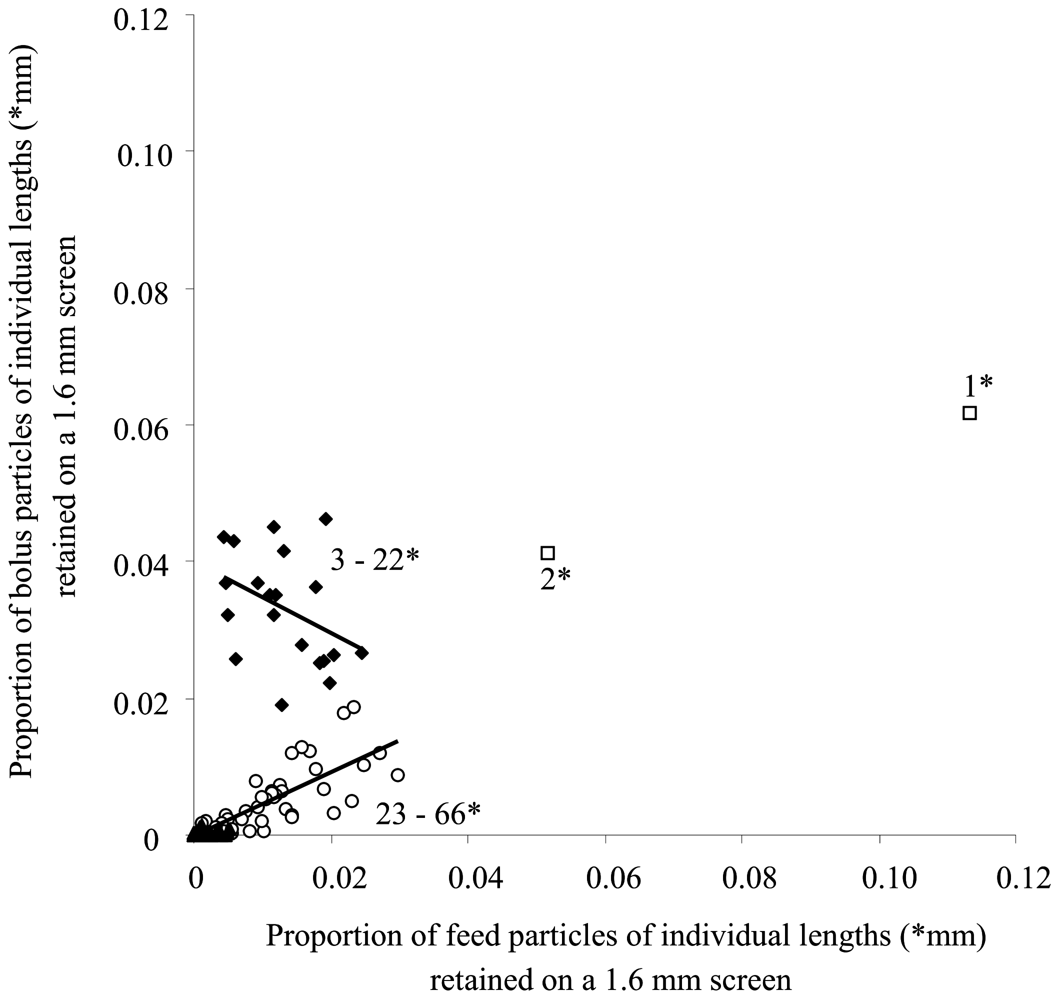


Figure 4.3.c. TMR particles passing a 19 mm screen but retained on a 8 mm screen.

◆ 1 – 13 mm particles; $y = 0.6011x + 0.0244$; $R^2 = 0.87$; $n = 13$.

○ 14 – 37 mm particles; $y = 0.7729x - 0.0003$; $R^2 = 0.96$; $n = 24$.

Less than 1% bolus particles were longer than 37 mm.

▲ 38 - 44 mm particles. Less than 1% feed particles were longer than 44 mm.

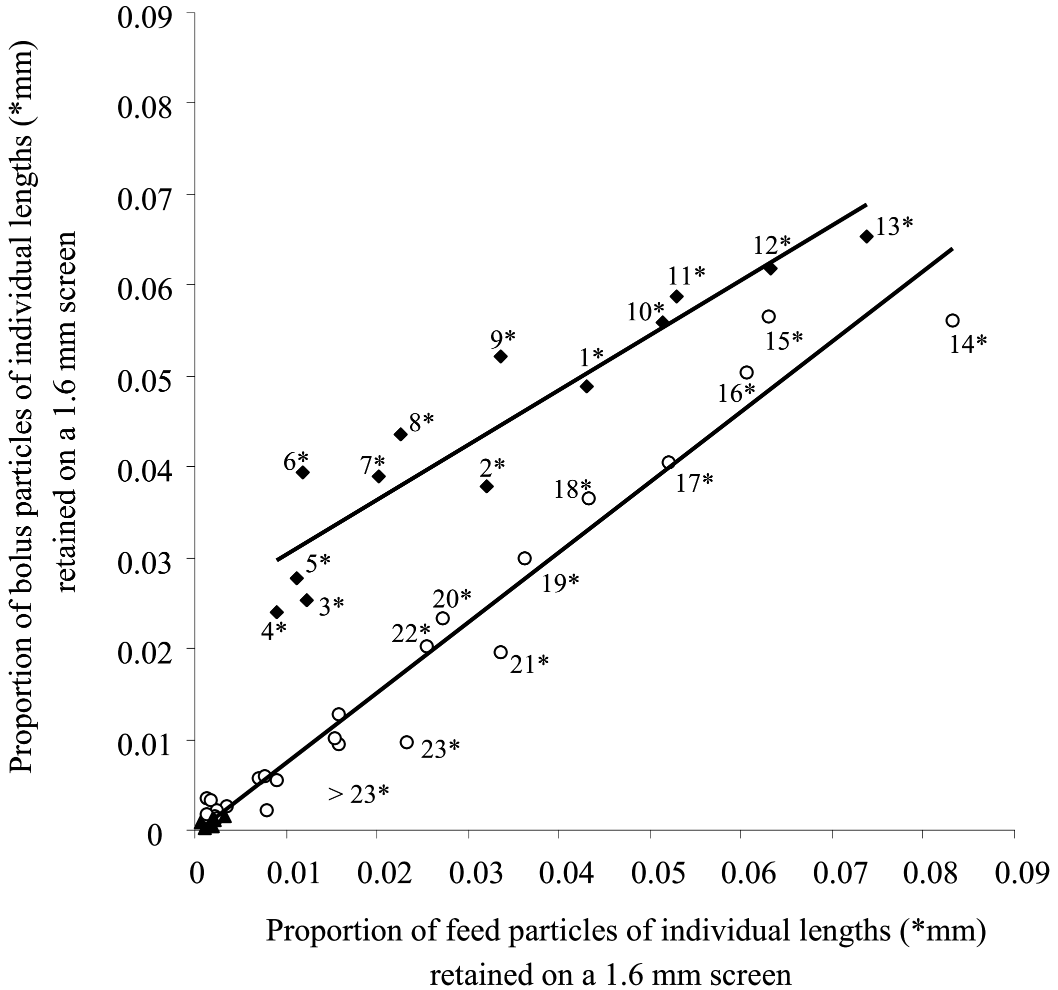


Figure 4.3.d. TMR particles passing a 8 mm screen but retained on a 2.5 mm screen.

◆ 1 – 22 mm particles; y = 0.8878x + 0.005; R^2 = 0.98; n = 22.

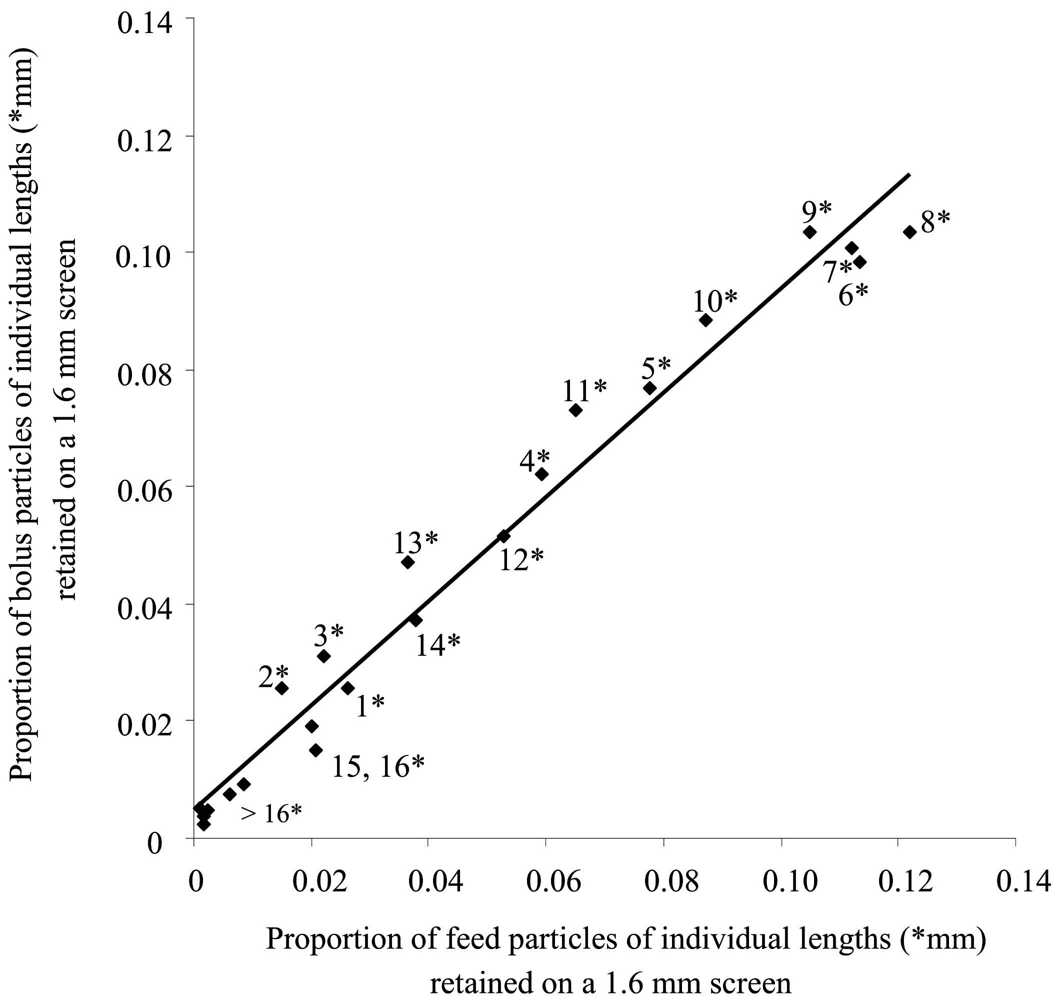
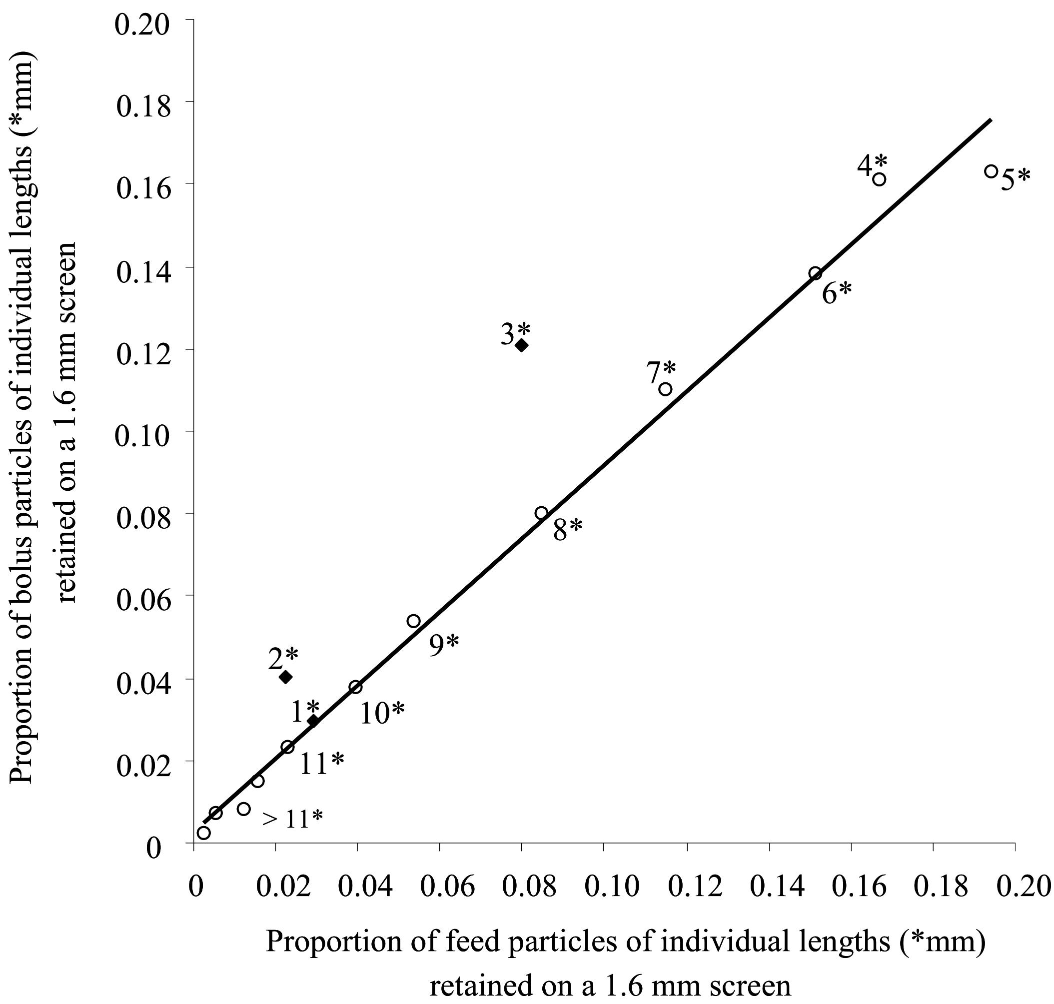


Figure 4.3.e. TMR particles passing a 2.5 mm screen but retained on a 1.18 mm screen.

◆ 1 – 3 mm particles.

○ 4 – 15 mm particles; y = 0.893x + 0.0025; R^2 = 0.99; n = 12.



Figures 4.4. Overall particle size reduction during the ingestive mastication.

Figure 4.4.a. Feed versus bolus dry matter proportions of particles retained on a 1.6 mm screen.

◆ TMR samples, y = 0.79x + 0.03; R^2 = 0.94; n = 65.

△ Corn silage and grass silage samples.

× Rye grass hay samples measured in the previous studies.

⁎ Rye grass hay samples from the previous studies which were not sieved.

○ Fecal samples, dry matter proportion of particles retained on a 1.6 mm screen (mean ± standard deviation) = 0.12 ± 0.04.

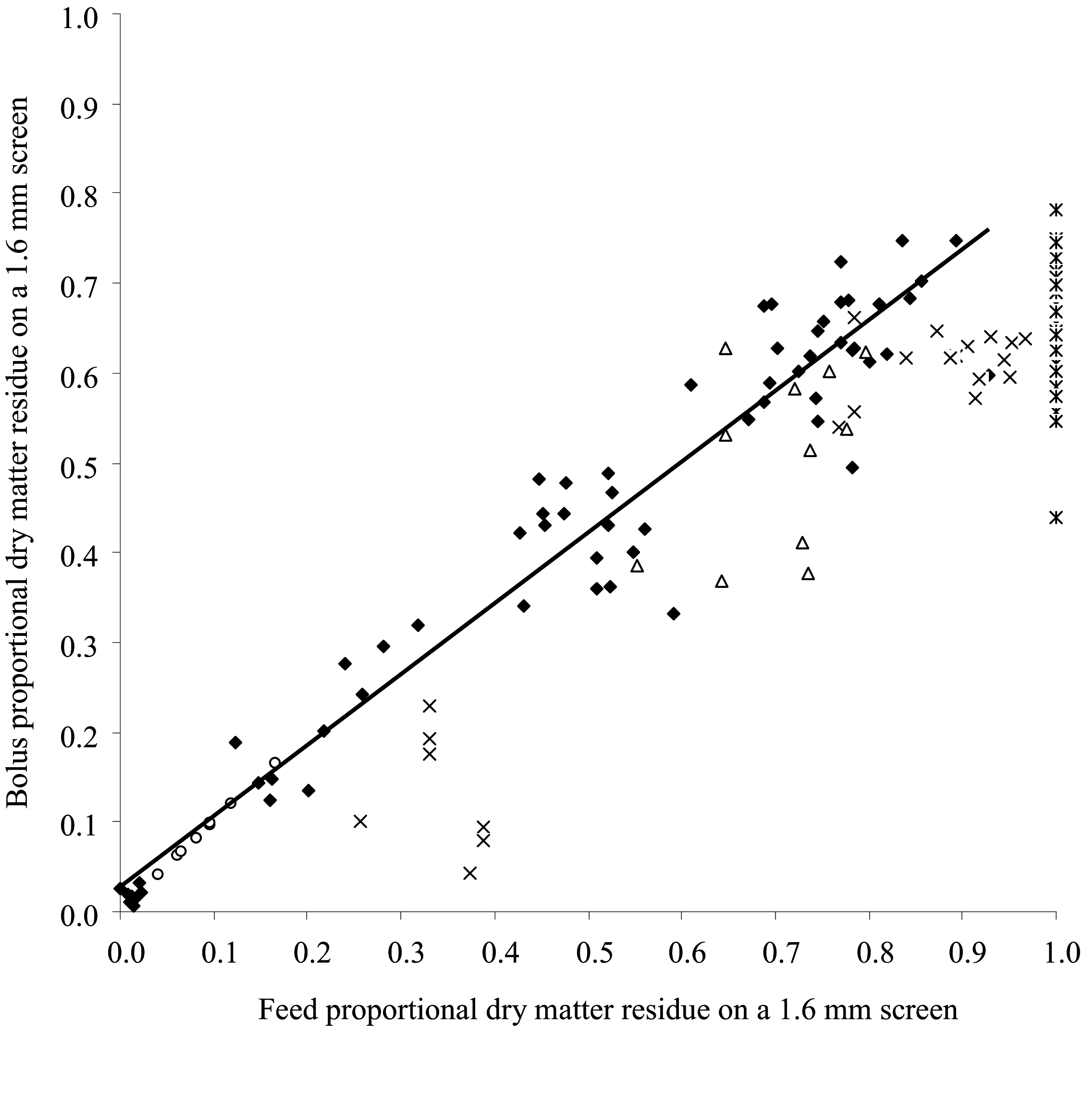


Figure 4.4.b. Feed versus bolus mean lengths. Mean lengths were calculated from particles retained on a 1.6 mm screen and ≥ 5 mm.

◆ TMR samples, mean length ≤ 20 mm, y = 0.76x +2.08; R^2 = 0.86; n = 47.

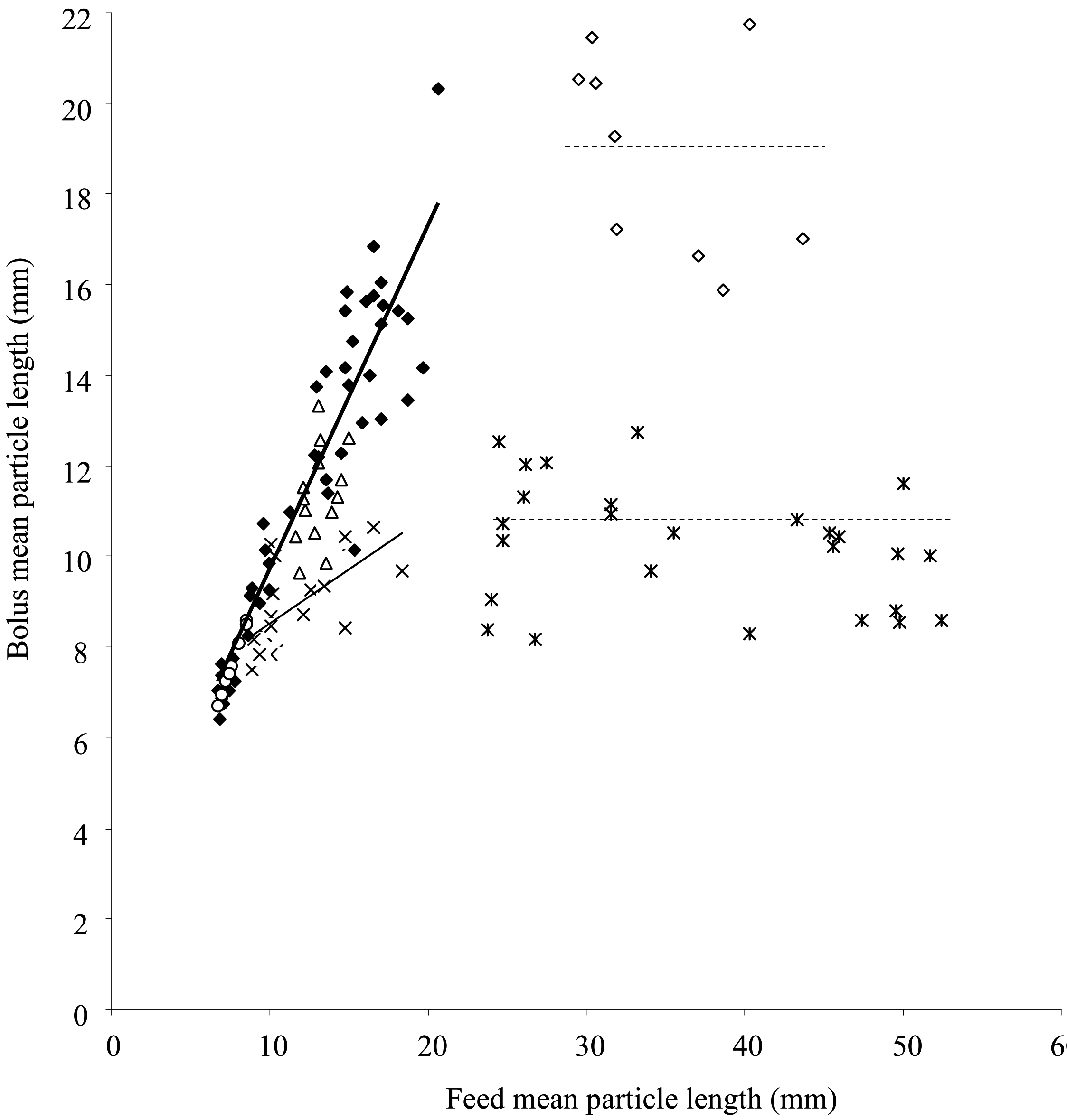
◇ TMR samples, mean length > 20 mm, bolus length (mean ± standard deviation) = 18.9 ± 2.3 mm

△ Corn silage and grass silage samples.

× Rye grass hay samples measured in the previous studies, mean length ≤ 20 mm, y = 0.24x + 6.13; R^2 = 0.43; n = 21.

⋇ Rye grass hay particles from the previous studies, mean length > 20 mm, bolus length (mean ± standard deviation) = 10.2 ± 1.4 mm.

○ Fecal particles, length (mean ± standard deviation) = 8.0 ± 0.80 mm.



Figures 4.5. Effect of chemical parameters (dry matter – DM, neutral detergent insoluble fiber – aNDF) on bolus mean particle length* (mm) relative to feed particle size.

Figure 4.5.a. Effect of feed DM on bolus mean particle length*.

△ TMR samples, feed particle length* (mean ± standard deviation) = 29.7 ± 9.1 mm

▲ Rye grass hay samples, feed particle length* (mean ± standard deviation) = 29.8 ± 7.8 mm

$y = -0.2064x + 28.777$, $R^2 = 0.86$, $n = 26$.

○ TMR samples, feed particle length* (mean ± standard deviation) = 9.5 ± 0.5 mm

● Rye grass hay samples, feed particle length* (mean ± standard deviation) = 9.5 ± 0.5 mm

$y = -0.0541x + 13.149$, $R^2 = 0.53$, $n = 16$.

* Mean length of particles retained on a 1.6 mm screen and ≥ 5 mm.

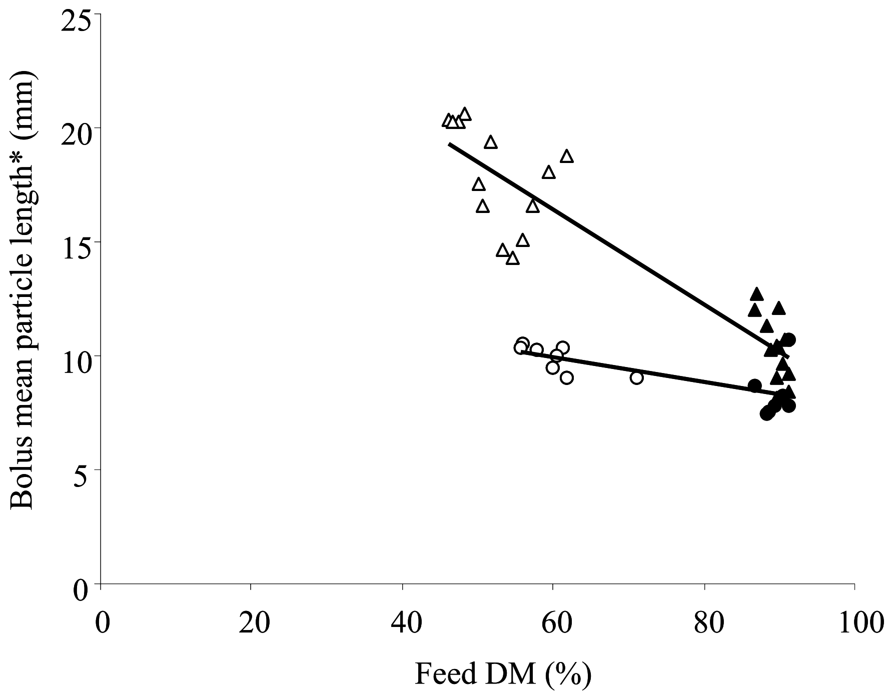


Figure 4.5.b. Effect of feed aNDF on bolus mean particle length*.

△ TMR samples, feed particle length* (mean ± standard deviation) = 29.7 ± 9.1 mm

▲ Rye grass hay samples, feed particle length* (mean ± standard deviation) = 29.8 ± 7.8 mm

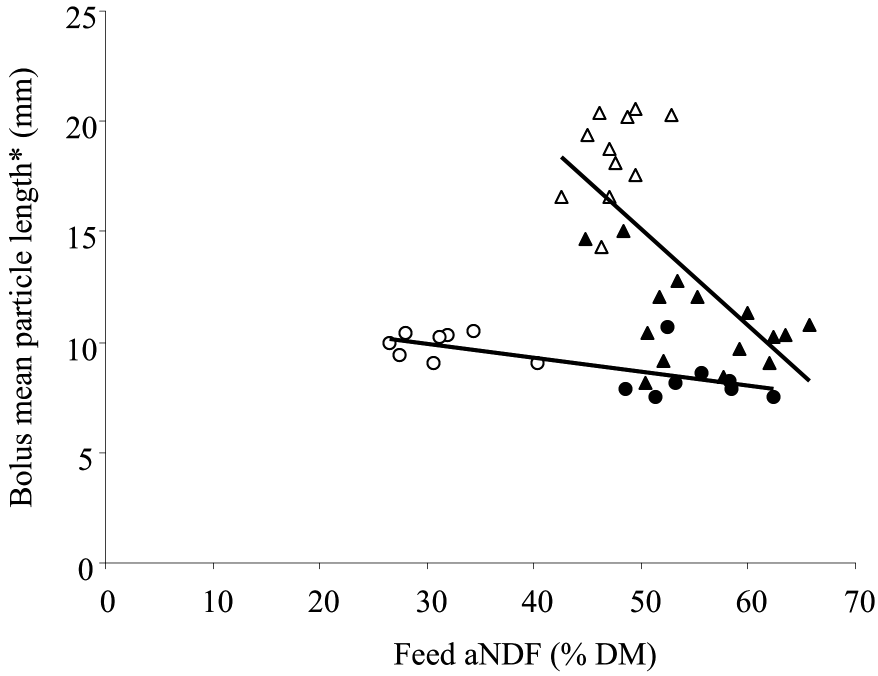
$y = -0.4379x + 37$, $R^2 = 0.44$, n = 26.

○ TMR samples, feed particle length* (mean ± standard deviation) = 9.5 ± 0.5 mm

● Rye grass hay samples, feed particle length* (mean ± standard deviation) = 9.5 ± 0.5 mm

$y = -0.0632x + 11.812$, $R^2 = 0.51$, n = 16.

* Mean length of particles retained on a 1.6 mm screen and ≥ 5 mm.



Figures 4.6. Effect of chemical parameters (dry matter – DM, neutral detergent insoluble fiber – aNDF) on bolus dry matter proportions on a 1.6 mm screen relative to feed particle size.

Figure 4.6.a. Effect of feed DM on bolus dry matter proportions on a 1.6 mm screen.

△ TMR samples,

feed dry matter proportion on a 1.6 mm screen (mean ± standard deviation) = 0.71 ± 0.06;

▲ Rye grass hay samples,

feed dry matter proportion on a 1.6 mm screen (mean ± standard deviation) = 0.80 ± 0.10;

$y = 9*10^{-5}x + 0.5939$, $R^2 = 0.00$, n = 16.

○ TMR samples,

feed dry matter proportion on a 1.6 mm screen (mean ± standard deviation) = 0.23 ± 0.06;

● Rye grass hay samples,

feed dry matter proportion on a 1.6 mm screen (mean ± standard deviation) = 0.34 ± 0.05;

$y = -0.0029x + 0.3931$, $R^2 = 0.25$, n = 14.

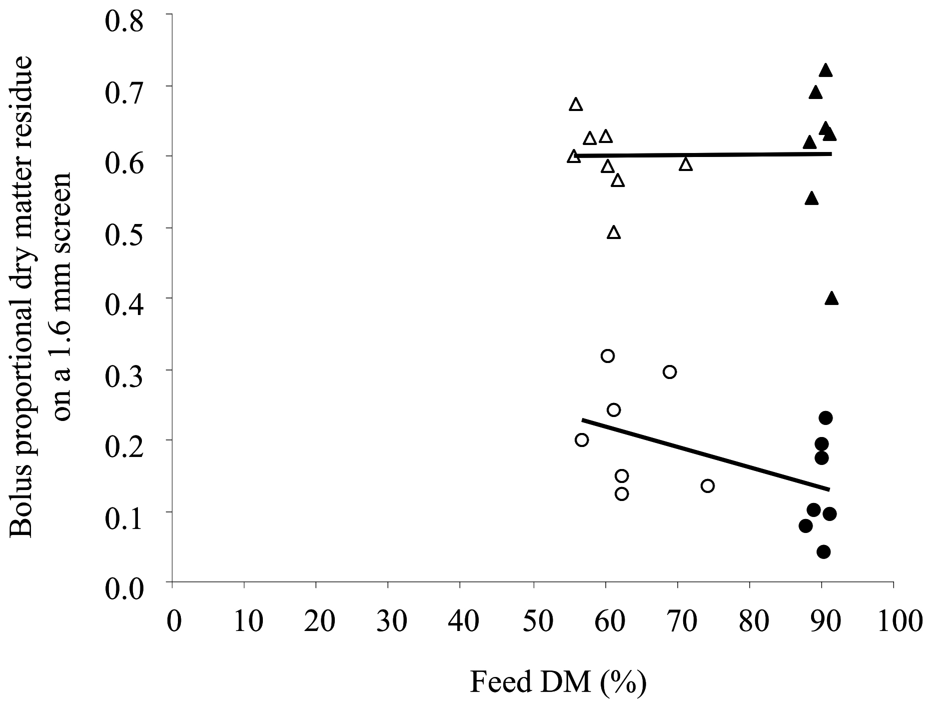


Figure 4.6.b. Effect of feed aNDF on bolus dry matter proportions on a 1.6 mm screen.

△ TMR samples,

feed dry matter proportion on a 1.6 mm screen (mean ± standard deviation) = 0.71 ± 0.06;

▲ Rye grass hay samples,

feed dry matter proportion on a 1.6 mm screen (mean ± standard deviation) = 0.80 ± 0.10;

$y = -0.0003x + 0.6112$, $R^2 = 0.00$, n = 16.

○ TMR samples,

feed dry matter proportion on a 1.6 mm screen (mean ± standard deviation) = 0.23 ± 0.06;

● Rye grass hay samples,

feed dry matter proportion on a 1.6 mm screen (mean ± standard deviation) = 0.34 ± 0.05;

$y = -0.0022x + 0.2518$, $R^2 = 0.23$, n = 14.

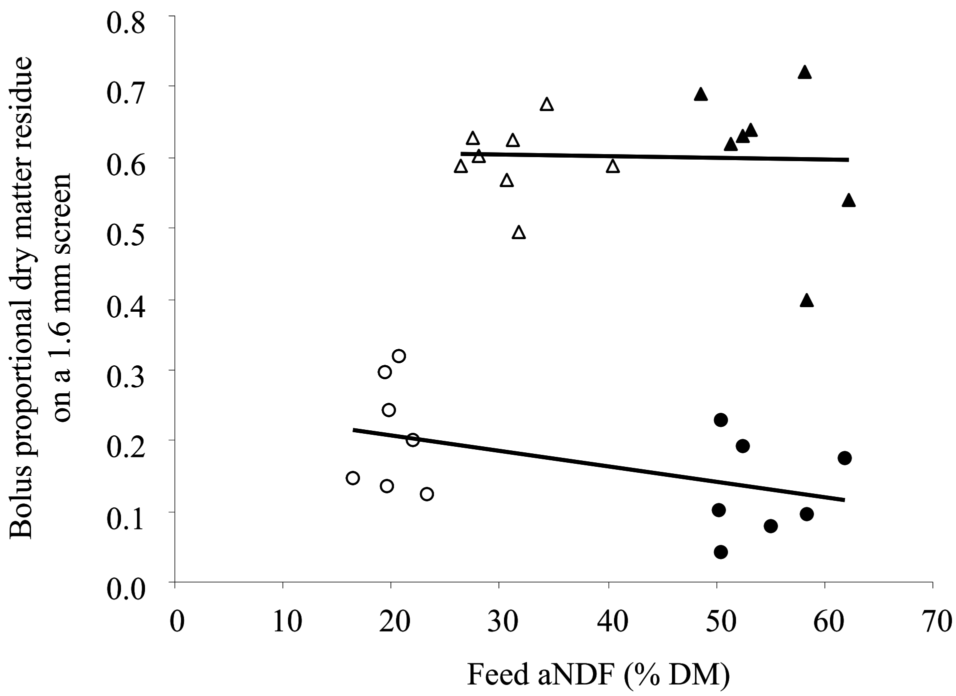


Figure 4.7. Effect of feed mean particle length* on bolus mean particle length* with feeds having different dry matter (DM), but similar crude protein (CP) and neutral detergent insoluble fiber (aNDF) contents.

◇ TMR samples, DM (mean ± standard deviation %) : 53.0 ± 5.7, aNDF (mean ± standard deviation % DM): 47.5 ± 2.2, CP (mean ± standard deviation % DM): 13.0 ± 3.2;
$y = 0.2341x + 10.669$, $R^2 = 0.71$, $n = 14$.

◆ Rye grass hay samples, DM (mean ± standard deviation %) : 89.9 ± 1.3, aNDF (mean ± standard deviation % DM): 51.0 ± 2.7, CP (mean ± standard deviation % DM): 12.8 ± 2.2;
$y = 0.1019x + 7.5442$, $R^2 = 0.41$, $n = 11$.

* Mean length of particles retained on a 1.6 mm screen and ≥ 5 mm.

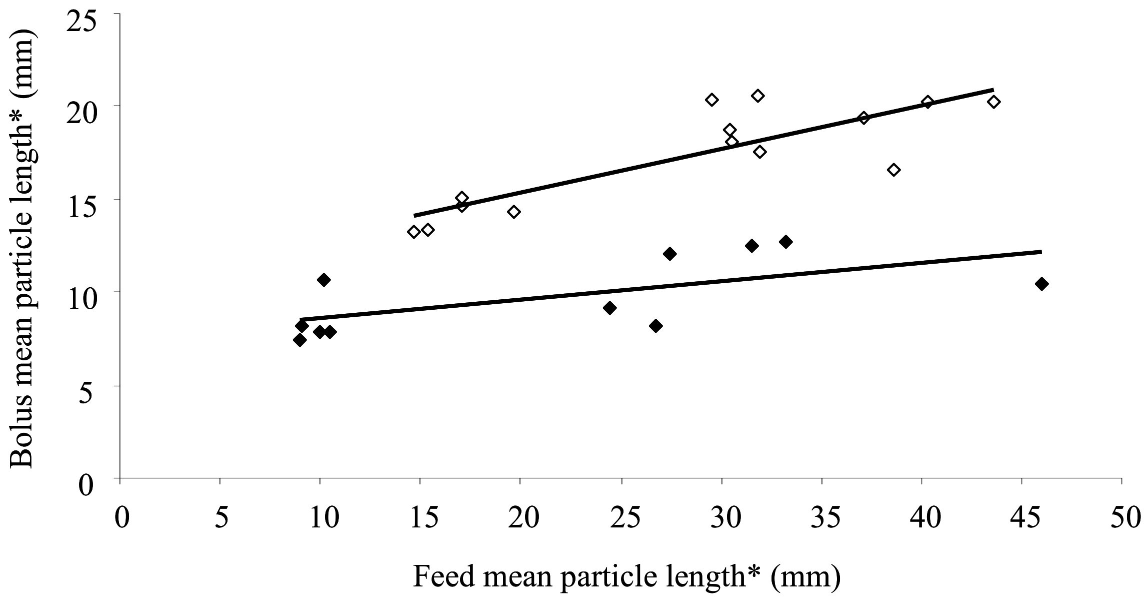


Table 4.1. Feed ingredients, chemical composition[1] and physical properties[2] of TMRs as well as respective milk production

Item	TMR1	TMR2	TMR3	TMR4	TMR5	TMR6	TMR7	TMR8	TMR9	TMR10
Ingredient, (% DM)										
Alfalfa hay										7.9
Alfalfa haylage										12.8
Vetch hay									7.6	
Trigonella hay			23.2							
Mixed legume grass hay[3]		25.0		15.5	19.0		18.9	21.4	7.3	8.4
Mixed legume grass haylage[3]					28.1					
Oat hay	31.9					7.7				
Triticale silage		24.5		13.3		10.4		23.6	25.1	10.6
Corn silage	13.9		25.1	16.0		21.9	34.2			
Soybean meal (44% CP)	11.3	12.1	11.4	14.3	16.4	16.0	8.9	9.4	12.9	11.4
Soybean flakes		4.2	3.9			5.7		4.0	5.4	5.8
Soypass®				2.4			3.3			
Corn gluten meal							0.8	1.4		
Sunflower meal								2.5		
Maize meal	21.0	20.3	25.1	25.7	28.0	25.8	19.8	31.4	28.1	23.0
Barley meal		11.5	3.8	5.6			8.0		10.5	17.5
Citrus pulp	9.8									
Carob pulp			1.1							
Sugar-beet pulp			3.9	2.1		5.9				
Wheat bran	9.0			0.8	6.5	3.7	3.9	2.3		
Mix[4]	3.2	2.4	2.4	4.2	2.1	2.9	1.9	3.8	3.1	2.8
Proportional residues (as fed) on screens[2]:										
19 mm	0.20	0.13	0.09	0.05	0.17	0.07	0.03	0.13	0.22	0.16
8 mm	0.28	0.31	0.26	0.34	0.30	0.38	0.45	0.33	0.25	0.28
2.5 mm	0.14	0.17	0.21	0.12	0.15	0.11	0.13	0.13	0.13	0.10
1.18 mm	0.18	0.21	0.25	0.24	0.17	0.25	0.23	0.22	0.21	0.31
Bottom pan	0.19	0.17	0.20	0.25	0.21	0.19	0.16	0.18	0.19	0.16

Table 4.1. Continued.

Item	TMR1	TMR2	TMR3	TMR4	TMR5	TMR6	TMR7	TMR8	TMR9	TMR10
Chemical composition										
DM (%)	50.1	51.5	63.8	55.0	60.4	51.6	63.3	48.5	52.1	54.2
OM (% DM)	91.0	92.2	91.2	93.0	91.0	93.8	93.8	93.6	91.4	92.3
CP (% DM)	16.0	15.9	17.3	17.5	14.8	25.0	21.3	17.6	17.1	23.0
aNDF (% DM)	34.6	35.0	29.6	30.7	33.9	30.1	32.3	28.9	26.2	30.5
Milk production										
Yield (kg / d)	19.7	21.9	26.3	26.6	28.7	30.4	31.9	32.0	35.2	38.8
Fat (%)	3.08	4.03	3.35	3.76	2.97	3.73	3.22	3.59	3.31	3.43
Protein (%)	3.16	3.41	3.32	3.12	3.21	3.27	3.23	3.27	3.20	3.21

[1] DM = dry matter, OM = organic matter, CP = crude protein, aNDF = neutral detergent insoluble fiber.
[2] Proportional residues as fed on sieves with apertures of 19, 8, 2.5 and 1.18 mm and on the bottom pan after a sequential, horizontal sieving procedure.
[3] Legume = either vetch or clover; grass = either oat or rye grass or triticale.
[4] Mineral, urea, vitamin, amino acid, molasses, fat supplement.

Table 4.2. Chemical composition[1] of treatment feeds

Item	DM	OM	CP	NDF
		Least square means		
	(%)	(% DM)		
Unprocessed TMR	55.1[c]	92.3b[c]	18.6[c]	31.2[c]
TMR fraction*				
19 mm	51.8[d]	92.0[c]	13.5[d]	48.4[a]
8 mm	56.2[c]	91.9[c]	14.7[d]	44.9[b]
2.5 mm	61.1[b]	92.3[c]	23.6[a]	31.8[c]
1.18 mm	63.6[a]	93.3[a]	24.0[a]	20.2[d]
Bottom pan	56.7[c]	92.9[ab]	21.6[b]	14.8[e]
SEM	0.72	0.21	0.45	0.63

[1] DM = dry matter, OM = organic matter, CP = crude protein, NDF = neutral detergent insoluble fiber.

*Sequential horizontal sieving technique. Fractions were residues on sieves with openings: 19 mm, 8 mm, 2.5 mm, 1.18 mm and on the bottom pan.

SEM = standard error of means.

Table 4.3. Particle size reduction during the ingestive mastication – Dry matter proportion of particles retained on a 1.6 mm screen (PROP_1.6) and mean length of these particles (≥ 5 mm considered) by *image analysis*.

Item	PROP_1.6					Mean length (mm)				
	Feed		Bolus		Feces	Feed		Bolus		Feces
	LSM	SEM	LSM	SEM	Mean ± STDEV	LSM	SEM	LSM	SEM	Mean ± STDEV
Unprocessed TMR	0.501^{a}	0.014	0.442^{b}	0.014	0.128 ± 0.044	16.9^{a}	0.58	15.7^{a}	0.60	8.48 ± 0.66
TMR fraction*										
19 mm	0.833^{a}	0.014	0.690^{b}	0.015		34.3^{a}	0.91	18.3^{b}	0.63	
8 mm	0.759^{a}	0.014	0.626^{b}	0.014		16.3^{a}	0.46	13.7^{b}	0.41	
2.5 mm	0.713^{a}	0.014	0.594^{b}	0.014		9.3^{a}	0.29	9.2^{a}	0.30	
1.18 mm	0.211^{a}	0.014	0.207^{a}	0.014		7.2^{a}	0.23	7.2^{a}	0.24	
Bottom pan	0.013^{a}	0.014	0.017^{a}	0.014		NE		NE		

*Sequential horizontal sieving technique. Fractions were residues on sieves with openings: 19 mm, 8 mm, 2.5 mm, 1.18 mm.

LSM = least square means; SEM = standard error of means; STDEV = standard deviation

Values within row and measure having different superscripts are different ($p < 0.01$).

Table 4.4. Percentage of 1 – 4 mm particles retained on a 1.6 mm screen.

Item	Percentage[a]		Weighted[b] percentage	
	Feed	Bolus	Feed	Bolus
	----- Mean ± standard deviation ------		----- Mean ± standard deviation ------	
Unprocessed TMR	12.4 ± 5.2	13.5 ± 2.8	2.0 ± 0.9	2.5 ± 1.0
TMR fraction*				
19 mm	19.2 ± 4.9	12.6 ± 3.6	1.1 ± 0.5	1.4 ± 0.7
8 mm	9.6 ± 2.8	10.6 ± 3.5	1.2 ± 0.5	1.7 ± 0.9
2.5 mm	12.3 ± 4.9	12.8 ± 4.8	4.3 ± 2.0	4.6 ± 2.3
1.18 mm	29.9 ± 4.9	32.7 ± 9.9	16.5 ± 3.6	18.7 ± 8.1

[a] Relative to total number of particles retained on the screen.

Percentage of particles (x) is related to dry matter proportion of particles retained on a 1.6 mm screen (y), y = - 27.09x + 31. 253, R^2 = 0.52, n = 106.

[b] Particles are weighted by their lengths. It is assumed that particle mass is proportional to particle length. Percentage is referred to weighted total.

Weighted percentage of particles (x) is related to dry matter proportion of particles retained on a 1.6 mm screen (y), y = - 20.395x + 16.257, R^2 = 0.64, n = 106.

Tables 4.5. Effect of chemical parameters (dry matter – DM, crude protein – CP, neutral detergent insoluble fiber – aNDF) on particle size reduction during ingestive mastication. - The test was performed on selected feeds, which had most similar particle size[a] but different chemical characteristics (Rye grass hay versus TMR fractions).

Table 4.5.a. Effect of chemical parameters on bolus ML* (mm). Chemical parameters are not related to feed ML*.

Item	Rye grass hay		TMR		Effect[a]		
	LSM	SEM	LSM	SEM	Feed[b]	PS[a]	Feed*PS
Feed							
Mean length* (mm)	19.66	1.52	19.58	1.52	ns	***	ns
DM (%)	89.53	0.82	56.57	0.82	***	***	***
CP (% DM)	13.02	0.60	18.72	0.60	***	***	***
aNDF (% DM)	56.11	0.96	39.30	0.96	***	***	***
Bolus							
Mean length* (mm)	9.33	0.36	13.87	0.36	***	***	***

LSM = Least square means; SEM = standard error of mean.

* Mean length of particles retained on a 1.6 mm screen and ≥ 5 mm.

[a] PS = particle size; feeds (hay / TMR fraction) were assigned to blocks - "long particles" and "mid size particles".

"Long particles": from samples retained on a 19 mm screen and from samples passing a 19 mm screen but retained on a 8 mm screen; 13 observations for each feed.

"Mid size particles": from samples passing a 8 mm screen but retained on a 1.18 mm screen (rye grass hay) and from samples passing a 8 mm screen but retained on a 2.5 mm screen (TMR); 8 observations for each feed.

[b] Rye grass hay versus TMR fraction

[c] *** = $p < 0.01$; ns = not significant, $p > 0.05$.

Table 4.5.b. Effect of chemical parameters on dry matter proportion retained on a 1.6 mm screen (PROP_1.6). Feed PROP_1.6 is not related to bolus PROP_1.6.

Item	Rye grass hay		TMR		Effect[c]		
	LSM	SEM	LSM	SEM	Feed[b]	PS[a]	Feed*PS
Feed							
PROP_1.6	0.57	0.02	0.48	0.02	***	***	ns
DM (%)	89.89	1.05	62.14	1.02	***	ns	ns
CP (% DM)	13.85	0.86	24.27	0.84	***	ns	ns
aNDF (% DM)	54.52	1.11	25.70	1.08	***	***	***
Bolus							
PROP_1.6	0.36	0.02	0.39	0.02	ns	***	ns

LSM = Least square means; SEM = standard error of mean.

* Mean length of particles retained on a 1.6 mm screen and ≥ 5 mm.

[a] PS = particle size, feeds (hay / TMR fraction) were assigned to blocks - “mid size particles” and “short particles”.

"Mid size particles": from samples passing a 8 mm screen but retained on a 1.18 mm screen (rye grass hay) and from samples passing a 8 mm screen but retained on a 2.5 mm screen (TMR); 8 observations for each feed.

"Short particles": from samples passing a 1.18 mm screen but retained on the bottom pan (rye grass hay) and from samples passing a 2.5 mm screen but retained on a 1.18 mm screen (TMR); 7 observations for each feed.

[b] Rye grass hay versus TMR fraction.

[c] *** = $p < 0.01$; ns = not significant, $p > 0.05$.

REFERENCES

Acosta, A., A. Boudon, and J.-L. Peyraud. 2007. Species and variety of herbage affects release of cell contents during ingestive mastication in dairy cows fed indoors. Animal Feed Science and Technology 132:28–48.

Allen, M.S. 1997. Relationship Between Fermentation Acid Production in the Rumen and the Requirement for Physically Effective Fiber. J. Dairy Sci. 80:1447–1462.

ANSI (American National Standards Institute). 1993. Method of determining and expressing particle size of chopped forage materials by screening. Pages 459-461 in ASAE Standards S424.1. 40th ed. Am. Soc. Agric. Eng., St. Joseph, MI.

Appleman, R. D., and D. G. Addis. 1960. Pelleted Hay Mixture Enables Dairy Cattle to Give More Milk with Less Butterfat. Dairy Science Abstrs., 22:603.

ASABE Standards, 2007. Method of Determining and Expressing Particle Size of Chopped Forage Materials by Screening - ANSI/ASAE S424.1 MAR1992 (R2007). ASABE, St. Joseph, MI.

Asadi Alamouti, A., M. Alikhani, G.R. Ghorbani, Q. Zebeli. 2009. Effects of inclusion of neutral detergent soluble fibre sources in diets varying in forage particle size on feed intake, digestive processes, and performance of mid-lactation Holstein cows. Animal Feed Science and Technology 154: 9–23.

Bailey, A. T., R. A. Erdman, L. W. Smith, and B. K. Sharma. 1990. Particle size reduction during initial mastication of forages by dairy cattle. J. Anim. Sci. 68:2084-2094.

Bal, M. A., R. D. Shaver, A. G. Jirovec, K. J. Shinners, and J. G. Coors. 2000. Crop Processing and Chop Length of Corn Silage: Effects on Intake, Digestion, and Milk Production by Dairy Cows. J. Dairy Sci. 83:1264–1273.

Bal, M.A., and E. B. Büyükünal Bal. 2010. Interaction between particle sizes of alfalfa hay and corn grain on milk yield, milk composition, chewing activity, and ruminal pH of dairy cows. Turk. J. Vet. Anim. Sci. 34(1): 83-89.

Balch, C. C. 1950. Factors affecting the utilization of food by dairy cows. I. The rate of passage of food through the digestive tract. Brit. J. Nutr. 4:361.

Balch, C. C., D. A. Balch, S. Bartlett, M. P. Bartrum, V. W. Johnson, S. J. Rowland and J. Turner. 1955. Studies on the secretion of milk of low fat content by cows on diets low in hay and high in concentrates. VI. The effects on the physical and biochemical processes of the reticulo-rumen. J. Dairy Sci. 22:270.

Baldwin, R. L., L. J. Koong, and M. J. Ulyatt. 1977. A dynamic model of ruminant digestion for evaluation of factors affecting nutritive value. Agr. Systems 2:255.

Baumgardt, B. R. 1967. Efficiency of Nutrient Utilization for Milk Production: Nutritional and

Physiological Aspects. J. Anim. Sci. 26:1186-1194.
Beardsley, D.W. 1964. Symposium on forage utilization: Nutritive value of forages as affected by physical form. Part II. Beef cattle and sheep studies. J. Anim. Sci. 23:239-245.
Beauchemin, K. A., W. Z. Yang, and L. M. Rode. 2003. Effects of Particle Size of Alfalfa-Based Dairy Cow Diets on Chewing Activity, Ruminal Fermentation, and Milk Production. J. Dairy Sci. 86:630–643.
Beauchemin, K. A., and W. Z. Yang. 2005. Effects of Physically Effective Fiber on Intake, Chewing Activity, and Ruminal Acidosis for Dairy Cows Fed Diets Based on Corn Silage. J. Dairy Sci. 88:2117–2129.
Belyea, R. L., F. A. Martz, and G. A. Mbagaya.1989. Effect of Particle Size of Alfalfa Hay on Intake, Digestibility, Milk Yield, and Ruminal Cell Wall of Dairy Cattle. J. Dairy Sci. 72:958-963.
Bhandari, S. K., K. H. Ominski, K. M. Wittenberg, and J. C. Plaizier. 2007. Effects of Chop Length of Alfalfa and Corn Silage on Milk Productio and Rumen Fermentation of Dairy Cows. J. Dairy Sci. 90:2355–2366.
Bhandari, S. K., S. Li, K. H. Ominski, K. M. Wittenberg, and J. C. Plaizier. 2008. Effects of the Chop Lengths of Alfalfa Silage and Oat Silage on Feed Intake, Milk Production, Feeding Behavior, and Rumen Fermentation of Dairy Cows. J. Dairy Sci. 91:1942–1958.
Bide, R. W., and W. J. Dorward. 1975. Clinical chemistry of grain-fed cattle. II. Liver functions. Can. J. Anim. Sci. 55:23.
Blaxter, K. L., and N. McC. Graham. 1956. The effect of grinding and cubing on the utilization of the energy of dried grass. J. Agr. Sci. 47:207.
Blosser, T. H., F. R. Murdock, R. E. Lintott, R. E. Erb, and A. O. Shaw. 1952. The use of dehydrated forages in dairy cattle rations. II. Comparative values of finely ground, chopped and pelleted dehydrated alfalfa as grain replacements for lactating dairy cows. J. Dairy Sci. 35:515-523.
Bowman, J. G., and J. L. Firkins. 1993. Effects of forage species and particle size on bacterial cellulolytic activity and colonization in situ. J. Anim. Sci. 71:1623-1633.
Brent, B. E. 1976. Relationship of acidosis to other feedlot ailments. J. Anim. Sci. 43:930.
Breukink, H. J., and T. deRuyter. 1976. Abomasal displacement in cattle: Influence of concentrates in the ration of fatty acid concentrations in ruminal abomasal and duodenal contents. Amer. J. Vet. Res. 37:1181.
Brooks, O. L., W. J. Miller, E. R. Beaty, and C. M. Clifton. 1962. Performance of dairy cows fed pelleted and baled Coastal Bermudagrass and alfala hay. J. Dairy Sci. 45:1188–1191.

Cardoza, R. S. 1985. Threshold size and factors affecting fecal particle weight distribution. M.S. Thesis, Univ. Georgia, Athens.

Castle, E. J. 1956. The rate of passage of feedstuffs through the alimentary tract of the goat. I. Studies on adult animals fed on hay and concentrates. Brit. J. Nutr. 10:15.

Chalupa, W., G. D. O'Dell, A. J. Katches, and R. Lavaker. 1970. Supplemental corn silage or baled hay for correction of milk fat depressions produced by feeding pellets as the sole forage. J. Dairy Sci, 52:208.

Chandler, P.T., and H.W. Walker. 1972. Generation of Nutrient Specifications for Dairy Cattle for Computerized Least Cost Ration Formulation . J. Dairy Sci. 55:1741–1749.

Clark, J. H., and C. L. Davis. 1980. Some aspects of feeding high producing dairy cows. J. Dairy Sci. 63:873.

Clark, P. W., and L. E. Armentano. 1993. Effectiveness of neutral detergent fiber in whole cottonseed and dried distillers grains compared with alfalfa haylage. J. Dairy Sci. 76:2644.

Clark, P. W., and L. E. Armentano. 2002. Influence of Particle Size on the Effectiveness of the Fiber in Alfalfa Silage. J. Dairy Sci. 85:3000–3007.

Colenbrander, V. F., C. H. Noller, and R. J. Grant. 1991. Effect of Fiber Content and Particle Size of Alfalfa on Performance and Chewing Behavior J. Dairy Sci. 74:2681-2690.

Colucci, P. E., L. E. Chase, and P. J. Van Soest.1982. Feed Intake, Apparent Diet Digestibility, and Rate of Particulate Passage in Dairy Cattle. J. Dairy Sci. 65:1445-1456.

Colvin, H. W. Jr., R. D. Digesti, and J. A. Louvier. 1978. Effect of succulent and nonsucculent diets on rumen motility and pressure before, during and after eating. J. Dairy Sci. 61:1414.

Conrad, H. R., A. D. Pratt, and J. W. Hibbs. 1964. Regulation of feed intake in dairy cows. I. Changes in importance of physical and physiological factors with increasing digestibility. J.Dairy Sci. 47:54.

Coppock, C. E., D. L, Bath, and B. Harris, JR. 1981. From Feeding to Feeding Systems. J. Dairy Sci. 64:1230-1249.

Cotanch, K. W., J. D. Darrah, C. S. Ballard, and R. J. Grant. 2010. Modification of the Penn State Particle Separator with 3.18- or 4.76-mm perforated steel sieves to measure physically effective fiber. Abstract, J. Anim. Sci. Vol. 88, E-Suppl. 2, p. 147.

Couderc, J. J., D. H. Rearte, G. F. Schroeder, J. I. Ronchi, and F. J. Santini. 2006. Silage Chop Length and Hay Supplementation on Milk Yield, Chewing Activity, and Ruminal Digestion by Dairy Cows. J. Dairy Sci. 89:3599–3608.

Crampton, E. W. 1957. Interrelationships between digestible nutrient and energy content, voluntary dry matter intake and the overall feeding value of forages. J. Anim. Sci. 16:546.

Crawford Jr., R. J., W. H. Hoover, and P. H. Knowlton. 1980. Effects of Solids and Liquid Flows on Fermentation in Continuous Cultures. I. Dry Matter and Fiber Digestion, VFA Production and Protozoa Numbers. J. Anim. Sci. 51:975-985.

Dawson, L. J., E. P. Aalseth, L. E. Rice, and G. D. Adams. 1992. Influence of fiber form in a complete mixed ration on incidence of left displaced abomasum in postpartum dairy cows. JAVMA 200:1989.

De Boever, J.L., J.I. Andries, D.L. De Brabander, B.G. Cottyn, and F.X. Buysse. 1990. Chewing Activity of Ruminants as a Measure of Physical Structure - A Review of Factors Affecting It. Animal Feed Science and Technology 27: 281-291.

De Boever, J. L., A. M. De Smet, D. L. De Brabander, and C. V. Boucque. 1993a. Evaluation of Physical Structure. 1. Grass Silage. J. Dairy Sci. 76:140-153.

De Boever, J. L., D. L. De Brabander, A. M. De Smet, J. M. Vanacker, and C. V. Boucque. 1993b. Evaluation of Physical Structure. 2. Maize Silage. 1993b. J. Dairy Sci. 76:1624-1634.

Deswysen A. G., and W. C. Ellis. 1990. Fragmentation and ruminal escape of particles as related to variations in voluntary intake, chewing behavior and extent of digestion of potentially digestible NDF in heifers. J Anim. Sci. 68:3871-3879.

DeVries, T. J., M. A. G. von Keyserlingk, and K. A. Beauchemin. 2003a. Short Communication: Diurnal Feeding Pattern of Lactating Dairy Cows. J. Dairy Sci. 86:4079–4082.

DeVries, T. J., M. A. G. von Keyserlingk, D. M. Weary, and K. A. Beauchemin. 2003b. Measuring the Feeding Behavior of Lactating Dairy Cows in Early to Peak Lactation. J. Dairy Sci. 86:3354–3361.

Eaton, H. D., K. L. Dolge, R. D. Mochrie, J. E. Avampato, and L. A. Moore. 1952. Field-cured and Field-baled Alfalfa Hay versus Artificially-dried and Chopped and Pelleted Alfalfa Hays as a Source of Carotene and Roughage for Guernsey and Holstein Calves. J. Dairy Sci. 35:98.

Einarson, M. S., J. C. Plaizier, and K. M. Wittenberg. 2004. Effects of Barley Silage Chop Length on Productivity and Rumen Conditions of Lactating Dairy Cows Fed a Total Mixed Ration. J. Dairy Sci. 87:2987–2996.

Elliot, J. M., and J. K. Loosli. 1959. Relationship of Milk Production Efficiency to the Relative Proportions of the Rumen Volatile Fatty Acids. J. Dairy Sci. 42:843.

Ensor, W. L., J. C. Shaw, and H. F. Tellechea, 1959. Special Diets for the Production of Low Fat Milk and More Efficient Gains in Body Weight. J. Dairy Sci. 42:189.

Erdman, R. A., R. L. Botts, R. W. Hemken, and L. S. Bull, 1980. Effect of dietary bicarbonate and magnesium oxide on production and physiology in early lactation. J. Dairy Sci. 63:923.

Fernandez, I., C. Martin, M. Champion, and B. Michalet-Doreau. 2004. Effect of Corn Hybrid and

Chop Length of Whole-Plant Corn Silage on Digestion and Intake by Dairy Cows. J. Dairy Sci. 87:1298–1309.

Fisher, J. M., J. G. Buchanan-Smith, C. Campbell, D. G. Grieve, and O. B. Allen.1994. Effects of Forage Particle Size and Long Hay for Cows Fed Total Mixed Rations Based on Alfalfa and Corn. J. Dairy Sci. 77:217-229.

Fronk, T. J., L. H. Schultz, and A. R. Hardie. 1980. Effect of dry period over-conditioning on subsequent metabolic disorders and performance of dairy cows. J. Dairy Sci. 63:1080.

Gibb, M.J., C.A. Huckle, R. Nuthall, and A.J. Rook. 1999. The effect of physiological state (lactating or dry) and sward surface height on grazing behaviour and intake by dairy cows. Applied Animal Behaviour Science 63:269–287.

Gleaves, E. W., P. Q. Guyer, J. G. Kendrick, F. G. Owen, E. R. Peo, Jr., and T. W. Sullivan. 1973. Nebraska computer feed formulation data, feed ingredient analysis, and ration specifications and restrictions. Agric. Exp. Stn. Bull. MP24. Univ. Nebraska, Lincoln.

Grant, R. J., V. F. Colenbrander, and D. R. Mertens. 1990a. Milk Fat Depression in Dairy Cows: Role of Particle Size of Alfalfa Hay. J. Dairy Sci. 73:1823-1833.

Grant, R. J., V. F. Colenbrander, and D. R. Mertens. 1990b. Milk Fat Depression in Dairy Cows: Role of Silage Particle Size. J. Dairy Sci. 73:1834-1842.

Grant, R. J. and J. L. Albright. 1995. Feeding behavior and management factors during the transition period in dairy cattle. J. Anim. Sci. 73:2791-2803.

Haenlein, G. F. W., C. R. Richards, and W. H. Mitchell. 1962. Effect of the Size of Grind and the Level of Intake of Pelleted Alfalfa Hay on Its Nutritive Value in Cows and Sheep. J. Dairy Sci. 45:693.

Harris, B. 1984. Feeds, feeding and nutrient requirements of dairy cattle. Florida Coop. Ext. Circ. 594, Gainesville.

Heinrichs J., and P.J. Kononoff. 2004. Evaluating particle size of forages and TMRs using the new Penn State forage particle separator. DAS 02-42. Dept. of Animal Science, Penn State University, University Park, PA 16802. http://www.das.psu.edu/dairynutrition/documents/das0242.pdf.

Hope, E. B., R. E. Erb, T. H. Blosser, U. S. Ashworth, and A.O. Shaw. 1950. The Use of Dehydrated Forages in Dairy Cattle Rations. I. Grain Substitution with Finely Ground Material. J. Dairy Sci. 33:306-314.

Howard, W. T., J. L. Albright, M. D. Cunningham, R. B. Harrington, and C. H. Noller. 1968. Leastcost complete rations for dairy cows. J. Dairy Sci. 51:595-600.

Huffman, C. F. 1939. Roughage Quality and Quantity in the Dairy Ration. A Review. J. Dairy Sci.

22:889-980.

Hungate, R. E. 1966. The Rumen and Its Microbes. Academic Press. New York.

Igathinathane, C., L.O. Pordesimo, and W.D. Batchelor. 2008a. Ground Biomass Sieve Analysis Simulation by Image Processing and Experimental Verification of Particle Size Distribution. ASABE Paper No. 084126. ASABE, St. Joseph, MI.

Igathinathane, C., L.O. Pordesimo, E.P. Columbus, W.D. Batchelor, and S.R. Methuku. 2008b. Shape identification and particles size distribution from basic shape parameters using ImageJ. Comput. Electron. Agric. 63, 168–182.

Igathinathane, C., L.O. Pordesimo, E.P. Columbus, W.D. Batchelor, and S. Sokhansanj. 2009. Sieveless particle size distribution analysis of particulate materials through computer vision. Computers and Electronics in Agriculture 66:147–158.

Jaster, E. H., and M. R. Murphy. 1983 Effects of Varying Particle Size of Forage on Digestion and Chewing Behavior of Dairy Heifers. J. Dairy Sci. 66:802-810.

Jensen, L. S., and J. McGinnis, 1952. A comparison of feeding pelleted and unpelleted diets containing different levels of alfalfa to laying hens. Poultry Sci. 31:307–310.

Jones, I. R., B. F. Magill, and R. G. Peterson. 1958. Baled, Wafered and Pelleted Hay: Comparative Feeding Values for Milk Production. Oregon Agr. Expt. Sta., Bull. May.

Julien, W. E., and H. R. Conrad. 1977. Influence of dietary protein on susceptibility to alert downer syndrome. J. Dairy Sci. 60:210.

Kennedy, P. M.. 1985. Effect of Rumination on Reduction of Particle Size of Rumen Digesta by Cattle. Aust. J. Agric. Res. 36:819-828.

Keunen, J. E., J. C. Plaizier, L. Kyriazakis, T. F. Duffield, T. M. Widowski, M. I. Lindinger, and B. W. McBride. 2002. Effects of a subacute ruminal acidosis model on the diet selection of dairy cows. J. Dairy Sci. 85:3304–3313.

King, R. L., and R. W. Hemken.1962. Composition of Milk Produced on Pelleted Hay and Heated Corn. J. Dairy Sci. 45:1336-1342.

Kleen, J. L., G. A. Hooijer, J. Rehage, and J. P. Noordhuizen. 2003. Subacute ruminal acidosis (SARA): A review. J. Vet. Med. A Physiol. Pathol. Clin. Med. 50:406–414.

Kononoff, P. J., and A. J. Heinrichs. 2003. The Effect of Reducing Alfalfa Haylage Particle Size on Cows in Early Lactation. J. Dairy Sci. 86:1445–1457.

Kononoff, P. J., A. J. Heinrichs, and D. R. Buckmaster. 2003a. Modification of the Penn State Forage and Total Mixed Ration Particle Separator and the Effects of Moisture Content on its Measurements. J. Dairy Sci. 86:1858–1863.

Kononoff, P. J., A. J. Heinrichs, and H. A. Lehman. 2003b. The Effect of Corn Silage Particle Size on Eating Behavior, Chewing Activities, and Rumen Fermentation in Lactating Dairy Cows. J. Dairy Sci. 86:3343–3353.

Kovács, P.L., K.-H. Südekum, and M. Stangassinger. 1997. Rumen contents and ruminal and faecal particle size distribution in steers fed a mixed diet at three amounts of intake. Animal Feed Science Technology 64:143-154.

Kowsar, R., G. R. Ghorbani, M. Alikhani, M. Khorvash, and A. Nikkhah. 2008. Corn Silage Partially Replacing Short Alfalfa Hay to Optimize Forage Use in Total Mixed Rations for Lactating Cows. J. Dairy Sci. 91:4755–4764.

Krause, K. M., D. K. Combs, and K. A. Beauchemin. 2002a. Effects of forage particle size and grain fermentability in midlactation cows. I. Milk production and diet digestibility. J. Dairy Sci. 85:1936–1946.

Krause, K. M., D. K. Combs, and K. A. Beauchemin. 2002b. Effects of forage particle size and grain fermentability in midlactation cows. II. Ruminal pH and chewing activity. J. Dairy Sci. 85:1947–1957.

Krause, K. M., and D. K. Combs. 2003. Effects of Forage Particle Size, Forage Source, and Grain Fermentability on Performance and Ruminal pH in Midlactation Cows. J. Dairy Sci. 86:1382–1397.

Krause, K.M., and G.R. Oetzel. 2006. Understanding and preventing subacute ruminal acidosis in dairy cattle: a review. Anim. Feed Sci. Technol. 126:215–236.

Lammers, B. P., D. R. Buckmaster, and A. J. Heinrichs. 1996. A Simple Method for the Analysis of Particle Sizes of Forage and Total Mixed Rations. J. Dairy Sci. 79:922-928.

Latham, M. J., J. D. Sutton, and M. E. Sharpe. 1974. Fermentation and microorganisms in rumen and content of fat in the milk of cows given low roughage rations. J. Dairy Sci. 57:803.

Lee, J. A., and G. R. Pearce. 1984. The Effectiveness of Chewing during Eating on Particle Size Reduction of Roughages by Cattle. Aust. J. Agric. Res. 35:609-618.

Le Liboux, S., and J.L. Peyraud. 1998. Effect of forage particle size and intake level on fermentation patterns and sites and extent of digestion in dairy cows fed mixed diets. Animal Feed Science and Technology 73:131-150.

Le Liboux, S., and J.L. Peyraud. 1999. Effect of forage particle size and feeding frequency on fermentation patterns and sites and extent of digestion in dairy cows fed mixed diets. Animal Feed Science and Technology 76: 297-319.

Leonardi, C., and L. E. Armentano. 2003. Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows. J. Dairy Sci. 86:557–564.

Leonardi, C., K. J. Shinners, and L. E. Armentano. 2005. Effect of Different Dietary Geometric

Mean Particle Length and Particle Size Distribution of Oat Silage on Feeding Behavior and Productive Performance of Dairy Cattle. J. Dairy Sci. 88:698–710.

Licitra G., M. Caccamo, I. Schadt, J. D. Ferguson, G. Gennuso, G. Azzaro. 2005. Method to Measure feed particles by image analysis. Abstract, J. Dairy Sci. Vol. 88, Suppl. 1: 252.

Luginbuhl, J.-M., K. R. Pond and J. C. Bums. 1990. Changes in ruminal and fecal particle weight distribution of steers fed coastal bermudagrass hay at four levels. J. Anim. Sci. 68:2864-2873.

McBride, B. W., L. P. Milligan, and B. V. Turner. 1984. Endoscopic observations of digesta transfer from the reticulo rumen to omasum of cattle. Can. J. Anim. Sci. 64 (Suppl.):84-85.

McCoy, G. C., H. S. Thurmon, H. H. Olson, and A. Reed. 1966. Complete feed rations for lactating dairy cows. J. Dairy Sci. 49:1058-1063.

Mertens, D. R. 1973. Application of theoretical mathematical models to cell wall digestion and forage intake in ruminants. Ph.D. Thesis. Cornell Univ.

Mertens, D. R., and L. O. Ely. 1979. A Dynamic Model of Fiber Digestion and Passage in the Ruminant for Evaluating Forage Quality. J. Anim. Sci. 49:1085-1095.

Mertens, D. R., T. L. Strawn, and R. S. Cardoza. 1984. Modeling ruminal particle size reduction: Its relationship to particle size description in Techniques in Particle Size Analysis of Feed and Digesta in Ruminants. P. M. Kennedy (ed.). Can. Soc. Anim. Sci. Occ. Pub. No. 1.

Mertens, D. R. 1986. Effect of physical characteristics, forage particle size and density on forage utilization. Page 91 *in* Proc. Nutr. Symp., St. Louis, MO. Am. Feed Ind. Assoc., Arlington,VA.

Mertens, D.R. 1997. Creating a System for Meeting the Fiber Requirements of Dairy Cows. J. Dairy Sci. 80:1463–1481.

Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds using refluxing in beakers or crucibles: Collaborative study. J. AOAC 85:1217-1240.

Mertens, D. R. 2005. Particle size, fragmentation index, and effective fiber: Tools for evaluating the physical attributes of corn silages. Pages 211-220 *in* Proc. Four-State Dairy Nutr. & Mgmt. Conf. MWPS-4SD18. Dubuque, IA.

Milligan, R. A., L. E. Chase, C. J. Sniffen, and W. A. Knoblauch. 1981. Least-cost balanced dairy rations. Newplan program 13. Agric. Econ. Ext. Circ. 81–24. Cornell Univ., Ithaca, NY.

Moore, L.A. 1964. Symposium on Forage Utilization: Nutritive Value of Forage as Affected by Physical Form. Part I. General Principles Involved with Ruminants and Effect of Feeding Pelleted or Wafered Forage to Dairy Cattle. J. Anim. Sci. 23:230-238.

Morrow, D. A. 1976. Fat cow syndrome. J. Dairy Sci. 59:1625.

Murdock, F. R., and A. S. Hodgson. 1979. Effects of roughage type and texture on milk fat by Lactating dairy cows. J. Dairy Sci. 62:505.

Nelson, M. L. 1988. Factors affecting the particle size distribution of grazed forage due to ingestive mastication by steers and wethers. J. Anim. Sci. 66:1256-1266.

Nocek, J. E., and E. M. Kesler. 1980. Growth and rumen characteristics of holstein steers fed pelleted or conventional diets. J. Dairy Sci. 63:249.

Nocek, J. E., K. A. Cummins, C. E. Polan, and J. H. Herbein. 1980. Influence of ration physical form and nitrogen availability on growth, ruminal fermentation, rumen morphology and in vitro acetate and propionate transport rates. J. Dairy Sci. 63 (Suppl.):131.

Nocek, J. E., R. L. Steele, and D. G. Braud. 1986. Performance of Dairy Cows Fed Forage and Grain Separately Versus a Total Mixed Ration. J Dairy Sci. 69: 2140 – 2147.

O'Dell, G. D., W. A. King, W. C. Cook, and W. A. Balk. 1964. Effects of forage supplementation and frequency of feeding of pelleted Coastal Bermudagrass hay on milk and milk fat production. J. Dairy Sci. 47:648.

O'Dell, G. D., W. A. King, and W. C. Cook. 1968. Effect of grinding, pelleting, and frequency of feeding of forage on fat percentage of milk and milk production of dairy cows. J. Dairy Sci. 51: 50-55.

Okine, E. K., and G. W. Mathison. 1991. Effects of feed intake on particle distribution, passage of digesta, and extent of digestion in the gastrointestinal tract of cattle. J. Anim. Sci. 69:3435-3445.

Olson, H. H. 1965. What do we know about complete feeds for dairy cattle? Proc. Cornell Nutr. Conf 80.

Onetti, S. G., R. D. Shaver, S. J. Bertics, and R. R. Grummer. 2003. Influence of Corn Silage Particle Length on the Performance of Lactating Dairy Cows Fed Supplemental Tallow. J. Dairy Sci. 86:2949–2957.

Paladines, O. L., J. T. Reid, B. D. H. Van Niekerk, and A. Bensadoun. 1964. Energy utilization by sheep as influenced by the physical form, composition and level of intake of diet. J. Nutr. 83:49.

Palmquist, D. L., and M. J. Ronning, 1961. Some Effects Caused by Feeding Ground, Pelleted Hay as Compared to Feeding Long Hay to Dairy Cows. J. Dairy Sci. 44:2237.

Palmquist, D.L., L.M. Smith, and M. Ronning. 1964. Effect of time of feeding concentrates and ground, pelleted alfalfa hay on milk fat percentage and fatty acid composition. J. Dairy Sci. 47:516-520.

Pan, J., S. Koike, T. Suzuki, K. Ueda, Y. Kobayashi, K. Tanaka, and M. Okubo. 2003. Effect of mastication on degradation of orchardgrass hay stem by rumen microbes: fibrolytic enzyme activities and microbial attachment. Animal Feed Science and Technology 106:69–79.

Peréz-Barberìa, F. J., and I. J Gordon. 1998. Factors affecting food comminution during chewing in ruminants: a review. Biological Joumal of the Linnean Society 63: 233-256.

Poppi, D. P., R. E. Hendricksen, and D. J. Minson. 1985. The relative resistance to escape of leaf and stem particles from the rumen of cattle and sheep. J. Agric. Sci. 105:9-14.

Porter, G. H., R. E. Johnson, H. D. Eaton, F. I. Elliott, and L. A. Moore. 1953. Relative Value for Milk Production of Field-cured and Field-baled, Artificially Dried-chopped, Artificially Dried-ground and Artificially Dried-pelleted Alfalfa when Fed as the Sole Source of Roughage to Dairy Cattle. J. Dairy Sci. 35:1140-1149.

Powell, E. B. 1939. Some Relations of Roughage Intake to the Composition of Milk. J. Dairy Sci. 22: 453.

Rinne, M., P. Huhtanen, and S. Jaakkola. 2002. Digestive processes of dairy cows fed silages harvested at four stages of grass maturity. J. Anim. Sci. 80:1986-1998.

Rodrique, C. B., and N. N. Allen, 1960. The Effect of Fine Grinding of Hay on Ration Digestibility, Rate of Passage, and Fat Content of Milk. Canadian J. Anim. Sci. 40:23-29.

Ronning, M., J. H. Meyer, and G. T. Clark, 1959. Pelleted Alfalfa Hay for Milk Production. J. Dairy Sci. 42:1373–1376.

Russell, J. B., and D. B. Dombrowski. 1980. Effect of pH on the efficiency of growth by pure cultures of rumen bacteria in continuous culture. Appl. Environ. Microbiol. 39: 604-610.

Rustas, B.-O., P. Nørgaard, A. R. Jalali, and E. Nadeau. 2010. Effects of physical form and stage of maturity at harvest of whole-crop barley silage on intake, chewing activity, diet selection and faecal particle size of dairy steers. Animal 4:1, pp 67–75.

Rustomo, B., O. AlZahal, N. E. Odongo, T. F. Duffield, and B. W. McBride. 2006. Effects of Rumen Acid Load from Feed and Forage Particle Size on Ruminal pH and Dry Matter Intake in the Lactating Dairy Cow. J. Dairy Sci. 89:4758–4768.

Santini, F. J., A. R. Hardie, N. A. Jorgensen, and M. F. Finner. 1983. Proposed use of adjusted intake based on forage particle length for calculation of roughage indexes. J. Dairy Sci. 66:811-820.

Schneider, B. H., and H.H. Brugman.1950. Pelleting Feed for Pigs. Wash. Agr. Expt. Sta.Circ. 115.

Schwab, E. C., R. D. Shaver, K. J. Shinners, J. G. Lauer, and J. G. Coors. 2002. Processing and Chop Length Effects in Brown-Midrib Corn Silage on Intake, Digestion, and Milk Production by Dairy Cows. J. Dairy Sci. 85:613–623.

Shahin, M.A., and S.J. Symons. 2005. Seed sizing from images of non-singulated grain samples.

Can. Biosyst. Eng. 47:49–54.
Shahin, M., S.J.S. Symons, and V. Poysa. 2006. Determining soya bean seed size uniformity with image analysis. Biosyst. Eng. 94:191–198.
Shaver, R. D., A. J. Nytes, L. D. Satter, and N. A. Jorgensen. 1986. Influence of Amount of Feed Intake and Forage Physical Form on Digestion and Passage of Prebloom Alfalfa Hay in Dairy Cows. J. Dairy Sci. 69:1545-1559.
Shaver, R. D., A. J. Nytes, L. D. Satter, and N. A. Jorgensen. 1988. Influence of Feed Intake, Forage Physical Form, and Forage Fiber Content on Particle Size of Masticated Forage, Ruminal Digesta, and Feces of Dairy Cows. J. Dairy Sci. 71:1566-1572.
Shaver, R. D. 1990. Forage particle length in dairy rations. Page 58 in Proc. Northeast Reg. Agric. Eng. Serv. Dairy Feeding Sys. Symp., Harrisburg, PA. Northeast Reg. Agric. Eng. Serv., Ithaca, NY.
Shaver, R.D.1997. Nutritional Risk Factors in the Etiology of Left Displaced Abomasum in Dairy Cows: A Review. J. Dairy Sci. 80:2449–2453.
Smith, L. W., and D. R. Waldo. 1969.Method for sizing forage cell wall particles.J. Dairy Sei., 52: 2051-2053.
Soita, H. W., M. Fehr, D. A Christensen, and T. Mutsvangwa. 2005. Effects of Corn Silage Particle Length and Forage:Concentrate Ratio on Milk Fatty Acid Composition in Dairy Cows Fed Supplemental Flaxseed. J. Dairy Sci. 88:2813–2819.
Stewart, C. S. 1977. Factors affecting the cellulolytic activity of rumen contents. Appl. Environ. Microbiol. 33:497-502.
Stone, W.C. 2004. Nutritional Approaches to Minimize Subacute Ruminal Acidosis and Laminitis in Dairy Cattle. J. Dairy Sci. 87:(E. Suppl.):E13–E26.
Sudweeks, E. M., and M. R. Holmes. 1975. Chewing time and volatile fatty acid production in steers. Proc. West. Sect. Am. Soc. Anim. Sci. 26:133.
Sudweeks, E. M., L. O. Ely, and L. R. Sisk. 1980. Effect of intake on chewing activity of steers. J. Dairy Sci. 63:152.
Swain, S. M., and L. E. Armentano. 1994. Quantitative evaluation of fiber from nonforage sources used to replace alfalfa silage. J. Dairy Sci. 77:2318.
Tafaj, M., Q. Zebeli, Ch. Baes, H. Steingass, and W. Drochner. 2007. A meta-analysis examining effects of particle size of total mixed rations on intake, rumen digestion and milk production in high-yielding dairy cows in early lactationAnimal Feed Science and Technology 138:137–161.
Teimouri Yansari, A., R. Valizadeh, A. Naserian, D. Christensen, and F. Eftekhari Shahroodi 2004.

Effects of alfalfa particle size and specific gravity on chewing activity, digestibility, and performance of Holstein dairy cows. J. Dairy Sci. 87:3912–3924.

Teimouri Yansari, A., and R. Primohammadi. 2009. Effect of particle size of alfalfa hay and reconstitution with water on intake, digestion and milk production in Holstein dairy cows. Animal 3:2, pp 218–227.

Thomas, P. C. 1975. Diet and milk secretion in the ruminant. World Rev. Anim. Prod. 11 (4):33.

Thorton, R. F. and D. J. Minson. 1972. The relationship between voluntary intake and mean apparent retention time in the rumen. Australian J. Agr. Res. 23:871.

Tomoko Oshita,, Kazuhisa Nonaka, Shinichi Kume, Tadashi Nakui. 2004. Effects of forage type on particle size distribution of ruminal digesta and faeces of non-lactating cows fed high quality forage. Livestock Production Science 91:107–115.

Troelsen, J. E., and F. W. Bigsby. 1964. Artificial Mastication-A New Approach for Predicting. J. Anim.Sci. 23:1139-1142.

Ulyatt, M. J., R. L. Baldwin, and L. J. Koong. 1976. The basis of nutritive value - A modeling approach. Proc. New Zealand Soc. Anim. Prod. 36:140.

Van Soest, P. J. 1955. Interrelationships between composition and physical condition of feeds, rumen fermentation products, blood constituents and milk fat of ruminants. Thesis, Univ. Wisconsin, Madison, Wis.

Van Soest, P. J. 1966. Forage intake in relation to chemical composition and digestibility. I. Some new concepts. Prec. 23rd Southern Pasture Forage Crop Improvement Conf., Blacksburg, Virginia. p. 24.

Van Soest, P.J., 1994. Nutritional Ecology of the Ruminant, 2nd ed. Cornell University Press, Ithaca, NY.

Vincent, J.F.V. 1983. The influente of water-content on the stiffness and fracture properties of grass leaves. Grass and Forage Science 38: 107-114.

Visen, N.S., J. Paliwal, D.S. Jayas, and N.D.G. White. 2004. Image analysis of bulk grain samples using neural networks. Can. Agric. Eng. 46:11–15.

Waghorn, G. C., I. D. Shelton, and V. J. Thomas. 1989. Particle breakdown and rumen digestion of fresh ryegrass (Lolium perenne L.) and lucerne (Medicago sativa L.) fed to cows during a restricted feeding period. British Journal of Nutrition 61:409-423.

Waldo, D. R. 1969. Factors influencing the voluntary intake of forages. Proc. Natl. Conf. Forage Qual. Eval. Util. p. E-1.

Waldo, D. R., L. W. Smith and E. L. Cox. 1972. Model of cellulose disappearance from the rumen. J. Dairy Sci. 55:125.

Welch, J. G., and A. M. Smith. 1969. Influence of forage quality on rumination time in sheep. J. Anim. Sci. 28:813.

Womac, A.R., C. Igathinathane, P. Bitra, P. Miu, T. Yang, S. Sokhansanj, and S. Narayan. 2007. Biomass Pre-processing Size Reduction with Instrumented Mills. ASABE Paper No. 076046. ASABE, St. Joseph, MI.

Woodford, S. T., and M. R. Murphy.1988. Effect of Forage Physical Form on Chewing Activity, Dry Matter Intake, and Rumen Function of Dairy Cows in Early Lactation. J. Dairy Sci. 71:674-686.

Woodman, H. E., J. W., Bee, and G. Griffith. 1930. Nutritive Value of Pasture. V. Pasture Grass Conservation: The Influence of Artificial Drying on the Digestibility of Pasture Herbage. J. Agr. Sci., 20: 53-62.

Yang, W. Z., K. A. Beauchemin, and L. M. Rode. 2001. Effects of Grain Processing, Forage to Concentrate Ratio, and Forage Particle Size on Rumen pH and Digestion by Dairy Cows. J. Dairy Sci. 84:2203–2216.

Yang, W. Z., and K. A. Beauchemin. 2005. Effects of Physically Effective Fiber on Digestion and Milk Production by Dairy Cows Fed Diets Based on Corn Silage. J. Dairy Sci. 88:1090–1098.

Yang W. Z., and K. A. Beauchemin. 2006a. Increasing the Physically Effective Fiber Content of Dairy Cow Diets May Lower Efficiency of Feed Use. J. Dairy Sci. 89:2694–2704.

Yang W. Z., and K. A. Beauchemin. 2006b. Effects of Physically Effective Fiber on Chewing Activity and Ruminal pH of Dairy Cows Fed Diets Based on Barley Silage. J. Dairy Sci. 89:217–228.

Yang, W. Z., and K. A. Beauchemin. 2007a. Altering Physically Effective Fiber Intake Through Forage Proportion and Particle Length: Chewing and Ruminal pH. J. Dairy Sci. 90:2826–2838.

Yang, W. Z., and K. A. Beauchemin. 2007b. Altering Physically Effective Fiber Intake Through Forage Proportion and Particle Length: Digestion and Milk Production. J. Dairy Sci. 90:3410–3421.

Yang, W. Z., and K. A. Beauchemin. 2009. Increasing physically effective fiber content of dairy cow diets through forage proportion versus forage chop length: Chewing and ruminal pH. J. Dairy Sci. 92:1603–1615.

Zebeli, Q., M. Tafaj, H. Steingass, B. Metzler, and W. Drochner. 2006. Effects of physically effective fiber on digestive processes and milk fat content in early lactating dairy cows fed total mixed rations. J. Dairy Sci. 89:651–668.

Zebeli, Q., M. Tafaj, I. Weber, J. Dijkstra, H. Steingass, and W. Drochner. 2007. Effects of Varying Dietary Forage Particle Size in Two Concentrate Levels on Chewing Activity, Ruminal Mat Characteristics, and Passage in Dairy Cows. J. Dairy Sci. 90:1929–1942.

Zebeli, Q., M. Tafaj, I. Weber, H. Steingass, W. Drochner. 2008. Effects of dietary forage particle size and concentrate level on fermentation profile, in vitro degradation characteristics and concentration of liquid- or solid-associated bacterial mass in the rumen of dairy cows. Animal Feed Science and Technology. 140:307–325.

Zebeli, Q., B.N. Ametaj, B. Junck, and W. Drochner. 2009. Maize silage particle length modulates feeding patterns and milk composition in loose-housed lactating Holstein cows. Livestock Science 124:33–40.