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The present thesis is a partial fulfilment of the requirements for a Master of Science Degree in Veterinary Medicine for International Students at The Swedish University of Agricultural Sciences (SLU), in the field of Animal Nutrition and Management.

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This work is dedicated to God almighty for his special favour and support throughout the study.

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Abstract

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There is limited information available on the effects of different forages on faecal characteristics in early-lactation dairy cows. In addition, information regarding relationships among dietary variables, production and faecal characteristics is limited. The aim of the present investigation includes 1) determination of the effects of diets, when differing in forage source, on faecal particle size distribution, consistency, pH and nutrient content as well as on production and body condition scores of early-lactation dairy cows; 2) investigation of potential correlations among dietary, production and faecal variables; and 3) understanding the principle involved in the use of image analysis to characterize particles from faeces of early lactating dairy cows. Twenty-six herds in Sweden comprising dairy cows (37.4±17.9 days in milk) of Swedish Red and Swedish Holstein breeds were used in the study. Nine herds used grass-clover silage (GCS) as the sole forage, eight herds used GCS combined with whole crop cereal silage (WCCS), four herds used GCS combined with whole-crop maize silage (MS) and five herds used GCS combined with ensiled pressed beet pulp (EPBP). The chemical composition of forages, nutrient contents of the diets and the formulated intakes were registered. Data were analysed by ANOVA using the GLM procedure of SAS (2001) with herd as the experimental unit. Visit was treated as repeated measure on a herd level. Because no significant treatment by visit interactions were found, data were analyzed as a mean over the two visits. Pearson partial correlation coefficients, with consideration to treatment effects, were estimated among dietary, production and faecal variables on a herd level basis (n=26) using the CORR procedure of SAS. Cows fed GCS alone or GCS combined with EPBP tended to have a higher yield of energy-corrected milk (ECM) than cows fed WCCS ($P<0.059$). For cows fed EPBP in addition to GCS, the higher ECM yield can be related to their higher formulated DM intake ($P<0.033$) and their improved feed conversion ($P<0.040$). Cows fed GCS combined with MS or WCCS had a higher faecal NDF concentration in dry matter than cows fed GCS alone or in combination with EPBP ($P<0.034$). The higher faecal NDF concentration in cows fed GCS and MS was associated with the improved faecal consistency score value by cows fed this diet ($P<0.016$). Increased formulated starch intake from concentrate and

diet increased the number of long faecal particles (r=0.439* and r=0.390†, respectively). Increased formulated DM intake of forage was associated with decreased faecal DM concentration (r=-0.541**) whereas increasing formulated DM intake of concentrate increased faecal DM concentration (r=0.630**). The strong positive linear correlation between number of grain kernels in the faeces and faecal starch concentration could be used as a tool to predict faecal starch content (r=0.827***). For the particle size determination by image analysis, gamma distribution curves (density plots) and accumulated distribution curves described and characterized the different particle dimensions in faeces into arithmetic mean particle length, arithmetic mean particle width, mode particle length, mode particle width, median particle length, median particle width, geometric mean particle length, geometric mean particle width and a 95-percentile fraction. The median length and width values, as well as the 95% length and width values were estimated using cumulative distribution function CDF in SAS. The 95-percentile fraction shows the maximum length or width for 95% of the faecal particles and describes the capacity of rumen to retain large particles. Image analysis can be used for measuring the characteristics, the length and width dimension values, of washed faecal particles.

Keywords: Dairy Cow, Faeces, Particle Size, Forages, Image Analysis

Introduction

Forage composition and the level of concentrate supplementation in dairy cow feeding have variable effects on intake, rumen fermentation, milk production and milk quality (Sutton, 1989). Changes in milk constituents have been reported to be due to the effects of diets on rumen fermentation pattern and quantity of glucose absorbed from the small intestine (Thomas and Chamberlain, 1984). Healthy rumen function in dairy cows can be ensured by feeding diets containing adequate amounts of physical structure (Yang and Beauchemin, 2005). Feeding dairy cows with diets rich in highly fermentable carbohydrates and finely chopped silages does not only encourage maximum milk yield, but also reduces fibre digestion and predispose the cows to metabolic diseases, such as subclinical ruminal acidosis, milk fat depression and displaced abomasum (NRC, 2001). Such feeding strategy results in extensive hindgut fermentation and consequently, presence of undigested long fibre particles in the feaces (Hall, 2002a). Fahey and Berger (1988) reported that cows consuming sufficient amounts of NDF but insufficient amounts of long particles have the tendency to have the same metabolic disorders as cows consuming diets deficient in chemical fibre. This led to the concept of physically effective neutral detergent fibre (peNDF; Mertens, 1997).The peNDF is the characteristic of forage that stimulates chewing and ruminal mat formation (Mertens, 1997). A healthy rumen environment is maintained if the roughage can stimulate chewing to produce salivary buffers sufficient enough to neutralize the effect of the fermentation acids produced in the rumen (Emery et al., 1960; Bailey and Balch, 1961; Yang et al., 2001a; Krause et al., 2002a; Beauchemin et al., 2003; Kononoff et al., 2003). Acids in the rumen are derived from the fermentation of carbohydrates which accounts for over 65% of the dry matter (DM) of dairy cattle diets (Mertens, 1997). An acidic rumen environment decreases fibre digestion (Mould et al., 1983). This is probably due to the destructive effect of the rumen acids on the fibre degrading micro organisms (Bonhomie, 1990; Yang and Varga, 1993). Particle size reduction is achieved through mastication, chemical and microbial degradation (Sahlin, 2006). Ruminants, which are unable to produce fibre degrading enzyme systems of their own, resort to the vast population of ruminal microbes which range from bacteria to protozoa and anaerobic fungi (Hume and Sakaguchil, 1991).When the rumen microbial population is adversely affected by a low pH, rumen residence time is reduced and undigested fibre particles pass quickly to the large intestine and ceacum, resulting in hindgut fermentation (Hall, 2002a). Hindgut fermentation has less nutritional relevance to the dairy cows because only VFA is absorbed whereas microbial proteins are lost in faeces as undigested particles (Hall, 2002a).

Different sieving techniques, such as wet and dry sieving, have been used to determine the size of particles in ingested feeds and faeces. Reports by Kennedy (1984) and Ulyatt et al. (1986) have shown that the wet-sieving procedure is the most widely accepted sieving method for particle-size determination. Although the method is widely accepted, it still lacks standardization in its measurement with respect to equipment, sieving time, degree of agitation and mass of particles applied to the sieves. The sieving techniques have given more insight to the concept of critical particle size (CPS). Particles that pass a sieve of a mesh size of 150 μm are fine enough to behave like solutes (Hungate, 1966; Weston and Hogan, 1967; Kennedy, 1984). However, in the rumen, only a few of them flow in the fluid phase, whereas a large number of them are trapped in the filter bed of the reticulorumen digesta mass (Faichney, 1986; Bernard et al., 2000). On the other hand, particles above a certain size are retained in the reticulorumen (Ulyatt et al., 1986). Therefore, this suggests that there is a certain size, CPS, above which large particles have a low probability of passing through the rumen. Poppi et al. (1980) suggested a CPS of 1.18 mm for cattle and sheep whereas Nørgaard and Sechic (2003) suggested a CPS of 5 mm for cattle. Van Soest (1982) stated that the CPS is not constant and increases when hay is ground and when the level of intake increases. This statement was challenged by Faichney (1986) because it was based on a mean particle size of the faeces, which is a measurement that gives no information about the CPS in contrast to the 95 percentile value. In recent times, new techniques that appear to be more reliable and describing have been developed. These include the use of separators with screens (Lammers et al., 1996), image analysis (Luginbuhl et al., 1984, Nørgaard and Bendixen, 2002), and laser diffractions (Olaisen et al., 2001). These new methods give information on both the size and shape of the particles in both ingested feed and faeces (Dijkstra et al., 2005).

Aims of the Investigation

- To determine the effects of diets, when differing in forage source, on faecal particle size distribution, consistency, pH and nutrient content as well as on production and body condition of early-lactation dairy cows.

- To investigate potential correlations among dietary, production and faecal variables.

- To understand the principle involved in the use of image analysis to characterize particles from faeces of early lactating dairy cows.

Study of Literature

Diet Composition and Intake by Dairy Cows

Effective utilisation and intake of DM by dairy cows depend on both the chemical composition and physical characteristics of the ration. Carbohydrates, as the major energy source for high-producing lactating dairy cows, account for 60 to 65% of the total DM in dairy rations (Mertens, 1997). The major carbohydrates in forages include cellulose and hemicellulose whereas the concentrates mainly contain starch, sugars and pectin. Recommendations by the National Research Council stated that the diet should contain relatively low amounts of fibre (NRC, 2001). This practice maximizes milk production but at the same time predisposes the lactating cows to metabolic disorders such as subclinical ruminal acidosis, milk fat depression, displaced abomasum and laminitis (NRC, 2001). Studies by others (McCullough, 1973; Mertens, 1985; Weiss and Shockey, 1991) have shown the importance of an optimal F:C ratio on the productivity of dairy cows. According to Mertens (1985), an NDF intake of 1.2 ± 0.1 percentage of live weight with 70 to 80 % of neutral detergent fibre (NDF) supplied from forage is optimal for early-lactating dairy cows. Mertens (1987, 1992) also proposed the use of NDF as a tool for establishing the upper limit for the F:C ratio of the dairy rations.

By definition, NDF is the partially or the slowly digested part of the ingesta. It measures the total chemical fibre in the feed. The NDF has been related to intake (Sudweeks et al., 1981; Allen, 1997; Mertens, 1994). Also, studies by others (Oba and Allen, 2000; Tafaj et al., 2004; Tafaj et al.,, 2005) have shown that increasing the amount of digestible fibre of hay or maize silage in dairy rations increases digesta stratification, particle breakdown in the rumen as well as digesta turnover, forage intake and fibre digestibility. Others have shown that increasing the non-fibre carbohydrates (NFC) of dairy rations increases the DM intake by dairy cows (Nocek and Russell, 1988; Batajoo and Shaver, 1994). This could probably be due to the palatable nature of the NFC.

Feeding cows with rations severely lacking in fibre or too high in non-structural carbohydrates, such as finely ground maize, has been shown to cause extensive hindgut fermentation resulting in negative effects on cow health and production (Hall, 2002a). Hindgut fermentation, which is a normal digestive process in horses and rabbits, encourages the loss of microbial proteins which is the source of essential amino acids to the ruminants (Varga, 2003). In addition, there is less buffering capacity in the hindgut than in the rumen, resulting in a more acidic environment, causing epithelial damage of the large intestine and long mucins in faeces, which is an indication

of hindgut fermentation (Hall, 2002b). This calls for a generally accepted standard to create a balance between forages and concentrates in diets in order to encourage both productivity and health of lactating dairy cows.

Physical Structure of Feeds and Chewing Activity by Ruminants

Chewing activity has been shown to be a response associated with the peNDF of a feed (Welch and Smith, 1969; Welch and Smith, 1970; Camell and Osbourn, 1972; Mertens, 1997). The peNDF is defined in relation to the physical characteristics of fibre that influence chewing and the bi-phasic nature of the rumen environment (Mertens, 1997). It is calculated as the physically effectiveness factor × the NDF concentration of the feed. The ef ranges from 0 to1, where long straw that requires much chewing has an ef of 1 and finely ground concentrate, that is not capable of stimulating chewing, has an ef of 0 (Mertens, 1997). The effective NDF (eNDF), on the other hand, is the NDF needed in a diet to maintain the percentage of milk fat produced by the cow (Mertens, 1997). Cattle have been reported to show a rhythm of chewing activity when fed long hay, whereas an evenly distributed chewing activity is exhibited by cows fed chopped hay (Jester and Murphy, 1983). Chewing occurs during 8 to 20 periods per day, which are evenly distributed throughout the day. Each chewing period lasts from five minutes up to two hours (Nørgaard, 2003). Chewing activity per kg of DM is an attribute that varies with the fibre content and particle size of the feed, level of intake and the physical state of the animal (Jaster and Murphy, 1983; Mertens, 1986; Sauvant et al., 1987; Nørgaard, 2003). When there is a high intake of feed, cattle spend less time chewing and ruminating per unit of feed (Welch and Hooper, 1988; Kovacs et al., 1997). Particle size and the NDF content of feed are more reliable indicators of chewing activity than the NDF content of the forage alone (Yang et al., 2001a). Studies have shown that group-fed cows tend to eat more than separately fed cows (Albright and Arave, 1997).This is because, cattle may be stimulated to start eating by imitating the first one that resumes eating after a break (Sahlin, 2006).

Increased amounts of fibre in dairy rations stimulate chewing activity and decrease the acid environment in the rumen (Yang et al., 2001a; Krause et al., 2002a; Beauchemin et al., 2003; Plaizer, 2004). Yang and Beauchemin (2006) reported that increased chewing time due to differences in dietary particle size does not always increase ruminal pH. The neutralizing effect of the salivary buffers, secreted by dairy cows during chewing, when they are fed diets containing adequate amounts of peNDF, levels out and neutralizes ruminal pH (Bailey and Balch, 1961; Allen, 1997; Maekawa et al., 2002). When too small amounts of peNDF is fed to dairy cows, there will be a

decreased chewing activity, resulting in less salivary buffer secretion, which results in a decreased ruminal pH. This results in alteration of the ruminal fermentation patterns with a low ratio of acetate to propionate and, consequently, to modified animal metabolism and reduced milk fat synthesis (Mertens, 1997). The fermentation acids produced have been shown to be the major cause of ruminal acidosis (Owens et al., 1998). To decrease the risks of ruminal acidosis, chewing time should be increased by adjusting the level of particle size and NDF concentration in the diet resulting in an improved ruminal pH status (Yang et al., 2001; Krause et al., 2002). However, the amount of peNDF that provides the most optimal chewing, saliva production and buffering of rumen environment has not been clearly stated (Einarson et al., 2004). This inconsistency probably could be due to the differences in measurements, definition of dietary peNDF and interactions among levels of concentrate inclusion, forage and grain sources and animal response variables (Zebeli et al., 2006). Santini et al. (1983) proposed that fibre or roughage intake can be adjusted by mean particle length to create a roughage index that more closely corresponds to total chewing time (TCT). The TCT is the sum of the rumination time and the eating time (Nørgaard, 2003). Nørgaard (2005) created a chewing index (CI) for dairy rations to be used in the new Nordic Feed Evaluation system "NorFor". The CI is the sum of the eating index (EI) and the ruminating index (RI) (Nørgaard, 2005). The National Research Council (2001) has also given a minimum NDF requirement from forage to be 15-19 % of dietary DM intake depending on dietary NDF concentration in order to allow for sufficient peNDF content in the diet.

The time spent chewing and ruminating is a function of animal and dietary factors (Beauchemin, 1991). Body weight has been described as a major determinant of rumination and chewing, as large animals tend to be more efficient in rumination than smaller animals (Bae et al., 1983). The efficiency of rumination is increased until a maximum value is reached after two years of age (Welch and Hooper, 1988). Weston et al. (1989) related rumination to particle size and intake. According to them, the duration of rumination increases with dietary intake and fibre content to a maximum of at least 12 h/day. Rumination times of cows at high intakes of forages of low feeding values can be 10 h/day (Dulphy et al., 1980). Longer particle sizes of feed influences the time spent eating, the time spent ruminating, the number of ruminating periods, the length of ruminating periods and the frequency of double contraction cycles in the reticulorumen (Nørgaard, 1989). Rumination time decreases when animals are fed diets with small amounts of roughage or when the roughage is finely chopped as finely chopped diets might not provide enough stimuli to evoke rumination (Nørgaard, 1989). Normal rumination requires forage particles greater than 5 mm to be retained in the rumen (Nørgaard, 1989). Increased intakes reduce

time spent ruminating per gram of feed, which explains the increase in average faecal particle size at higher intakes (Van Soest, 1994). Pseudorumination may occur if the feed particles are longer than 300 mm, such as long hay and silages and this may cause delayed and less efficient rumination activity (Deswysen and Vanbelle, 1978; Leek, 2004). Rumination takes up to 20 to 40% more time than eating (Nørgaard, 2003) and the rate varies in different animals during eating (Dulphy et al., 1980; Weston et al., 1989). During eating, rumination performs the primary function of comminution as well as facilitating the clearance of digested particles from the reticulo-rumen by reducing the particle size and positioning them at the zone of escape (adjacent to the reticulo-omasal orifice; Ulyatt et al., 1986; Waghorn et al., 1986; Ellis et al., 1999).

Ruminal pH, Milk Production and Milk Fat Percentage

Relationships among ruminal pH, milk production and milk fat percentage have been studied to a great extent. Beauchemin et al. (2003) and Krause and Comb (2003) recorded an increased milk production but a decreased milk fat percentage when cows were fed forages with low particle size. They also showed a reduction in ruminal pH to a relatively low level when ruminally fermentable carbohydrates were fed to lactating dairy cows. The studies suggest that a decrease in ruminal pH encourages high milk production but decreases milk fat yield. The decreased milk fat percentage has been reported to be due to the low proportion of acetate to propionate in the rumen (Mertens, 1997; Beauchemin et al., 2003). Mertens (1997) related milk fat percentage to eNDF. According to him, milk fat percentage is the animal's response that is associated with eNDF. Allen (1997) reported that milk fat percentage of cows in early lactation is less responsive to the diet than cows later in lactation. Therefore he suggested that the ruminal pH should be used as a response variable for determining the fibre requirements by dairy cows. Beauchemin et al. (2003) and Nelson and Satter (1990) also reported an increase in milk production due to high DMI when diets containing high proportion of alfalfa silage were fed to lactating dairy cows. This is because of the relatively low content of NDF in alfalfa and its high rate of digestion (Nelson and Satter, 1990; Beauchemin et al. 2003). Maintaining the ruminal pH at a normal status is important in dairy production because a decrease in ruminal pH decreases appetite (Britton and Stock, 1987), ruminal motility (Ash, 1959), fibre digestion (Mould et al., 1983), microbial yield (Hoover, 1986) and milk fat percentage (Mertens, 1997).

The volatile fatty acids (VFA), which are produced during ruminal fermentation, are absorbed across the rumen wall. Butyric acid, during its passage across the rumen wall converts into ß-hydroxybutyric acid (BHBA; McDonald et al., 1988). Acetic and

propionic acid on the other hand, pass almost unchanged across the rumen wall into the portal blood and are carried, together with the BHBA, to the liver (McDonald et al., 1988). The utilisation of the produced acetate is strongly insulin dependent (Kaneko, 1980). Both acetic and BHBA pass from the liver by blood circulation to various organs and tissues, where they are used as sources for energy and fatty acids (McDonald et al., 1988). Propionate is converted to glucose in the liver and joins the liver glucose pool. Some part of it may be converted to glycogen, and stored in the muscles or to fatty acids, reduced coenzymes and L-glycerol-3-phosphate and used for triacyl-glycerol synthesis (McDonald et al., 1988). Part of the glucose is circulated by the blood to various body tissues, where it may be used as energy source or as a source of reduced co-enzyme in the synthesis of fatty acids and glycerol.

Rumen Microbes and Nutrient Digestion

The rumen environment has a vast population of microorganisms, which have adapted the ruminant animals to various ecological niches and they are able to convert low quality feeds to high quality proteins (Varga et al., 1997). These rumen microbes attach, degrade and ferment structural carbohydrates in forage cell walls, thereby providing volatile fatty acids and protein to the host animal (Varga et al., 1997). Studies by Cheng et al. (1991) showed that ruminal fibre digestion depend on the following factors; plant structure and composition, which regulate bacterial access to nutrients; nature of the population densities of the predominant fibre digesting micro-organisms; microbial factors that control adhesion and hydrolysis by complexes of hydrolytic enzymes of the adherent microbial population and animal factors that increase the availability of nutrients through mastication, salivation and digesta kinetics. The major ruminal microbes include the cellulolytic bacteria *Ruminococcus albus, Ruminococcus flavefaciens* and *Fibrobacter succinogenes*, which account for 0.3-4 % of the bacterial population (Krause et al., 1999; Weimer et al., 1999), fungi, which accounts for 8% of the microbial biomass (Orpin, 1983) and a limited number of protozoal genera, which can digest 5-21% of the cellulosic material dependent on the diet (Dijkstra and Tamminga, 1995). The fibrolytic bacteria tend to degrade the more readily digestible structures, such as the mesophyll cells, but *F. succinogenes* also digests parenchyma bundle sheats, epidermal cell walls and leaf sclerenchyma (Akin, 1989). Varga et al. (1997) showed that these bacteria have an array of hydrolytic enzymes including cellulase and hemicellulase. The fungi degrade the more recalcitrant cell wall materials, including the sclerenchyma cells and the vascular tissue (Akin, 1989). Fungi have also been shown to have cutinase effects because of their ability to penetrate the cuticle and cell wall of lignified tissues (Kolattukudy,

1984). Studies by Gijzen et al. (1988) have shown that 19-28% of the total cellulase activity in fibre digestion can be attributed to protozoa. Akin (1989) however, suggested that digestion by protozoa seems to be limited to very susceptible tissues, such as the mesophyll cells.

Fermentation Patterns and Effects of Ruminal pH on Microbial Fermentation

The pattern of fermentation is influenced by the composition of the microbial population which in turn is determined by the basal diet, particularly the type of dietary carbohydrates and by the rate of depolarization of available substrates (review by Dijkstra, 1994). Diets rich in fibre encourage the growth of acetate-producing bacterial species and the acetate:propionate:butyrate molar proportions will typically be 70: 20:10 (Dijkstra et al., 2005). Feeding a starch-rich concentrate diet favours the development of propionate-producing bacteria. However, acetate is in most cases more abundantly produced than other acids (Dijkstra et al., 2005). Studies have shown that a large protozoal population could develop under certain conditions if a concentrate-rich diet is fed to ruminants, which will result in a shift from propionate to butyrate production (Williams and Coleman, 1997). Fermentation patterns can shift from acetic acid to propionic acid if the amount of substrates available for fermentation is high, either as a result of high intake or due to increased rates of depolarization of substrates (Dijkstra, 1994). Studies have shown that ruminal fermentation patterns could be affected by factors, such as physical form of diet, level of intake, frequency of feeding and the use of chemical additives in feed processing (Nagaraja et al., 1997; Ørskov, 1981; Thomas and Rook, 1981).

Effects of ruminal pH on rumen microbial activity have been demonstrated by various researchers. Hoover (1986) showed that when the ruminal pH was less than 6, the structural carbohydrate-fermenting microbes were usually limited. Russell and Wilson (1996) supported this finding after carrying out an in-vitro investigation of microbial activity at a decreased pH. From their studies, ruminal microbial activity was compromised when the rumen pH decreased below 6.2. Strobel and Russell (1986) also showed reduced microbial yield in vitro when the ruminal pH was low. The drop in rumen pH has been reported to be due to excess fermentation of starch to VFA, which overwhelms the buffering and absorptive capacity of the cow (Krause et al., 2003). Rapidly fermenting carbohydrates, such as sugars, soluble fibres and some starches, have the potential to decrease ruminal pH rapidly by virtue of the sheer mass of organic acids produced in the rumen in a

relatively short period of time. Sugars and starch are fermented to lactic acid, which is a stronger acid than acetic and butyric acids. However, sugars (mono- and disaccharides) may not be as prone to causing ruminal acidosis as starch, possibly due to the conversion of some portion of sugars to glycogen, as opposed to its immediate fermentation (Thomas, 1960).

A decrease in rumen pH due to the excess VFA has been shown to decrease fibre digestion (Mould et al., 1983) probably due to the defaunating effect of the ruminal acids (Bonhomie, 1990; Yang and Varga 1993). The ruminal acids kill or destroy the fibre digesting micro organisms, thereby interrupting the mechanism involved in fibre digestion. This results to the presence of undigested fibre particles greater than 0.5 inches in faeces (Hall, 2002a). The ruminal acids also cause some ulceration on the intestinal epithelium and this has been evidenced by the presence of mucin and fibrin cast in the faeces (Hall, 2002b).The undigested feed particles by-pass the rumen to the large intestine and ceacum thereby producing foamy, sticky and acidic faeces. This form of fermentation is of reduced nutritional value to the dairy cow since the microbial protein is lost in faeces although the VFA probably is absorbed (Van Soest, 1994). These findings suggest that effective fibre digestion is enhanced in a rumen environment with adequate rumen flora and at a normal and even pH level.

Ruminal Acidosis and Displaced Abomasum

Acidosis has been defined by Stedman (1982) as a decrease in the alkali (base excess) in the body fluids relative to the acid (hydrogen ion) content (Stedman, 1982). Ruminal acidosis is a production problem that usually occurs when ruminants ingest an excessive amount of readily fermented carbohydrates (Owen et al., 1998). It has been found to occur both in acute and chronic (subacute) forms (Owen et al., 1998). Acute ruminal acidosis is a condition that occurs when the ruminal pH is aproximately less than 5 to 5.2, whereas the subacute ruminal acidosis (SARA) is defined as a ruminal pH of approximately 5.2 to 5.6 (Mishra, 1970; Owen et al., 1998; Keunen et al., 2002). Acute ruminal acidosis is marked with increased osmolality, ruminal acidity and peracute ruminitis, with high accumulation of acids and glucose in the blood whereas chronic ruminal acidosis causes reduced performance and intake probably due to hypertonicity of the digesta (Radostits et al., 1994; Owen et al.,1998).

The etiology of ruminal and systemic acidosis has been described in excellent reviews by Britton and Stock (1987), Huntington (1988), Elanco (1993) and Hermon (1996). Carbohydrates in the rumen and ceacum are fermented to VFA and lactate by anaerobic microbes. These organic acids are absorbed by herbivores for metabolism in

tissues (Owen et al., 1998). However, when the carbohydrate supply is increased abruptly; following grain engorgement or during adaptation to high concentrate diets, the supply of total acids and the prevalence of lactate increases, which results in metabolic disturbances (Owen et al., 1998). These metabolic disturbances have been variously described as D-lactic acidosis; overeating, acute impaction, grain founder and grain overload (Dunlop and Hammond, 1965). Owen et al. (1998) showed that the occurrence of ruminal acidosis could be influenced by factors, such as grain source, grain processing and starch type, because of the effect of these factors on the rate of cleavage of starch to glucose. For example, wheat has a more readily extractable starch than maize, which has less surface area for microbial attack. Heat and pressure processing, particle size reduction, and high moisture storage of grain increase starch availability and propensity for acidosis (Reinhardt et al., 1997; Britton and Stock, 1987). Studies by Hungate (1966) showed that the release of free glucose in the rumen during fermentation encourages the growth of *Streptococcus bovis*, which thrives only when free glucose is available and has been incriminated as a major cause of lactic acidosis. *Streptococcus bovis* is a good lactate producer and the rate of production of lactate by this organism depends on the ruminal pH (Stone, 2004). Leedle (1993), however, showed that the concentration of these organisms is very low in the rumen of cattle fed high- concentrate diets. Studies by others (Mishra et al., 1970; Oetzel et al., 1996; Oba and Allen, 2000) showed that dairy cows with SARA have a low level of lactate.

Heat stress also has been reported to be one of the causes of ruminal acidosis (Dale and Brody, 1954). Heat stress alters a cow's acid-base balance when a cow pants and exhales carbondioxide and the total amount of buffering capacity within her system decreases (Dale and Brody, 1954). Clinically, acidosis can be diagnosed by measuring ruminal or blood acidity, with a ruminal pH of 5.6 and 5.2 often being used as a benchmark for chronic and acute acidosis, respectively (Cooper and Klopfenstein, 1996). Britton et al. (1991) has used variations in feed intake among days as an index for subclinical or chronic acidosis based on the concept that an increased variability from day to day in feed intake by an individual animal is associated with feeding acidotic diets (Britton and Stock, 1987).

Acidosis can be prevented by observing some good management practices. Feeding more roughage or modulating intake of starch by dairy cows reduces the incidence of acidosis (Owen et al., 1997). In addition, including monensin in the diets of ruminants has proved to decrease the incidence of digestive deaths in pens of feedlot cattle (Parrott, 1993; Vogel, 1996). This is probably due to the inhibition of certain lactate-producing bacteria and reduced daily variation in feed intake (Cooper and Klopfenstein, 1996).

Reports have indicated that the intensification in milk production in the dairy industry has an increasing effect on the incidence of displaced abomasums (DA). In Sweden for instance, there was an increased incidence of DA from 0.3 to 0.7 % between 1993 and 1999 and the corresponding increase in average annual milk production during the same period was from 7 740 to 8 300 kg of energy-corrected milk (ECM: 0.25 × kg of milk + 12.2 × kg of fat +7.7 kg of protein)/cow (Stengärde and Pehrson, 2002). The same study showed that multiparious cows were more prone to DA than the primiparious cows and that the Swedish Friesians were more predisposed to DA than the Swedish Red cows. The incidence of DA in lactating dairy cows has been suggested to be caused by conditions, such as, decreased forage to concentrate ratio (Coppock et al.,1972), presence of volatile fatty acids within the abomasum (Breukink, 1991), sorting occurring when feeding total mixed rations (Stengärde and Pehrson, 2002) and finely chopping of forages before ensiling (Shaver, 1997). The review by Shaver (1997) showed that when a high grain, low forage diet or finely chopped forages are fed to lactating dairy cows, there will be a reduction in the depth of the ruminal mat. The ruminal mat plays an important role in capturing grains so that they are fermented at the top of the ruminal fluid. The VFA produced at the top of the ruminal fluid are absorbed from the rumen with almost no VFA entering the abomasums in healthy cows. A thick ruminal mat is generally present during the dry period when cows are fed a high forage diet, but the depth of the ruminal mat is reduced drastically in early lactation, especially when cows experience pronounced declines in DMI (NRC, 2001). Although VFA within the abomasum can initiate abomasal displacement; the ruminal concentration of VFA is not highly correlated to the concentration in the abomasum (Breukink and de Ruyter, 1976). Other studies have shown that near parturition, a decline in plasma calcium up to 5 mg/dl can result in abomasal atony and distension with subsequent displacement of the abomasum (Hull and Wass, 1973; Curtis et al., 1983; Massey et al., 1993).

Clinical treatment of displaced abomasum has been studied by Oetzel, (1996). Oral administration of calcium chloride to cows with DA has proved to be an effective measure of preventing the metabolic disorder (Oetzel, 1996). Stengärde and Pehrson (2002) suggested a management approach, where the rumen fill is maintained by feeding forages rich in physically effective fibre both before and after calving.

Factors Affecting Faecal Particle Size and Consistency

Faecal particle size and consistency have been reported to be affected by the peNDF content of the feed that maintains rumen function and the impact of the type of non-fibre carbohydrates NFC

on ruminal pH (Hall, 2002a). When diets less in peNDF or high in digestible NFC is fed to lactating dairy cows, ruminal residence time is shortened and proper ruminal fermentation process will be disturbed (Varga, 2003). This results in the presence of long fibre particles in faeces (Hall, 2002a). Large fibre particles in faeces is an indication of short retention time of feed in the rumen and poor reduction in size of particles by rumination and microbial fermentation (Hall, 2002a) High-producing dairy cows show high DMI with subsequent increases in rates of passage of feed through the rumen. This results in more of the feed being fermented in the small intestine, caecum and large intestine. Inadequate intake of peNDF results in insufficient formation of the ruminal mat, which is not effective enough to retain large particles in the rumen (Mertens, 1997). Decreased feed particle size can increase faecal particle size if there is insufficient peNDF in the diet (Poppi et al., 1980). Long pieces of coarse fibre in faeces suggest sorting of feed whereas the presence of grain in manure is an indication of poor grinding or insufficient consumption of peNDF (Hall, 2002a). When cows are fed a well mixed TMR, there is a tendency of proper rumen fermentation and a better nutrient utilization than when cows are fed concentrates and forages separately (NRC, 2001). Animals' access to feed and sequence of feeding also have a role to play in the consistency and particle size of the faeces (NRC, 2001). Robinson and McQueen (1994) reported an increase in both ruminal pH and propionate concentration when number of feedings was increased from two to five per day. Klusmeyer et al. (1990) indicated that increasing feeding from two to four times per day did not improve ruminal fermentation patterns and milk production. In a related investigation, Sniffen and Robbinson (1984) hypothesized that feeding highly fermentable carbohydrates to cows that have been without feed for over 6 hrs could cause ruminal acidosis; and at the same time depress feed intake and fibre digestion. Therefore, they suggested that feeding forages as the first feed in the morning before other feedstuffs would encourage the formation of fibre mat as well as providing a buffering capacity in the rumen by salivary production associated with chewing.

The consistency of manure is a function of the feed moisture content and the mean retention time of the feed in the digestive tract of the animal (Varga, 2003). A normal faecal consistency consists of manure that has a medium porridge-like appearance and forms a dome-shaped pile, 2.5 to 5.0 cm high when dropped on the ground (Varga, 2003). Loose manure may be due to excessive intakes of protein or high levels of rumen degradable protein (Varga, 2003). This is probably because of increased water consumption in an attempt to excrete excess nitrogen through the urine. Extensive hindgut fermentation of carbohydrates and increased acid production may cause diarrhea. Also, poisoning, bacterial and parasitic

infections could result in diarrhea. Reports have also indicated that during periods of heat stress, cows produce large quantities of loose manure (Hall, 2002a). Studies have shown that high-producing cows excrete a more liquid faeces (Shellenberger and Kesler, 1961). Restricted water or protein intake and severe dehydration could result in firmer faeces (Varga, 2003).

Particle Size, Sieving Technique and Image Analysis

Particle size determination is usually performed either by a wet sieving or a dry sieving technique. Wet sieving has been described in the works of Kennedy (1984) and Ulyatt et al. (1986) as the most generally accepted procedure for identifying long particles in faeces. The technique has helped to understand the concept of the CPS of 1.18 mm, below which particles can pass through the reticulo-omasal orifice (Ulyatt et al., 1976; Reid et al., 1977; Poppi et al., 1980, 1985; Mertens, 1997). The wet sieving technique also was used by Tomoko et al. (2004) to explain the effect of different forage types on particle size distribution of ruminal digesta and faeces of non-lactating dairy cows fed high-quality forages. From their findings, the CPS for escape from the reticulo rumen was between 2 and 4 mm for cows fed round-baled silages and long orchardgrass hay. They concluded that particle size reduction and passage from the rumen could be attributed to intrinsic factors of the fed forages. These results agree with the findings by Nørgaard and Sechic (2003), who observed that 95% of washed faeces particles from cattle fed grass silage at maintenance level were shorter than 5 mm. Washing of faecal samples, which is one of the preliminary steps to dry sieving techniques and image analysis, has been described by Nørgaard et al. (2004). Dry sieving has been applied in several studies to understand the different characteristics of plant materials fed to ruminant animals (Robertson and Van Soest, 1982; Kennedy and Poppi, 1984; Ulyatt et al., 1986; Faichney and Brown, 1991; Nørgaard et al., 2004). The particles were separated into different fractions based on their ability to pass through apertures of different sizes. Luginbuhl et al. (1988) observed that materials retained by any sieve represent a range of sizes determined by the resistance of that sieve to onward passage of particles and by the mesh size of the sieve situated immediately above it. Luginbuhl et al. (1985) observed a pronounced inverse relationship in the length to mesh size ratio of scanned particles and they suggested that the smaller the particles the more likely it is to be presented end on for passage through the sieve. This suggests the manner in which particles are reduced in size during ingestive chewing, comminution and the method of positioning at the zone of escape from the rumen. Large particles are chewed during eating until they are reduced to a certain size, which is appropriate for bolus formation and subsequent swallowing. The swallowed

particles are ruminated and fermented to small sizes that fall to the ventral rumen. Following series of double contraction cycles at the reticulo-omasal orifice, small particles below 1.18 mm pass through to the abomasum (Ulyatt et al., 1976; Reid et al., 1977; Poppi et al., 1980, 1985).

Image analysis has also been used in some studies to understand the different characteristics of forage samples fed to ruminant animals (Grenet et al. 1989; Nørgaard and Bendixen, 2002; Nørgaard and Sehic, 2003). Grenet et al. (1989) investigated, by use of image analysis, the different morphological characteristics of ruminal digesta particles in sheep fed mixed grass hay. They concluded that a precise assessment of particle length, width, area and shape could be made by use of image analysis. Nørgaard and Sehic (2003) reported a particle length distribution in the faeces of cows fed roughages and concentrates from different diet sources to be similar to their observations in lambs fed the same diets. Also, the distribution of particle length and width of sieved forage samples have been charaterized by mode, median, arithmetic mean, geometric mean and 95 percentile values (Nørgaard and Bendixen, 2002; Nørgaard and Sehic, 2003; Nørgaard et al., 2004;).

The use of logarithmic (log) normal curves to describe the weight of sieved particles has been adopted by the American Society of Animal Science (1969), the American Society of Dairy Science (1970) and Waldo et al. (1971). Waldo et al. (1971) suggested that the log normal distribution gives a thorough description of the weight distribution of sieved digesta from the rumen and faeces of cows fed chopped and pelleted hay. However, data from image analysis has shown that forages are not fragmented into spherical or cuboidal shapes (Luginbuhl et al., 1988); as a result, there is likely a deviation from the log normal distribution of the lengths of sieved particles according to Waldo et al. (1971). Gamma distribution curves and cumulative distribution curves have been used to describe the length and width distribution of particles from both feed and faeces samples (Nørgaard and Bendixen, 2002; Nørgaard and Sechic, 2003; Nørgaard et al., 2004). The shape of the gamma distribution curve explains rumen fermentation (Nørgaard et al., 2004). A flat curve indicates many long particles whereas a steep curve indicates many short particles in faeces (Nørgaard et al., 2004). Many small particles in faeces describe good rumen fermentation (Nørgaard et al., 2004). However, many long particles in faeces indicate a diet that has not been properly fermented in the rumen (Nørgaard et al., 2004).

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Faecal characteristics and production of early-lactation dairy cows fed diets differing in forage source

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Abstract

Effects of feeding grass-clover silage alone or in combination with another forage source on faecal characteristics and production of early-lactation dairy cows were studied in 26 herds comprising cows of Swedish Red and Swedish Holstein breeds. The study also involved investigations of potential correlations among dietary, production and faecal variables. Nine herds used grass-clover silage (GCS) as the sole forage, eight herds used GCS combined with whole crop cereal silage (WCCS), four herds used GCS combined with whole-crop maize silage (MS) and five herds used GCS combined with ensiled pressed beet pulp (EPBP). The chemical composition of forages, nutrient contents of the diets and the formulated intakes were registered. Data were analysed by ANOVA using the GLM procedure of SAS (2001) with herd as the experimental unit. Visit was treated as repeated measure on a herd level. Because no significant treatment by visit interactions were found, data were analyzed as a mean over the two visits. Because breed and housing system did not have significant effects on any of the variables studied, they were not used as covariates in the model. Pearson partial correlation coefficients, with consideration to treatment effects, were estimated among dietary, production and faecal variables on a herd level basis (n=26) using the CORR procedure of SAS. Cows fed GCS alone or GCS combined with EPBP tended to have a higher yield of energy-corrected milk (ECM) than cows fed WCCS (P=0.059). For cows fed EPBP in addition to GCS, the higher ECM yield can be related to their higher formulated DM intake (P=0.033). Cows fed GCS combined with MS or WCCS had a higher faecal NDF concentration in dry matter than cows fed GCS alone or in combination with EPBP (P=0.034). The higher faecal NDF concentration in cows fed GCS and MS was associated

with the improved faecal consistency score value by cows fed this diet (P=0.016). Increased formulated starch intake from concentrate and diet increased the number of long faecal particles (r=0.439* and r=0.390†, respectively). Increased formulated DM intake of forage was associated with decreased faecal DM concentration (r=-0.541**) whereas increasing formulated DM intake of concentrate increased faecal DM concentration (r=0.631**). The strong positive linear correlation between number of grain kernels in the faeces and faecal starch concentration could be used as a tool to predict faecal starch content (r=0.827***).

Keywords: Dairy Cow, Faeces, Particle Size, Forages

Introduction

Dairy diets are formulated to achieve high intake and productivity by balancing fibre and non-fibre carbohydrates with the protein needs of the cow (McCullough, 1973; Weiss and Shockey, 1991). A low forage to concentrate ratio in dairy diets may decrease feed efficiency and increase the risk of digestive disorders (Nocek, 1997). Evaluating the faecal characteristics of dairy cows provides information about the general health, rumen fermentation and digestive functions of the cows. A low intake of dietary physically effective neutral detergent fibre (peNDF) may compromise the function of the ruminal mat of longer particles, rumen motoric and fibre digestion (Weidner and Grant, 1994; Mertens, 1997). Tafaj et al. (1999, 2001) observed that dairy cows fed a ground - (2.6 mm) vs. a long (28.7 mm) or a chopped (9.2 mm) hay, had impaired rumen conditions, including a 10-h shorter mean rumen retention time of digesta and a decreased fibre digestibility. According to Hall (2002), such feeding regimes may result in undesirable rumen fermentation and presence of undigested fibre particles in faeces. Failure of the rumen to selectively retain particles could result in passage of potentially digestible feed particles to the small and large intestine, which, consequently, might cause faeces with a loose and foamy consistency (Hall, 2006). On the contrary, a firm faeces characteristic indicates less hindgut fermentation and a low intake of dietary protein (Hall, 2006).

The presence of large fibre particles and grain kernels in the faeces indicates a too short retention of feed particles in the ruminal system to achieve a proper particle size reduction during rumination and microbial degradation (Hall, 2002). This may be due to an inadequate intake of peNDF for stimulating rumination and maintaining a normal ruminal pH (Mertens, 1997). Dairy cows in early lactation have a high intake and a high passage rate of feeds out of the rumen. Furthermore, cows in early lactation require high-energy diets to minimize body condition losses and to fulfil energy demands for high milk yields. A high grain intake is required to meet such energy demands. Nørgaard et al. (2004) observed that whole grain in the rumen combined with a starchy diet, low in peNDF, might reduce the capacity of the rumen system to retain large particles. However, increasing the amount of digestible fibre of hay or maize silage in dairy rations increases digesta stratification, particle breakdown in the rumen as well as digesta turnover, forage intake and fibre digestibility (Oba and Allen, 2000; Tafaj et al., 2005). Maize silage has a lower NDF concentration than grass silage because of its high grain concentration (Allen et al., 2003). In addition, maize silage and whole crop cereal silage contain NDF that has a lower rate of potential degradability in the rumen than grass

silage (Quirke et al., 2002; Bååth Jacobsson, 2005). Ensiled pressed beet pulp has a relatively high rate of fibre degradation in rumen because of its content of the soluble fibre fraction pectin (Mara et al., 1999; Micard and Thibault, 1999; Hartnell et al., 2005).

The use of wet sieving technique for particle size determination in faeces has been documented in the works by Poppi et al. (1980), Kennedy (1984) and Ulyatt et al. (1986). The technique has a wide application and has given information on the dynamics involved in ruminal digesta flow and particle communition (Kennedy, 1984; Ulyatt et al., 1986). Kennedy and Poppi (1984) have tried to develop a dynamic model of ruminal digestion based on the critical particle theory. Particles leaving the reticulo-rumen are reduced to a certain size (critical particle size (CPS)) small enough to pass through the reticulo-omasal orifice (Kennedy and Poppi, 1984).

There is limited information available on the effects of different forages on faecal characteristics in early-lactation dairy cows. In addition, information regarding relationships among dietary variables, production and faecal characteristics is limited. The aim of this study was to determine effects of diets, when differing in forage source, on faecal particle size, consistency, pH and nutrient content as well as on production, body condition and cleanliness of early-lactation dairy cows. The study also involved investigations of potential correlations among dietary, production and faecal variables.

Materials and Methods

Animals and management

This experiment was conducted in 29 dairy herds comprising cows of Swedish Red breed and Swedish Holstein breed (Table 1). The herds were visited twice; the first visit occurred during the period from November 2004 to January 2005 and the second visit occurred from February to April, 2005. The diets were changed between visits in two of the herds and one herd was visited only once, resulting in 26 herds for data analysis. Twenty-three of the herds were located in the south-west region and the remaining three were located in Uppland in the east of Sweden.

From each of the 26 herds, 5 cows in early lactation, less than 78 days in milk (37.4±17.9 DIM) were randomly selected at each visit, because cows at this stage of lactation are more prone to nutritional disorders. The number of milking cows and rolling herd average are shown in Table 1. The cows were milked twice a day and the milk yield and composition (fat, protein, and urea) were recorded once a month at the regular test day. The energy-corrected milk (ECM) was calculated according to Sjaunja et al. (1991).

Table 1. Feeding, production and management characteristics of the studied herds.

Forage	Herd	Breed	Number of cows milking	Loose Housing	Robot	ECM[1] (kg)
GCS[2]	1	SR[6]	49			9208
GCS	2	SR/SH[7]	90	Yes		8792
GCS	3	SR/SH	149	Yes		9403
GCS	4	SR/SH	49			8999
GCS	5	SH	93			9559
GCS	6	SH	48	Yes		10075
GCS	7	SR	73	Yes	Yes	9844
GCS	8	SR	46			11031
GCS	9	SH	58	Yes	Yes	11560
GCS+WCCS[3]	10	SH	54	Yes		7502
GCS+WCCS	11	SR	81	Yes	Yes	10117
GCS+WCCS	12	SH	62	Yes		9189
GCS+WCCS	13	SR/SH	61			8155
GCS+WCCS	14	SR/SH	129	Yes		9612
GCS+WCCS	15	SR	55			9428
GCS+WCCS	16	SR/SH	58	Yes		8734
GCS+WCCS	17	SR	60			10365
GCS+MS[4]	18	SR/SH	104	Yes		10290
GCS+MS	19	SR/SH	77			11043
GCS+MS	20	SH	37			10770
GCS+MS	21	SR	48			10916
GCS + EPBP[5]	22	SR	76	Yes		10870
GCS + EPBP	23	SR/SH	128	Yes		9190
GCS + EPBP	24	SR/SH	121	Yes		9596
GCS + EPBP	25	SH	41			11606
GCS + EPBP	26	SH	76	Yes		10036

[1]ECM=energy-corrected milk on an average annual basis in the herd (rolling herd average)
[2]GCS=grass-clover silage
[3]WCCS=whole-crop cereal silage
[4]MS=maize silage
[5]EPBP=ensiled pressed beet pulp
[6]SR=Swedish Red Breed
[7]SH=Swedish Holstein Breed

Feeds and feeding

The harvested forages were treated with additives, such as acids, salts, inoculants or sugar sources, in 81% of the herds before being stored in silos, round bales, piles or tubes. Out of the 26 selected

herds, 9 herds were fed grass-clover silage as the sole forage, 8 herds were fed grass-clover silage and whole crop cereal silage, 4 herds were fed grass-clover silage and whole crop maize silage and 5 herds were fed grass-clover silage and ensiled pressed sugar beet pulp. The concentrates were fed either separately or mixed with forages to a mixed ration. Concentrates were complete feed or small grains and purchased protein concentrates or rapeseed, flaxseed, peas, rapeseed cake and field beans. Feeding strategies in the herds are shown in Table 2.

Registrations

During each of the two visits in a herd, herd level registrations were made for feed storage and quality as well as milk production. Chemical composition of the different forages fed is shown in Table 3. Whole-crop cereal silage consisted of barley, oats and wheat in pure stands or in mixtures with peas or field beans.

Individual feed rations and milk production from 5 cows in early lactation were registered in all the herds visited. Feed rations used in the herds were reformulated according to milk yield registered from the latest monthly test day. Formulated intake, that is the expected feed intake according to milk yield, was registered (Table 4). Each of these 5 cows were checked for body condition scores (BCS) by visual observation and palpation according to Edmonson et al. (1984), cleanliness according to Cook (2002) and general health. The BCS was determined on a scale from 1 to 5 with a 0.5-unit precision, where 1 is extremely thin and 5 is excessively fat (Edmonson et al., 1984). Cleanliness of the cows was determined on a scale from 1 to 4, where 1 is completely clean and 4 represents dried manure covering legs, udder and tighs (Cook, 2002). General health of the cows was checked.

Table 2. Feeding strategies in the herds.

Forage	Herd	Feeding regime	TCL[1] of ensiled forages (cm)	Frequency of daily allocation of forage or mixed ration of forage	Frequency of daily concentrate feeding
GCS[2]	1	Separate[6]	4	4	4
GCS	2	Separate	4	2	AF[9]
GCS	3	Separate	4	2	AF
GCS	4	PMR[7]	7	5	2
GCS	5	Separate	4.5	4	6
GCS	6	PMR	27.5	2	AF
GCS	7	Separate	3	2	AF
GCS	8	Separate	2	2	AF
GCS	9	Separate	7	2.5	AF
GCS+WCCS[3]	10	PMR	15	2	2
GCS+WCCS	11	PMR	2	2	AF
GCS+WCCS	12	PMR	2	4	AF
GCS+WCCS	13	PMR	-	3	6
GCS+WCCS	14	TMR[8]	2	3	
GCS+WCCS	15	Separate	2	8	8
GCS+WCCS	16	Separate	4.5	2	2
GCS+WCCS	17	PMR	6	4	6
GCS+MS[4]	18	TMR	3	2	
GCS+MS	19	Separate	2.5	4	5
GCS+MS	20	Separate	4.5	2	5
GCS+MS	21	PMR	7	7	7
GCS + EPBP[5]	22	PMR	3.5	3	AF
GCS + EPBP	23	PMR	2.5	7	AF
GCS + EPBP	24	PMR	5.5	8	AF
GCS + EPBP	25	PMR	4	6	6
GCS + EPBP	26	PMR	4	3	AF

[1]TCL=theoretical chopping length
[2]GCS=grass-clover silage
[3]WCCS=whole-crop cereal silage
[4]MS=maize silage
[5]EPBP=ensiled pressed beet pulp
[6]Separate=Forage and concentrate fed separately
[7]PMR= Partially mixed ration
[8]TMR=Totally mixed ration
[9]AF=Automated feeding

Table 3. Chemical composition in g/kg DM, unless stated otherwise, of forages fed in the herds (*n*=no. of herds).

	GCS[1] Harvest 1 *(n=19)*		GCS Harvest 2 *(n=19)*		GCS Harvest 3 *(n=12)*		WCCS[2] *(n=8)*		MS[3] *(n=4)*		EPBP[4] *(n=5)*	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
DM[5], %	36.0	7.5	32.4	7.7	34.3	14.1	34.9	10.3	31.9	2.3	27	-
NDF[6]	469	60	502	52	460	57	476	34	417	66	307	21
CP[7]	164	25	148	15	166	14	108	34	79	7	100	5
AAT[8]	72	1.3	70	1.6	71	1.3	52	22	80	-	100	-
PBV[9]	38	26	27	15	44	14	1	29	-65	4.6	-68	-
ME[10]	11.4	0.6	10.5	0.6	10.7	0.5	9.5	0.3	11.0	0.1	12.8	-
Starch	-	-	-	-	-	-	83	59	256	98	5.0	-

[1]GCS=grass-clover silage
[2]WCCS=whole-crop cereal silage
[3]MS=maize silage
[4]EPBP=ensiled pressed beet pulp
[5]DM=dry matter
[6]NDF=neutral detergent fibre
[7]CP=crude protein
[8]AAT=amino acids absorbed in small intestine
[9]PBV=protein balance in rumen
[10]ME= metabolizable energy expressed in megajoule (MJ)/kg DM

Table 4. Formulated intakes of forages and concentrates fed to dairy cows in early lactation. Values are averaged over herds within treatment (*n*=no. of herds).

	Treatment							
	GCS[1] *(n=9)*		GCS + WCCS[2] *(n=8)*		GCS + MS[3] *(n=4)*		GCS +EPBP[4] *(n=5)*	
Dietary Ingredient	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
GCS, kg DM	10.0	2.4	8.0	1.9	6.7	1.0	8.0	0.9
WCCS, kg DM	-	-	2.8	1.5	-	-	-	-
MS, kg DM	-	-	-	-	3.4	0.4	-	-
EPBP, kg DM	-	-	-	-	-	-	4.1	2.1
Total forage, kg DM	10.0	2.4	10.8	2.1	10.1	0.8	10.0	1.5
Concentrate, kg DM	12.0	3.0	10.8	3.1	11.1	3.1	14.3	2.8
Forages, % of total feed	45.8	12.8	50.6	11.1	48.8	8.9	41.7	6.7

[1]GCS=grass-clover silage
[2]WCCS=whole-crop cereal silage
[3]MS=maize silage
[4]EPBP=ensiled pressed beet pulp

Nutrient concentrations of rations given to cows in early lactation are shown in Table 5.

Table 5. Nutrient concentrations of the diets fed to dairy cows in early lactation. Values are averaged over herds within treatment (*n*=no. of herds).

	Treatment							
	GCS[1] *(n=9)*		GCS + WCCS[2] *(n=8)*		GCS + MS[3] *(n=4)*		GCS +EPBP[4] *(n=5)*	
Nutrient	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
CP[5], g/kg DM	183	13	171	22	188	14	206	18
AAT[6], g/MJ ME	7.8	0.7	7.5	1.1	8.1	0.6	8.7	1.3
PBV[7], g/day	551	171	455	186	408	180	343	185
NDF[8], g/kg DM	344	26	340	41	336	36	353	17
Starch, g/kg DM	160	38	174	44	186	31	146	30
ME[9], MJ/kg DM	12.1	0.8	11.5	1.0	11.9	1.1	11.5	1.6

[1]GCS=grass-clover silage
[2]WCCS=whole-crop cereal silage
[3]MS=maize silage
[4]EPBP=ensiled pressed beet pulp
[5]CP=crude protein
[6]AAT =amino acids absorbed in the small intestine per megajoule (MJ) of metabolisable energy (ME)
[7]PBV=difference between the amount of rumen degradable protein and the amount of microbial protein synthesized in the rumen
[8]NDF=neutral detergent fibre.
[9]ME=metabolizable energy expressed in megajoule (MJ)/kg DM

Sample collection

Faecal samples were collected from each of the 5 cows in a bucket placed directly behind the rectum and the cows were allowed to drop the faeces at will. Consistency of the faeces was determined during the excretion as described by Zaaijer and Noordhuizen (2003) and modified by Steen (2004). The consistency was determined on a scale from 1 to 5 with 0.5-unit precision, where 1 represented runny faeces and 5 hard and dry faeces (Steen, 2004). Faecal colour was scored on a scale from 1 to 3 where 1 represented dark brown, 2 was brown and 3 was yellowish brown (Steen, 2004). Faecal pH was determined with litmus paper (pH range 6.4-8, Merck KgaA, Darmstadt, Germany). A composite faecal sample of 300-400 g from the 5 cows was collected and frozen for later analysis of contents of DM, neutral detergent fibre (NDF), and starch as well as particles size. In addition, samples of silages being fed to the herds at the time of the visits were taken for analysis of chemical composition if not already analysed by the farmer.

Chemical analysis

The DM content of the silage was determined at 60ºC for 24 h, whereas the DM content of faeces was determined at 55-60ºC for 48 h. The crude protein content of silage was determined as the total N concentration by the Kjeldahl technique in a Tecator Kjeltec Auto Sample 1035Analyser (Tecator Inc., Höganäs, Sweden). The NDF concentration of silage and faeces was determined according to Goering and Van Soest (1970), with the inclusion of $NaSO_4$ after heat treatment of the ND solution, which followed amylase treatment. Whole-crop cereal silage and maize silage were analysed enzymatically for starch content, including maltodextrin, according to Larsson and Bengtsson (1983). The starch content of the faeces was analysed with the same method but without correction for free glucose because of its insignificant content in faeces. Amino acids absorbed in small intestine (AAT) and protein balance in rumen (PBV) were calculated from standards according to Madsen et al. (2005).

Wet sieving of faeces

Each composite faecal sample was put in a plastic bucket and mixed thoroughly with a spoon in order to distribute the particles evenly. A duplicated 100-g faecal sample was used for wet sieving of each composite sample. Samples were wet sieved with a 2.36-mm sieve placed in a plastic container under running tap water until it was 2/3 full with water. The faeces were dissolved in the sieve, which was shaken gently with two hands to ensure that all the

particles were thoroughly washed. The dark green water resulting from the dirty samples was discarded and then the sample was rewashed 3 to 4 times until only the visible particles and kernels remained.

The sieve with the particles on it was placed on a surface and particles >1cm were removed with an artery forceps. The length of the particles was measured to ensure their sizes being longer than 1 cm. The particles >1cm and the grain kernels were counted and their numbers were recorded. The counted particles and kernels were dried in an oven at 105˚C for 24 h. Weight of the dried particles were recorded. Average number of particles >1 cm and grain kernels, as well as average dry weights of long particles, were calculated from the duplicates of each faecal sample. Also the percentage of long particles (>1 cm) on a dry weight basis was calculated for each duplicate and then averaged over the duplicates for each faecal sample.

Statistical analysis

Data from 26 herds were analysed by analysis of variance using the GLM procedure of SAS version 8.2 (2001) and herd as the experimental unit. Sources of variation in the model were forage treatments and visits. Visit was treated as a repeated measure on herd level. Because no significant treatment by visit interactions were found, data were analyzed as a mean over the two visits. Because breed and housing system did not have significant effects on any of the variables studied, they were not used as covariates in the model. Number of herds (*n*) per treatment were 9, 8, 4 and 5 for grass-clover silage, grass-clover silage + whole-crop cereal silage, grass-clover silage + maize silage and grass-clover silage + ensiled pressed beet pulp, respectively. When a significant *F*-value was detected at $P<0.05$, unless stated otherwise, significant differences among treatment means were tested by using a t-test (LSD) with a 95% confidence interval. Pearson partial correlation coefficients, with considerations to treatment effects, were estimated among dietary, production and faecal variables on a herd level basis (n=26) using the CORR procedure of SAS (2001). Linear correlations were declared significant at $P<0.05$ and tendencies to significant correlations were discussed at $P<0.10$. Data describing diet composition averaged over herds within treatment are shown as means and standard deviations (S.D.).

Results

Feed intake, milk production and faecal characteristics

Cows fed grass-clover silage combined with ensiled pressed beet pulp had higher formulated DM and NDF intakes than cows fed the other dietary treatments (Table 6). Furthermore, cows fed grass-clover silage combined with ensiled pressed beet pulp had a higher formulated intake of amino acids absorbed in the small intestine than cows fed whole-crop cereal silage in combination with grass-clover silage (Table 6). No differences in the theoretical length of cut of the silages were found among the dietary treatments (data not shown).

Table 6. Formulated daily intakes of dry matter (DM) and nutrients as well as feed efficiency of early-lactation dairy cows fed different dietary treatments. Values are averaged over herds within treatment (*n*=no. of herds).

	Treatment				Significance	
	GCS[1] *(n=9)*	GCS + WCCS[2] *(n=8)*	GCS + MS[3] *(n=4)*	GCS + EPBP[4] *(n=5)*	*P*-value	S.D.
Total DM, kg	22.0[b]	21.6[b]	21.2[b]	24.3[a]	0.033	1.69
Total CP[5], kg	4.03	3.71	3.99	4.38	0.111	0.46
Total AAT[6], kg	2.07[a,b]	1.88[b]	2.08[a,b]	2.40[a]	0.036	0.29
Total NDF[7], kg	7.58[b]	7.30[b]	7.10[b]	8.58[a]	0.054	0.86
Total starch, kg	3.53	3.78	3.98	3.54	0.821	0.89
Total ME[8], MJ	268	247	253	280	0.198	27.7
FC[9], MJ ME/kg ECM[10]	7.00	7.19	6.50	6.88	0.561	0.78
FC, kg DM/kg ECM	0.57	0.62	0.54	0.59	0.176	0.64

[a,b]Values in the same row with different superscripts differ significantly at $P<0.05$.
[1]GCS=grass-clover silage
[2]WCCS=whole-crop cereal silage
[3]MS=maize silage
[4]EPBP=ensiled pressed beet pulp
[5]CP=crude protein
[6]AAT=amino acids absorbed in small intestine
[7]NDF=neutral detergent fibre
[8]ME=metabolizable energy expressed in megajoule (MJ)
[9]FC=feed conversion expressed in megajoule (MJ) metabolizable energy (ME)/kg ECM
[10]ECM=energy-corrected milk

Cows fed a diet containing grass-clover silage alone or in combination with ensiled pressed beet pulp tended to yield more milk in kg ECM and also yielded more milk protein compared to cows fed grass-clover silage combined with whole-crop cereal silage (Table 7). Yield of milk fat was higher for cows fed grass-clover silage combined with maize silage or fresh sugar beet pulp than for cows fed grass-clover silage combined with whole-crop cereal silage. Cows fed grass-clover silage combined with maize silage tended to have a higher content of milk urea than cows fed the other dietary treatments.

Faecal consistency was improved when feeding a diet containing grass-clover silage combined with maize silage compared to feeding the other dietary treatments. Cows fed grass-clover silage combined with maize silage or whole-crop cereal silage had a higher faecal NDF content than cows fed grass-clover silage alone or in combination with ensiled pressed beet pulp (Table 7). There were no differences in the contents of long particles or grain in faeces from cows fed the different dietary treatments (Table 7). Cows were in good general health (data not shown).

Table 7. Milk production, body condition, cleanliness and faecal characteristics of early-lactation dairy cows fed different forage treatments. Values are averaged over herds within treatment (*n*=no. of herds).

	Treatment				Significance	
	GCS[1] *(n=9)*	GCS + WCCS[2] *(n=8)*	GCS + MS[3] *(n=4)*	GCS+ EPBP[4] *(n=5)*	*P*-value	S.D.
Lactation no.	2.52	2.39	2.35	2.10	0.320	0.19
Days in milk	38.8	35.4	32.5	42.2	0.459	9.88
Energy-corrected milk, kg	38.7[a]	34.6[b]	39.0[a,b]	40.9[a]	0.059	4.04
Milk, kg	38.4	34.8	38.0	41.5	0.102	4.54
Milk fat, %	4.16	4.11	4.38	4.04	0.339	0.29
Milk protein, %	3.29	3.23	3.28	3.19	0.612	0.15
Yield of milk fat, kg	1.59[a,b]	1.43[b]	1.66[a]	1.67[a]	0.050	0.17
Yield of milk protein, kg	1.26[a]	1.12[b]	1.24[a,b]	1.32[a]	0.048	0.12
Milk urea, m*M*	4.81[b]	4.66[b]	6.28[a]	4.95[b]	0.059	0.93
Cleanliness[5]	2.26	2.25	1.80	2.18	0.356	0.45
Body condition score[6]	2.66	2.79	3.03	2.70	0.442	0.38
Faecal colour[7]	2.08	2.09	2.28	2.57	0.106	0.37
Faecal consistency[8]	2.32[b]	2.55[b]	3.05[a]	2.39[b]	0.016	0.35
Faecal pH	7.13	7.23	7.24	7.49	0.324	0.34
Faecal dry matter, %	14.48	14.18	15.15	14.51	0.580	1.11
Faecal starch, % of DM	1.33	1.24	0.95	0.96	0.844	0.92
Faecal NDF, % of DM	41.41[b]	45.57[a]	46.21[a]	41.00[b]	0.034	3.59
Long faecal particles, no./100 g faeces	41.4	41.7	34.3	39.2	0.535	8.75
Long faecal particles, g DM/100 g faeces	0.12	0.15	0.11	0.13	0.487	0.04
Long faecal particles, % of DM	0.88	1.01	0.74	0.93	0.410	0.28
Faecal grains, no./100 g faeces	12.9	11.9	8.9	8.3	0.862	11.12

[a,b]Values in the same row with different superscripts differ significantly at $P<0.05$.
[1]GCS=grass-clover silage
[2]WCCS=whole-crop cereal silage
[3]MS=maize silage
[4]EPBP=ensiled pressed beet pulp
[5]Cleanliness was judged on a scale from 1 to 4, on which 1 is clean and 4 is very dirty (Cook, 2002).
[6]Body condition score was judged on a scale from 1 to 5, on which 1 is extremely lean and 5 is excessively fat (Edmonson et al., 1984).
[7]Faecal colour was scored on a scale from 1 to 3 where 1 represents dark brown, 2 is brown and 3 is yellowish brown (Steen, 2004).
[8]Faecal consistency was scored on a scale from 1 to 5 on which 1 represents runny faeces and 5 hard faeces (Steen, 2004).

Relationships among diet, production and faecal characteristics

Increaed formulated intakes of total DM, CP, AAT and metabolizable energy, as well as DM intake of concentrate, increased milk production in kg of ECM (Table 8). Increased formulated intakes of total CP and AAT and increased dietary CP concentration increased milk urea concentration but increased formulated intake of forage NDF decreased milk urea concentration. Increased formulated forage DM intake resulted in cleaner cows whereas increased formulated intake of concentrate DM resulted in dirtier cows.

Increased formulated intake of starch from diet and concentrate and increased dietary starch concentration increased the number of long particles in faeces (Table 8). Increased formulated intake of forage decreased faecal DM concentration whereas increased formulated intake of concentrate as well as total intakes of CP, AAT and metabolisable energy, as well as dietary CP concentration, increased faecal DM concentration. Increased formulated intake of forage NDF and increased dietary concentration of NDF increased faecal NDF concentration whereas increased formulated intake of starch from forage decreased faecal NDF concentration. Increased formulated intake of starch from forage increased faecal starch concentration whereas increased dietary NDF concentration decreased concentration of starch in faeces (Table 8).

Increased dietary NDF concentration increased faecal consistency but increased body condition score values decreased faecal consistency score values and faecal DM concentration (Table 9). Increased faecal DM concentration decreased the number of long particles in faeces. Increased number of grain in faeces increased faecal starch concentration but decreased number of long particles in faeces.

Table 8. Partial correlation coefficients between dietary factors and production and faecal characteristics. No. of herds=26.

Dietary factor	ECM[1], kg	Urea, mmole/l	Clean-liness[2]	Long faecal particles, no./100 g faeces	Faecal DM, %	Faecal starch, % of DM	Faecal NDF, % of DM
Forage, kg DM			-0.427*		-0.541**		
Forage, kg NDF[3]		-0.790***					0.535**
Forage, kg starch						0.444*	-0.596**
Concentrate, kg DM	0.465*		0.465*		0.631**		
Concentrate, kg starch				0.439*			
Total DM, kg/day	0.423*						
Total CP[4], kg/day	0.562**	0.595**			0.636**		
Total AAT[5], kg/day	0.498*	0.511*			0.550**		
Total starch, kg/day				0.390†			
Total ME, MJ /day	0.449*				0.439*		
CP, g/kg DM	0.566**	0.602**		-0.424*	0.569**		
NDF, g/kg DM						-0.415*	0.644***
Starch, g/kg DM				0.396†			

***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; †$P < 0.10$

[1]ECM=energy-corrected milk

[2]Cleanliness was judged on a scale from 1 to 4, on which 1 is clean and 4 is very dirty (Cook, 2002).

[3]NDF=neutral detergent fibre

[4]CP=crude protein

[5]AAT=amino acids absorbed in small intestine

Table 9. Partial correlation coefficients between diet, production and faecal characteristics. No. of herds=26.

	Faecal Consistency[1]	Faecal DM, %	Long faecal particles, no./100 g faeces	Long faecal particles, % of DM	Faecal grains, no./100 g faeces	Faecal pH
NDF, g/kg DM	0.431*					
ECM[2], kg/day		0.368†				
Milk, kg/day		0.367†				
Milk protein, %	-0.496*					
Body condition score[3]	-0.667***	-0.417*				
Faecal DM, %	0.401†		-0.442*	-0.377†		-0.370†
Faecal starch, % of DM		0.370†			0.827***	
Long faecal particles, no./100 g faeces					-0.464*	

***$P < 0.001$; *$P < 0.05$; †$P < 0.10$

[1]Faecal consistency was scored on a scale from 1 to 5 on which 1 represents runny faeces and 5 hard faeces (Steen, 2004).

[2]ECM=energy-corrected milk.

[3]Body condition score was judged on a scale from 1 to 5, on which 1 is extremely lean and 5 is excessively fat (Edmonson et al., 1984).

Means and variations of factors presented with partial correlation coefficients are shown in Table 10.

Table 10. Means and variations of factors presented with partial correlation coefficients in tables 8 and 9. No. of herds=26.

Factor	Mean	S.D.	Min	Max
Forage, kg DM	10.2	1.76	6.9	13.5
Forage, kg NDF[1]	4.7	0.74	3.7	5.9
Forage, kg starch	0.2	0.37	0	1.3
Concentrate, kg DM	12.0	2.58	5.5	16.4
Concentrate, kg starch	3.5	0.78	2.2	5.6
Total DM, kg	22.3	1.91	18.6	26.8
Total CP[2], kg	4.0	0.50	2.4	4.8
Total AAT[3], kg	2.1	0.33	1.2	2.6
Total starch, kg	3.7	0.82	2.2	5.6
Total energy, MJ	262	29.3	214	347
CP, g/kg DM	184	20.2	126	224
NDF, g/kg DM	344	27.9	264	405
Starch, g/kg DM	167	36.3	99	227
ECM[4], kg/day	37.5	3.97	26.2	47.0
Milk, kg/day	37.5	4.59	27.4	50.6
Milk protein, %	3.25	0.15	3.00	3.52
Milk urea, m*M*	5.04	1.04	3.30	6.80
BCS[5]	2.78	0.37	2.00	3.60
Cleanliness[6]	2.18	0.46	1.35	3.00
Faecal consistency[7]	2.51	0.42	1.80	3.80
Faecal pH	7.26	0.34	6.61	7.75
Faecal DM, %	14.5	1.11	12.6	16.6
Faecal starch, % of DM	1.20	0.88	0.26	3.69
Faecal NDF, % of DM	43.5	4.05	37.1	52.3
Long faecal particles, no./100 g faeces	40.0	8.80	22.5	53.2
Long faecal particles, g DM/100 g faeces	0.130	0.039	0.049	0.206
Long faecal particles, % of DM	0.913	0.284	0.451	1.529
Faecal grain, no./100 g faeces	11.5	10.65	0.3	42.3

[1]NDF=neutral detergent fibre.

[2]CP=crude protein.

[3]AAT=amino acids absorbed in small intestine.

[4]ECM=energy-corrected milk.

[5]BCS=body condition score; was judged on a scale from 1 to 5, on which 1 is extremely lean and 5 is excessively fat (Edmonson et al., 1984).

[6]Cleanliness was judged on a scale from 1 to 4, on which 1 is clean and 4 is very dirty (Cook, 2002).

[7]Faecal consistency was scored on a scale from 1 to 5 on which 1 represents runny faeces and 5 hard faeces (Steen, 2004).

Discussion

Increased faecal consistency score values were observed in cows fed diets containing grass-clover silage combined with maize silage, which is an indication of improved nutrient utilization (Varga, 2003). However, no differences in feed conversion among cows fed different dietary treatments were found in this study. Maize silage and whole-crop cereal silage contain NDF that has a lower rate of potential degradability in the rumen than grass silage (Quirke et al., 2002; Bååth Jacobsson, 2005). This was associated with a higher faecal concentration of NDF in cows fed maize silage or whole-crop cereal silage in combination with grass-clover silage than in cows fed grass-clover silage only or in combination with ensiled pressed beet pulp. Ensiled pressed beet pulp contains the soluble fibre fraction pectin (160 g/kg DM) that is easily degraded in the rumen (Mara, 1999; Micard and Thibault, 1999; Hartnell et al., 2005). Reasons for why the higher faecal NDF concentration resulted in higher faecal consistency in cows fed maize silage but not in cows fed whole-crop cereal silage are unclear.

The increased number of long faecal particles observed when both formulated intakes of starch from concentrate and starch from the total diet were increased or when the dietary starch concentration was increased, indicates a shortened ruminal residence time and a poor fermentation of the fibre particles due to a decrease in ruminal pH with a subsequent decrease in the number of fibre degrading micro-organisms (Hall, 2002; Varga, 2003; Nordqvist, 2006).

Faecal DM concentration decreased with increased formulated intake of forage DM but increased with increased formulated intakes of concentrate DM, dietary CP, AAT and energy as well as with increased dietary CP concentration. Similar results were achieved by Ireland-Perry and Stallings (1992), who determined the relationships among faecal DM concentration and intakes of forage, concentrate and metabolizable energy. The decreased faecal DM concentration with increased forage intake but the increased faecal DM concentration with increased concentrate intake can be explained by the waterholding capacity by fibrous materials (Marynard et al., 1979). The increased faecal DM concentration with increased formulated intake of concentrate could be due to a simultaneous decrease in NDF concentration as there was a tendency to a negative correlation between faecal DM concentration and faecal pH in the present study.

In this study, increased BCS values correlated with decreased faecal consistency score values indicating that increased fatness in cows might tend to result in a higher risk of imbalanced ruminal fermentation of nutrients. The positive correlation between formulated intake of forage NDF, dietary NDF concentration and

faecal NDF concentration is in agreement with results by Ireland-Perry and Stallings (1992). In the present study, increased number of grains in faeces correlated with increased faecal starch concentration but decreased the number of long particles in faeces. This could be an indication of feed sorting or poor processing of grain as have been reported by others (Hall, 2002). A large number of faecal grains could be an indication of unprocessed dietary cereal grain and/or insufficient dietary peNDF content (Poppi et al., 1980; Hall, 2002).

The higher yield of milk fat by cows fed grass-clover silage combined with ensiled pressed beet pulp than by cows fed grass-clover silage combined with whole-crop cereal silage probably was related to the higher content of soluble fibres, such as pectin, in ensiled pressed beet pulp (Ferris and Mayne, 1994). Mertens (1997) reported that milk fat concentration is closely correlated to the effective NDF (eNDF) of the diet. However, no significant correlation was observed in the present study between milk fat content and dietary NDF intake, possibly because milk fat percentage in early-lactation dairy cows is only partly sensitive to dietary composition (Allen, 1997).

Cows fed grass-clover silage alone or grass-clover silage combined with ensiled pressed beet pulp tended to have a higher milk yield in kg ECM than cows fed whole-crop cereal silage (Table 7). For cows fed fresh sugar beet pulp in addition to grass-clover silage, the higher ECM yield can be related to their higher formulated DM intake (Tables 6 and 7). In addition, yield of milk protein was higher in cows fed grass-clover silage alone or in combination with ensiled pressed beet pulp than for cows fed grass-clover silage combined with whole-crop cereal silage. For cows fed ensiled pressed beet pulp, the higher milk protein yield can be explained by their higher formulated intake of AAT compared to cows fed grass-clover silage in combination with whole-crop cereal silage. The tendency to a higher milk urea content in cows fed grass-clover silage combined with maize silage compared to cows fed the other forage sources might be explained by an energy release during carbohydrate degradation of the maize silage needed for an efficient microbial protein synthesis (De Campeneere et al., 2005). De Campeneere et al. (2005) reported a much higher milk urea concentration in cows fed maize silage to 50 or 100% of the dietary forage portion compared to cows fed prewilted grass silage as the sole forage source. They concluded that the low milk urea concentration in the cows fed the grass silage was due to a high rate of urinary loss of urea because grass silage increased urinary volume (De Campeneere et al., 2005). However, the study by Campeneere et al. (2005) and the present study did not determine the urinary urea concentration from the cows. Based on results from different feeding trials with cows fed maize-silage based diets and cows fed grass-silage based diets, a

higher urinary clearance rate of urea is evident in grass-silage based diets (Faverdin and Vérité, 1998).

Conclusions

Feeding maize silage in combination with grass-clover silage to dairy cows in early lactation improves faecal consistency compared to feeding grass-clover silage alone or in combination with whole-crop cereal silage or ensiled pressed beet pulp. However, daily milk yield in kg of ECM tended to be higher in cows fed grass-clover silage alone or combined with ensiled pressed beet pulp compared to cows fed whole-crop cereal silage in addition to grass-clover silage.

Increasing formulated starch intake from concentrate or from the total diet was associated with an increased number of long faecal particles, which might be explained by digestive disorders. Feeding more forages decreases faecal DM concentration, whereas feeding more concentrate increases faecal DM concentration. The close positive linear relationship between the number of grain kernels in faeces and faecal starch concentration appears as a potentially easy and cheap practical diagnostic dietary tool on farm level.

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Brief Communication

Image analysis methodology for particle-size determination of washed faeces from dairy cows in early lactation

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Abstract

Image analysis is a technique for measuring the dimensions of particles in feed and washed faeces. The new technique has thrown more light on the concept of the critical particle size (CPS) and the dynamics involved in particle size reduction in the rumen. The method involves several steps which include washing of faeceal samples in nylon bags, freeze drying before dry sieving in a set of four sieves and scanning of the particles. In this study, faecal sample (FcWCS181) from cows fed grass clover silage and whole crop cereal silage was analysed using image analysis. The distribution of the particle length and width in the individual sieving fraction from the sample (FcWCS181) was described using a gamma distribution function. An accumulated distribution curve was also used to describe the different characteristics of the particle length and width. The median length and width values, as well as the 95% length and width values were estimated using a cumulative distribution function CDF in SAS.

The distribution of the particle length in the sample (FcWCS181) showed that 95% of the particles were longer than 15 mm and this was taken to be the critical particle length (CPL), also 95% of the particles were thinner than 1.32 mm. The outstandingly high CPL value recorded in this study in sample (FcWCS181) was attributed to poor rumen capacity to retain large particles for sufficient fermentation to occur.

Keywords: Dairy Cow, Faeces, Particle Size, Image Analysis

Introduction

Image analysis is a new technique that characterizes particles according to length, width and area (Nørgaard et al., 2004). The technique also describes the various dimensions of the length and width of washed feed and faecal particles using terms such as mode, arithmetic means, geometric means and 95 percentile fractions (Nørgaard et al., 2004). The method has been used by some scholars to measure the different dimensions of sieved feed and faecal particles (Grenet et al., 1989). The findings by Grenet et al. (1989) showed that the precise assessment of particle shape, width, length and area could be ascertained by use of image analysis. Gamma distribution curves and cumulative distribution curves have been used to describe the length and width distribution of particles from feed (Nørgaard & Sehic 2003) and faeces samples Nørgaard et al. (2004). The 95 percentile length value reflects the capacity of the rumen system to retain large particles (Nørgaard et al., 2004). A flat curve indicates many long particles whereas a steep curve indicates many short particles in faeces (Nørgaard et al., 2004). Many small particles in faeces describe good rumen function (Nørgaard et al., 2004). However, many long particles in faeces indicate a diet that does not allow proper retention of large particles in the rumen (Nørgaard et al., 2004). Waldo et al. (1971) suggested the use of a log normal distribution to describe the length distribution of sieved digesta from the rumen and faeces of cows fed chopped and pelleted hay. However, data from image analysis has shown that forages are not fragmented into spherical or cuboidal shapes (Luginbuhl et al., 1988). As a result, there is likely a deviation from the log normal distribution of the lengths of sieved particles according to Waldo et al. (1971). Image analysis has also been used to explain the concept of the critical particle length (CPL; Nørgaard and Sechic, 2003). According to them, the CPL for cattle fed grass silage at maintenance level is 5 mm.

The aim of this study was 1) to understand the principle involved in the use of image analysis to characterize particles from faeces of early lactating dairy cows fed grass-clover silage and whole-crop cereal silage plus concentrate and 2) to determine the critical particle length in the faeces of cows fed this same diet, using image analysis.

Materials and Methods

Fifteen to twenty-four nylon bags of dimensions 150 x 90 mm and pore size of 10 µ were prepared for each washing procedure. Table salt was poured inside each bag and the bags were shaken thoroughly to observe if they had openings. The nylon bags were marked and three representative faecal samples of 10 g each were taken from one composite faecal sample from five cows in each herd. Each of the three samples was put in a nylon bag. Four milliliters of liquid soap was added to each bag before closure with rubber bands. The closed bags were placed in a washing machine (Brand AA Class ECO WASH) and washing was done at a temperature of 40˚C and 800 rev/min for 2 hrs. The washed faeces particles were put in preweighed aluminum containers and about 2 milliliters of distilled water was added to each container to dissolve the particles; the mixture was placed in a freezer until the content was completely frozen. The frozen particles were then freeze dried for 24 to 48 hrs at a pressure of 0.004 Pa and a temperature of -20 ˚C. Dry sieving of the particles followed after an estimation of the proportion of dry weight of retained particles in the three subsamples. Subsamples with nearly the same weights were mixed together for dry sieving. The dry sieving was done in a set of four sieves arranged in a descending order according to the following pore sizes 2.36 mm (O), 1.0 mm (M) 0.5 mm (S), 0.212 mm (D) and the bottom bowl (B) for collection of the smallest particles.

Figure I. Separation of clumped particles during the dry sieving process. (Photo by Elise Bostad)

Sieving of the particles was done at 1min intervals at 1500 rev/min until all the particles were completely separated into

different fractions, clumped particles were separated after each sieving interval as shown in Figure I.

Figure II. Different sieving fractions of the sample in pre-weighed aluminum containers. (Photo by Elise Bostad)

The amount of time spent on each sieving process depended on the kind of sample and level of manual separation. The largest particles (O fraction) and the kernels were both separated on the 2.36 mm sieve. The sieved particles were put into a set of preweighed aluminum containers and their weights were recorded (Figure II). Two sub samples, each with a weight of 0.0015 g from fractions D and B respectively, 0.008 g from fraction S and 0.02 g from fraction M were taken. These subsamples were distributed one after the other on a scanner (CannonScan9900F; Figure III). The whole O fraction and all the kernels from each sieved sample were scanned. The scanning was done against a blue background with the O fractions and the kernels scanned at 1200 dpi whereas the other fractions were scanned at 2400 dpi according to Nørgaard et al. (2004) as shown in Figure IV. The different fractions of the scanned sample are shown in Figures V, VI, VII, VIII, IX and X.

Fig III. Separation of particles on scanner. particles.
(Photo by Elise Bostad)

Figure IV. Scanning of particles (Photo by Elise Bostad)

Figure V. Scanned kernels and particles.

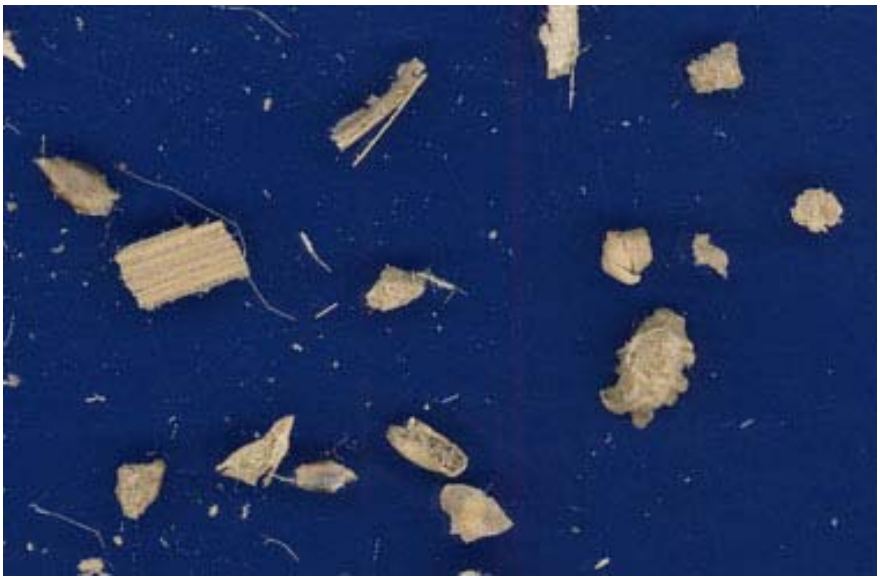

Figure VI. O Fraction, particles > 2.36 mm.

Figure VII. M Fraction, 2.36 mm < particles > 1.0 mm.

Figure VIII. S Fraction, 1.0 mm< particles> 0.5 mm.

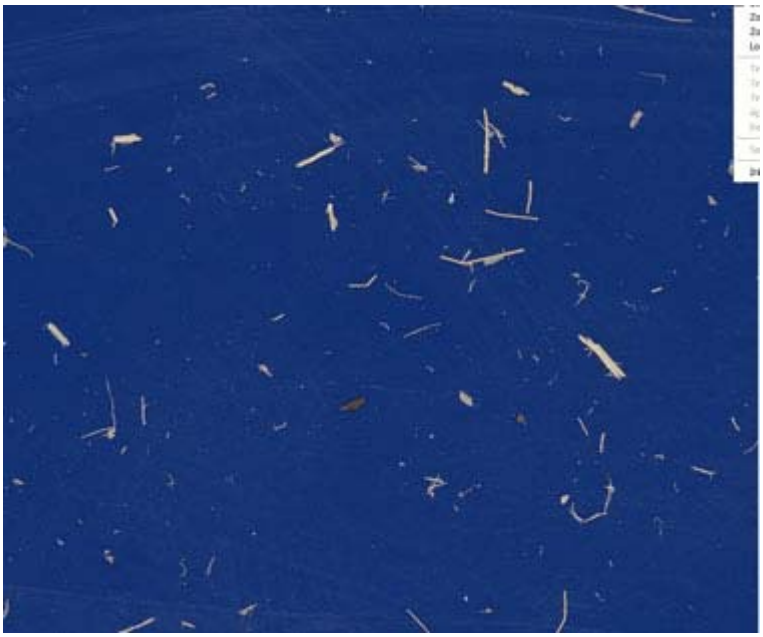

Figure IX. D Fraction, 0.5 mm< particles > 0.212 mm.

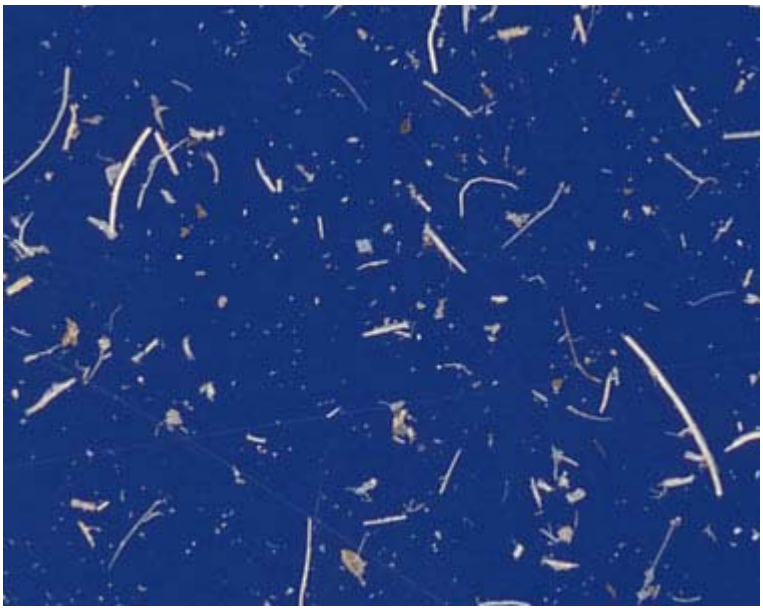

Figure X. B Fraction, particles < 0.212 mm.

The particles were separated with paint brush and forceps before scanning to avoid overlapping. Background segmentation was performed to enable the programme count and to identify the individual particles. The particles were identified individually and the areas (A), lengths (PL) and widths (PW) of the particles were measured by "Image Analysis": Image ProbPlus version 5.1, Media Cybernatics, http://www.mediacy.com (Nørgaard and Bendixen, 2002). Descriptive terminologies, such as arithmetic mean particle length (APL), arithmetic mean particle width (APW), mode particle length (Mode_PL), mode particle width (Mode_PW), median particle length (MPL), median particle width (MPW), geometric mean particle length (GPL), geometric mean particle width (GPW) and 95 percentile fraction (Frac_95%) described the different dimensions of the length and width of the particles and these were estimated by using the principles described by Nørgaard, (2006a).The mass proportion of particles retained on an individual sieve (Massep) was also calculated according to Nørgaard (2006a). The arithmetic mean length (APL) and the arithmetic particle width (APW) were estimated by using the weighed A values in each sieving fraction and by using the sieve mass proportions. Also, a gamma distribution function γ (x, α_i, $ß_i$) for PL and PW values from each sieving fraction was estimated by using the weighed A values. A composite distribution function ($C= m_M \times \gamma_M + m_S \times \gamma_{S1} + m_B \times \gamma_{B.....}$) was used to estimate the overall distribution of the particles from the different sieving fractions. Estimation of the mode length

and width values was done by a stepwise (0.01 mm) identification of the maximum C(PL) and C(PW) values (Tables I and II). The median length and width as well as the 95% length and width values were estimated using cumulative distribution function CDF in SAS.

Results and Discussion

The length and width dimensions of the pre-washed and freeze-dried faecal particles from cows fed grass-clover silage and whole-crop cereal silage plus concentrates are shown in Tables I and II, respectively. The percentages of particles retained on the 1.0 mm (M), 0.5 mm (S), 0.212 mm (D) sieves and on the bottom bowl (B) were 10, 30, 36 and 24%, respectively (Tables I and II). The D sieve retained the highest proportion of particles (36%) compared to the other fractions M, S and B. The particles retained on the M sieve fraction accounted for the smallest proportion of particles (10%). There were no particles retained on the 2.36 mm sieve (O fraction) in the sample FcWCS181, as can be seen from the two tables. There was a large difference between the length and the width dimensions of the particles from faeces of cows fed grass clover silage and whole-crop cereal silage (Tables I and II).

The APL, Mode_PL, MPL and GPL decreased as screen size decreased (Table I). Also, a similar result was obtained in the APW, Mode_PW, MPW and GPW (Table II). In this study, 95% of the length of washed faecal particles was shorter than 15 mm whereas 95% of the width of the particles was thinner than 1.32 mm (Tables I and II). Relating this finding to the sieve sizes, materials retained by each sieve was determined by the rate of separation of the clumped particles, their onward passage through the sieve openings and by the mesh size of the sieve situated immediately above the other. This agrees with the findings of Luginbuhl et al. (1988). Futhermore, the particle size is a factor of the mesh size (pore size) and the ability of the particles to pass through sieves of different pore sizes. Particles on each of the sieves have similar characteristics with regard to size and shape in the sample (FcWSC181). This suggests the manner in which particles are reduced in size during ingestive chewing, comminution and the method of positioning at the zone of escape from the rumen (Luginbuhl et al., 1988).

Table I. Characteristic length dimension values of washed and freeze-dried faeces particles from the individual sieving fractions in sample FcWCS181.

Sieving fraction	Pore size in sieve (mm)	Massep[a]	Descriptive terms and dimensions of the particle length (mm)				
			Mode_PL[b]	MPL[c]	GPL[d]	APL[e]	Frac_95%[f]
O	2.36	-	-	-	-		-
M	1.0	0.10	4.87	9.15	8.12	11.17	27.54
S	0.5	0.30	1.24	2.20	1.82	2.67	6.42
D	0.212	0.36	0.13	0.91	0.78	1.18	3.14
B	Bottom bowl	0.24	0.13	0.37	0.32	0.49	1.31
Overall		1.00	0.20	1.58	1.27	3.33	14.81

[a]Massep=proportion of particles retained on the individual sieve
[b]Mode_PL= most frequent particle length
[c]MPL= median particle length
[d]GPL= geometric mean particle length
[e]APL= arithmetric mean particle length
[f]Frac_95%= predicted 95 percentile value

Table II. Characteristic dimensions of the width of washed faeces particles in the individual sieving fractions from sample FcWCS181.

Sieving fraction	Pore size in sieve (mm)	Massep[a]	Descriptive terms and dimensions of the particle width (mm)				
			Mode_PW[b]	MPW[c]	GPW[d]	APW[e]	Frac_95%[f]
O	2.36	-	-	-	-		-
M	1.0	0.10	1.21	1.31	1.23	1.35	2.14
S	0.5	0.30	0.32	0.43	0.37	0.49	1.03
D	0.212	0.36	0.09	0.15	0.14	0.17	0.41
B	Bottom bowl	0.24	0.06	0.08	0.07	0.09	0.18
Overall		1.00	0.07	0.27	0.19	0.36	1.32

[a]Massep=proportion of particles retained on the individual sieve
[b]Mode_PW=most frequent particle width
[c]MPW=median particle width
[d]GPW=geometric mean particle width
[e]APW=arithmetric mean particle width
[f]Frac_95%=predicted 95 percentile value

Gamma distribution curves (density plots) and accumulated distribution curves to illustrate the distribution of the particle length and the particle width values in the individual sieving fractions are shown in Figures 1a and 2a (length) and in Figures 3a and 4a

(width), respectively. Also, curves showing the overall density distribution and the overall accumulated distribution of the particle length and width in faeces from the cows fed grass clover silage and whole crop cereal silage can be found in Figures 1b and 2b (length) and in Figures 3b and 4b (width), respectively. Among the five sieving fractions, the bottom bowl (B fraction) retained the largest number of short particles. This is illustrated by the sharp steep curve in Figure 1a. Ninty-five percent of the particles on the bottom bowl were shorter than 1.3 mm whereas the Frac_95% value for fractions M, S and D was 27, 6.4 and 3.1 mm (Table I). The Fra_95% value for the overall sample (FcWCS181) was estimated to be 15 mm which is 3 times longer than the CPL of 5 mm by Nørgaard and Sehic (2003). This could be due to poor retention of large particles in the rumen system. Nørgaard et al. (2004) attributed long particles that were longer than 5 mm in faeces to poor rumen fermentation. The long particles could also be related to the forage source and forage maturity stage Tomoko et al. (2004). Particle size reduction and passage of digesta from the rumen seemed to be due to the intrinsic factors associated with the forage (Tomoko et al., 2004).

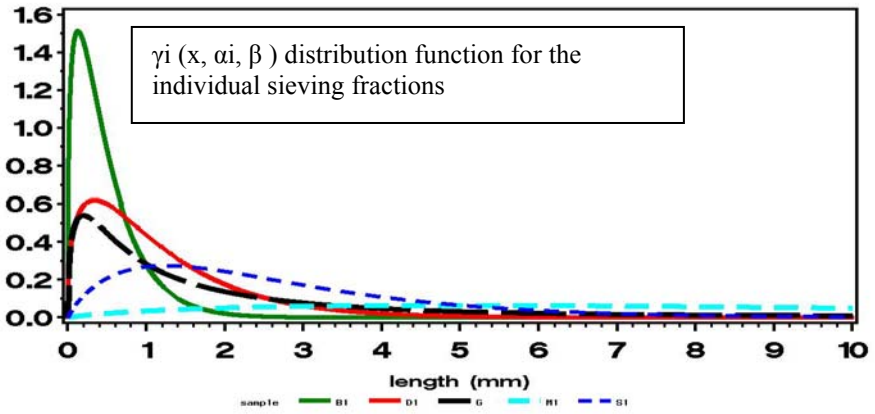

Figure 1a. Density distribution of particle length (PL) in the individual sieving fractions (B, D S and M) and the overall (G) from cows fed grass-clover silage and whole-crop cereal silage supplemented with concentrates.

Figure 1b shows the overall distribution of particle length values of washed faeces particles from sample FcWCS181. The most frequent particle length (Mode_PL) value was 0.2 mm. The arithmetic mean particle length value was 3.3 mm, which represents the APL value shown in Figure 1b. This value was above the CPS of 1.18 mm by Ulyatt et al. (1976). The geometric mean particle length (GPL) and the MPL values were 1.27 mm and 1.58 mm (dotted lines), respectively (Figure1b).

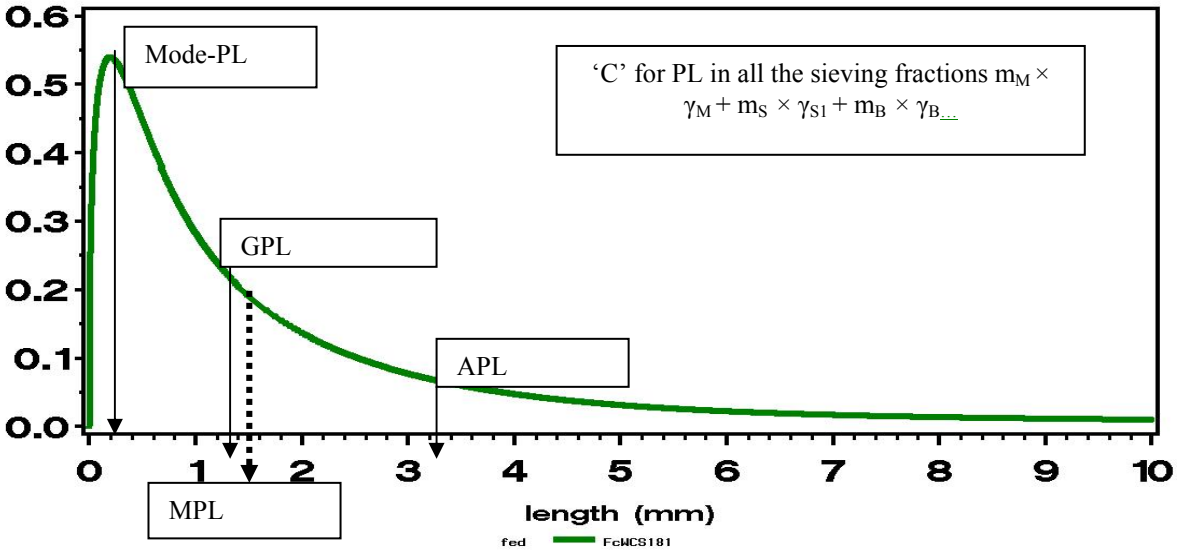

Figure 1b. Overall density distribution of particle length (PL) in the four sieving fractions (B,D,S and M) of washed faecal sample from cows fed grass-clover silage and whole-crop cereal silage supplemented with concentrates, with indication of mode particle length (Mode_PL), median particle length (MPL), geometric mean particle length (GPL) and arithmetic mean particle length (APL).

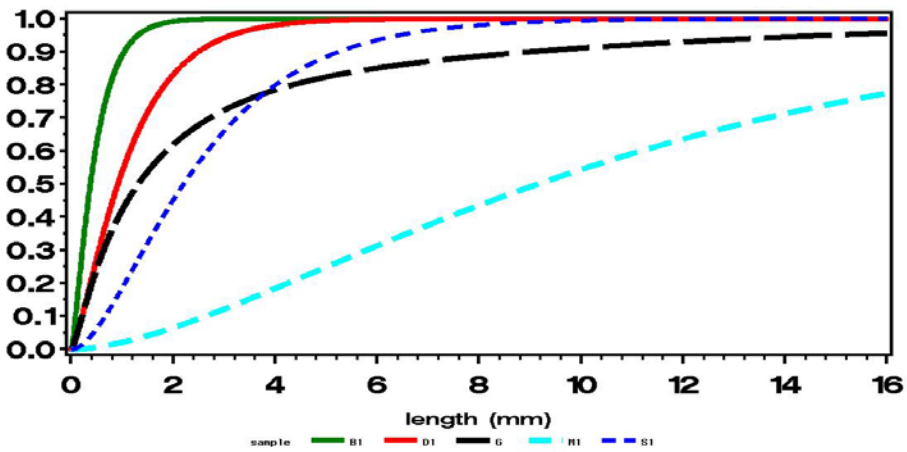

Figure 2a. Accumulated distribution of particle length (PL) in the individual sieving fractions (B, D, S and M) and the overall (G) distribution of washed faecal sample from cows fed grass-clover silage and whole-crop cereal silage supplemented with concentrates.

An accumulated distribution of the particle length in the entire sample (FcWCS181) of the washed faeces particles shows that 95% of the particles (Frac_95%) were shorter than 15 mm whereas 5% of the particles were 15 mm and above (Figure 2b).

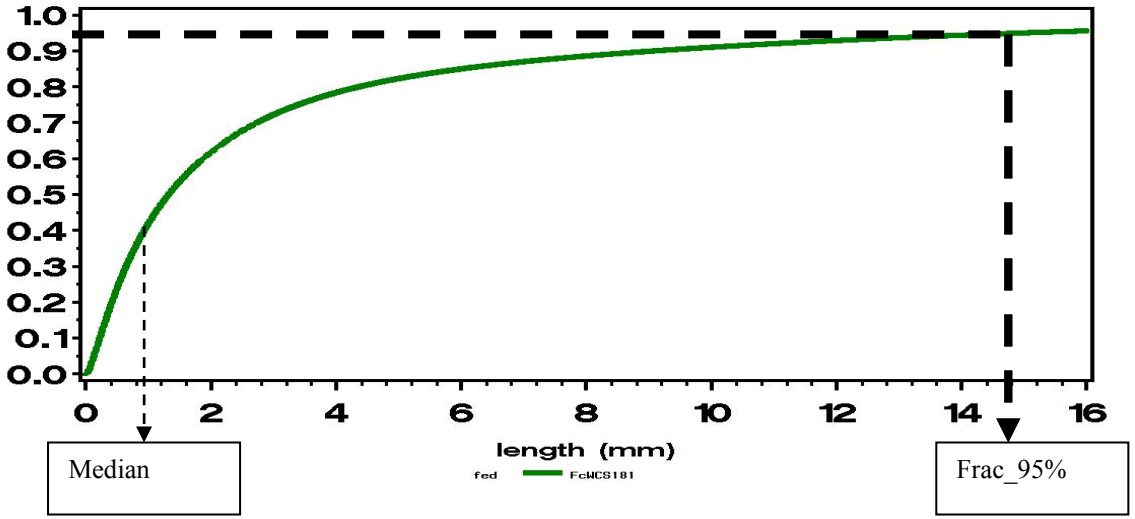

Figure 2b. Overall accumulated distribution of particle length (PL) of the four sieving fractions (B, D, S and M) of washed faecal sample from cows fed grass-clover silage and whole-crop cereal silage supplemented with concentrates, with indications of the median value (1.6 mm) and the 95 percentile fraction of the particle length (15 mm).

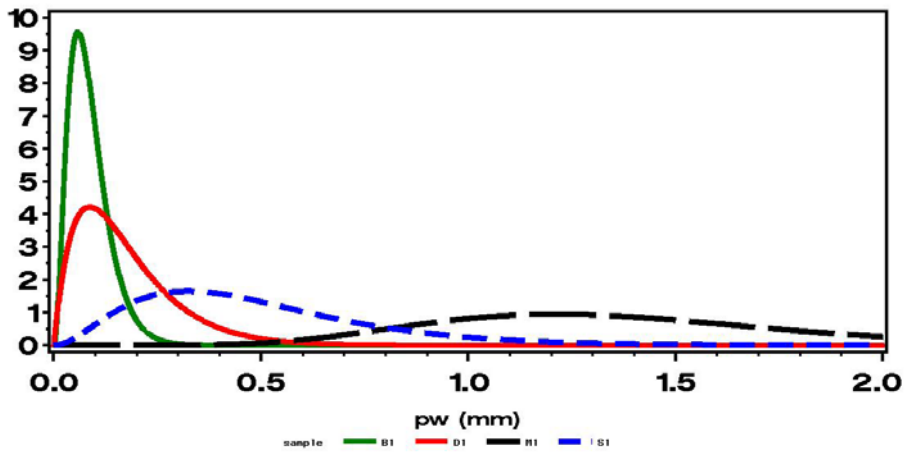

Figure 3a. Density distribution of particle width (PW) in the individual sieving fractions (B, D, S and M) of washed faecal sample from cows fed grass-clover silage and whole-crop cereal silage supplemented with concentrates.

Figure 3b shows the overall density distribution of the particle width (PW) values of washed faecal particles in sample FcWCS181. The distribution is left skewed with many thin and few wide particles. The most frequent mean width of particles (Mode-PW) was 0.07 mm illustrated by the peak of the steep curve. The APW value in the sample was 0.36 mm. The GPW value of the particle width was 0.19 mm whereas the MPW value was 0.27 mm.

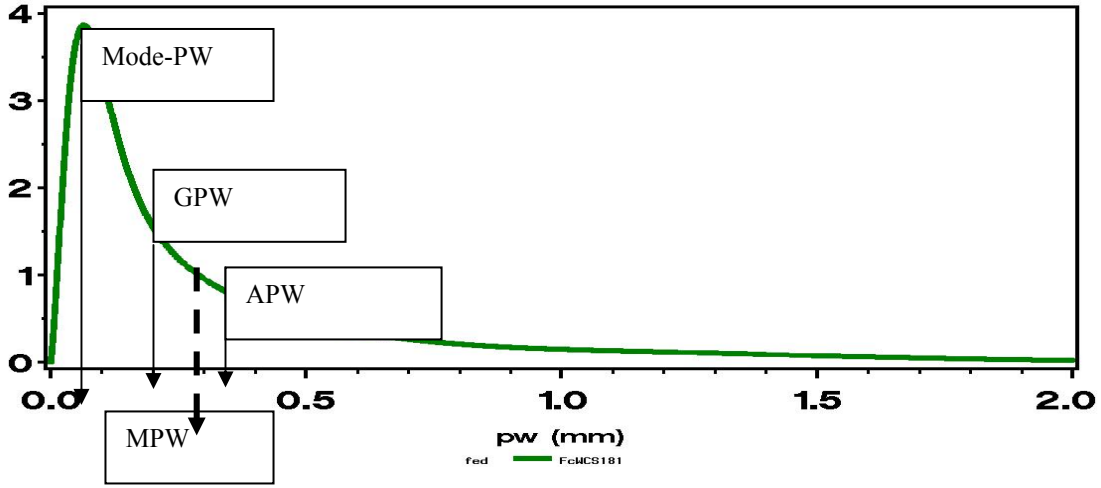

Figure 3b. Overall density distribution of particle width (PW) of the four sieving fractions (B, D, S and M) of washed faecal sample from cows fed grass-clover silage and whole-crop cereal silage supplemented with concentrates with the indication of the mode particle width (Mode PW), median particle width (MPW), geometric mean particle width (GPW) and arithmetic mean particle width (APW).

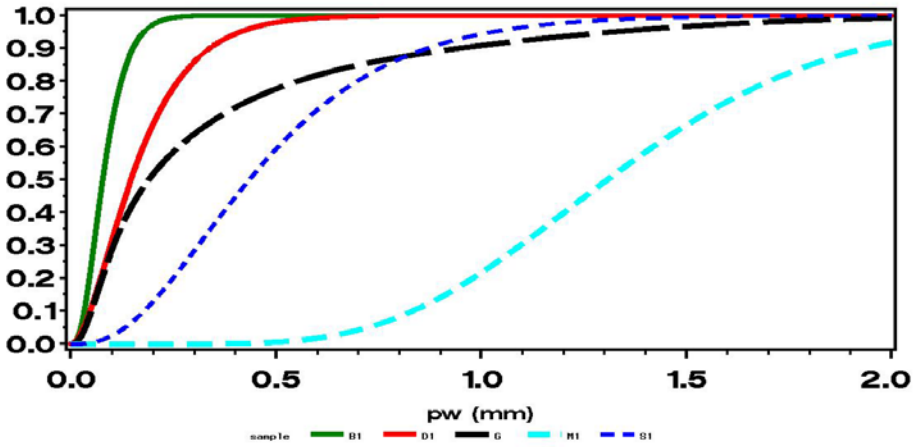

Figure 4a. Accumulated distribution of particle width (PW) in the individual sieving fractions (B, D, S and M) and the overall (G) distribution of washed faecal sample from cows fed grass-clover silage and whole crop cereal silage supplemented with concentrates.

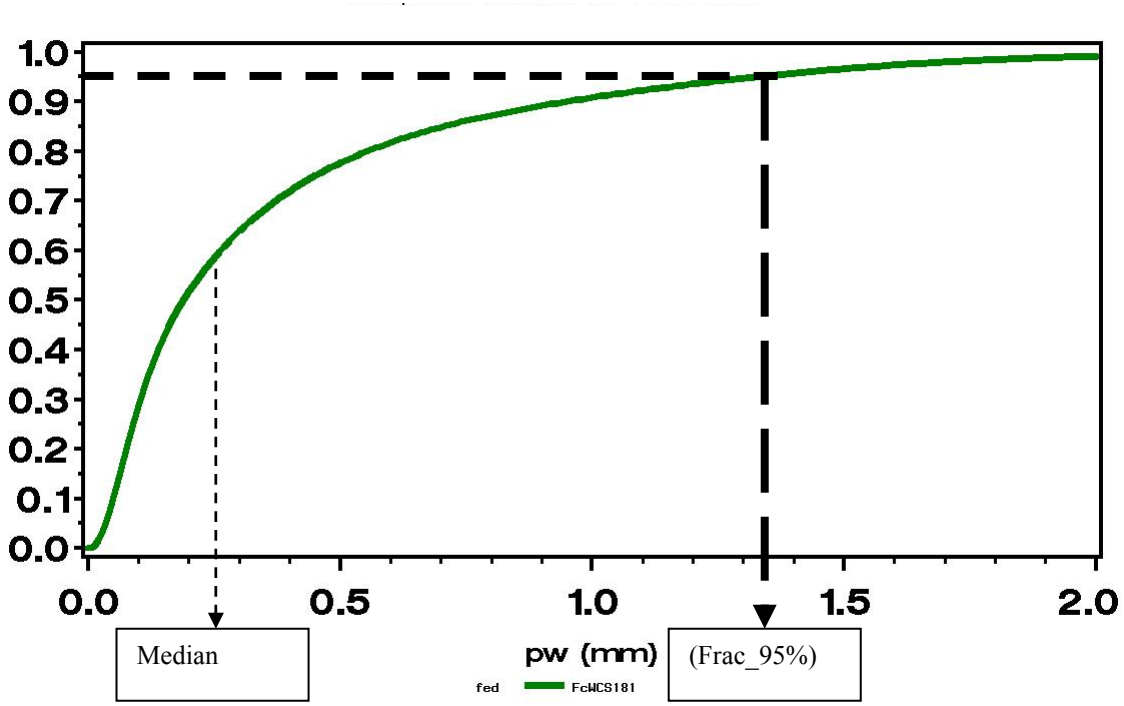

Figure 4b. Overall accumulated distribution of particle width (PW) in the in the four sieving fractions(B, D, S and M) of washed faecal sample from cows fed grass-clover silage and whole-crop cereal silage supplemented with concentrates with indication of the median value (0.27 mm) and the 95 percentile fraction of the particle width.

Figure 4a and Table II show that 95 percent of all the particles on the bottom bowl (B fraction) were thinner than 0.18 mm (Frac_95%) value whereas the Frac_95% value of the particles in the D sieving fraction was 0.41 mm. Particles in the M and S sieving fractions appeared to be thicker than those of B and D with thicknesses of 2.0 and 1.0 mm (Frac_95%), respectively.

Figure 4a shows the accumulated distribution of the particle width in each sieving fraction (Bottom bowl, D, S and M) and the overall (G) from sample FcWCS181. From the curve, 95% of the particles (Frac_95%) values from fractions B, D and S were below 1.32 mm in width whereas 5% of the particles had their width above 1.32 mm. Particles from fraction M appeared to deviate from the 95 percentile range. An overall accumulated distribution of the particle width showed that 95% of the particles (Frac_95%) in sample FcWCS181 were thinner than 1.5 mm in width (Figure 4b).

Conclusions

Image analysis can be used for measuring the characteristic, the length and width dimension values of washed faeces particles. The technique has successfully identified and characterized the different dimensions of the particles from faeces of cows fed grass-clover silage and whole-crop cereal silage plus concentrates. The method also can be used for identification of the 95 percentile length and width values, which in the sample used in the present study was higher than the CPL of 1.18 mm and 5 mm for cattle proposed by other researchers. The findings, therefore, suggest that, 95 percentile values might reflect the capacity of the rumen system to retain large particles.

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