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The physically effective fiber of total mixed rations and its effects on dairy cow performances

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To my husband Paolo

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INTRODUCTION

Fiber : chemical and physical characteristics

Fiber can be described as the structural polymer of plant cell walls. It contains carbohydrates (cellulose, hemicellulose, pectin, galactans, gums and mucilage), proteins lignified nitrogenous substances, polyphenols and minerals.

Cellulose is the main structural component of the cell plant, it is formed by a long chain of glucose sugar linked by β bonds. These bonds are resistant to mammalian digestive enzymes but can be broken by cellulolytic rumen bacteria. Hemicellulose

Hemicellulose is a complex of carbohydrate polymers composed of xylose, galactose and mannose.

Pectins are derived from the acid galacturonic and are characterized by high digestibility.

The lignin is a phenylpropanoid polymer. It is completely indigestible and varies between 2% and 12% of DM, increasing when the age of the plant increases (Van Soest, 1994).

The cell wall proteins are the only ones to have a structural function for the plant; they may account for 15% of DM and can be divided into four main categories: glycine- protein, proline-protein, hydroxyproline , and proteoglycans.

Minerals linked to the cell wall are mainly calcium carbonate ($CaCO_3$) and silica (SiO_2). They provide the plant with rigidity and sometimes fragility at the same time.

Cellulose and hemicellulose are the most representative fractions of plants. In forage, cellulose is 40-45% of the cell wall and 15-40% of the total dry plant, depending on the particular species and age of the plant. Hemicelluloses also increase with the age of plants and accounts for 12-25% of their DM. In general quality declines as the plant matures. This decline is different in the two main botanical families, legumes and grasses, and within the same plant as well. In legumes the stem has a function of support, and it is generally much more lignified than the leaves. This structural difference between the two parts of the plant tend to increase as the plant ages. Conversely, in grasses, where the leaves have an important structural as well as metabolic function, as the plant ages both stem and leaves are more lignified.

1.1. Chemical evaluation

The definition of fiber is to some extent arbitrary and differs if it is considered as an anatomical component of the plants or as a nutritional entity. In the first case it coincides with the plant cell walls. In nutritional terms Van Soest (1994) defined fiber as the fraction of the plant cell wall that is refractory to digestion by mammalian enzymes and that is slowly fermented or is not fermented at all by microorganisms of the digestive tract.

The fiber content of feed, as defined by Van Soest (1994), has been historically analyzed following the so called crude fiber or Weende method. This method has many limitations that cause large errors in the estimation of the fiber of the feeds. The most widely accepted and used methods for fiber determination are those proposed by Goering and Van Soest (1970), which provides a quantitative evaluation of different fiber fractions of the cell wall and involve the determination of the so called neutral detergent fiber, acid detergent fiber, acid detergent lignin (Figure 1).

The neutral detergent fiber (NDF) is a measure of the total content of cellulose, hemicelluloses, lignin and cutin. It is estimated submitting the feed to a treatment with detergents and a chelating agent at neutral pH. The acid detergent fiber (ADF) includes mainly cellulose, lignin, cutin and ashes and is estimated treating the feed with a diluted solution of sulfuric acid and chelating agents. The acid detergent lignin (ADL) is the residue after treatment with a concentrated solution of sulfuric acid and includes lignin and cutin .

1.2. Role of fiber in the rumen

Fiber is the main component of dairy cow diets. In general the diets consist mainly of fiber (hemicellulose, cellulose) and non fiber carbohydrates (sugars, starch, pectins). The amount of dietary fiber and its characteristics may affect the efficiency (productivity) and the metabolism of cows. The first important role of fiber and its physical characteristic is related to the ability of the fibrous particles to stimulate chewing activity and rumination.

In ruminants mastication and rumination contribute to decrease particles size, allowing them to pass through the rumen and from the rumen to the digestive tract. The particle size of digesta is considered to be a critical factor that affects the particle passage from the rumen. As particle size

is reduced the feed is more readily digested by the rumen microorganisms.

Additionally to chewing activity, fiber contributes to saliva production, which provides liquid for the microbial population, re-circulates nitrogen and minerals, lubricate the feed bolus to facilitate its passage through the esophagus. It also prevents excessive foaming, which is due to ruminal bloat, because it contains anti-foaming property and buffers the rumen. This is the major buffer which helps to maintain a rumen pH between 6.2 and 6.8.

Dietary fiber enable rumen and reticulum motility and the contraction. In fact, the rumen is always contracting and moving. Healthy cows will have one to two rumen contractions per minute. Contractions mix the contents of the rumen, bring microbes in contact with new feedstuffs, reduce flotation of solids, and move materials out of the rumen. Lack of or a decrease in the frequency of rumen movements is one way of diagnosing sick animals.

1.3. Fiber in dairy cow diets

Fiber was nutritionally defined by Mertens (1997) as "slowly digestible or indigestible fractions of feeds that occupy space in the gastrointestinal tract of animals".

Dairy cattle require a minimum concentration of fiber in their diet, but this minimum has not been clearly defined (NRC, 2001). Defining fiber requirements is very difficult because diets are made up with many interacting components (Varga et al., 1998).

Ruminants require forage fibre in coarse physical form but the NRC (2001) recommendations do not fully take into account the physical form or size of the NDF particles.

A lack of fiber in the ration, associated to very high energy levels may increase the risk of acidosis. Rations which are low in fiber may then be the cause of significant economic losses due to the alteration of rumen fermentation and metabolic disorders that can also be the cause of death for the animal. On the other hand, excessive amounts of long, coarse forage may limit intake and digestibility, ultimately affecting the energy balance of the animal and decrease milk yield (Allen, 1997).

Mertens (1987) proposed the use of NDF to establish the upper limit for the forage to concentrate (**F:C**) ratio in dairy diets. Although NDF is a valuable tool in formulating ration, it

refers only to the chemical characteristics of fiber and does not take into account the physical characteristic of the fiber, like density and particle size.

In fact, these physical fiber characteristics, particularly the size of particle in the rumen, influence rumen fermentation and the rate of passage. Furthermore, the amount and particle size of the ration influences the health and metabolism of the anima and affects milk yield and milk fat independently of the amount or composition of chemically measured NDF.

1.4. Fiber fibrousness

The physical proprieties of the fiber are affected by the F:C ratio, the type of forages used in rations and their concentration, the type of ingredients, the amount of ground non-forage fiber components, the processing used and the size of the particles. All these physical characteristics influence many aspects of the physiology and metabolism of the ruminant, such as its chewing activity, the proportion of volatile fatty acid in the rumen and rumen pH, which in turn affects milk fat production. Because the type and the amount of fiber source influence milk fat production, several authors developed the concept of fibrousness characteristic of the fiber.

Sudweeks et al. (1981) and Norgaard (1986) (cited by Mertens, 1997) studying the relationships between fiber component of the ration and chewing activity of the animals proposed to use time spent on chewing per 1 kg of dry matter (DM) as index value of fibrousness characteristics of forages. This chewing activity per kg DM was a characteristic attributed to a feed, but it actually varied depending also on some characteristics of the animal, such as its breed, body size, and level of intake, as well as particle size and fiber content of the feed.

Mertens (1997) proposed two distinct concepts for evaluating the physical characteristics of the fiber and related the effectiveness of the fiber to animal response. These are the physical effective NDF (peNDF) and the effective NDF (eNDF).

1.5. Effective NDF and physical effective NDF (peNDF)

PeNDF refers to the physical characteristic of a type of feed. The particle size stimulates chewing activity and forms the biphasic mat in the rumen, which consists of a fibrous mat of large particles floating in a pool of liquid and of small particles.

PeNDF is the fraction of fiber that would stimulate and predict chewing activity and would assure a rumen pH> 6. PeNDF gives a more appropriate measure of fiber effectiveness than the chewing activity per kg DM, because in addition to the total fiber NDF, it takes into account the particle size, minimizing all the effects related to the characteristics of the animal (intake, animal size).

PeNDF is the product of NDF concentration to the physical effectiveness factor (*pef)*. Pef varies from 0, when NDF in a feed fails to stimulate chewing and 1 when NDF stimulates the maximum chewing activity. PeNDF is a very critical factor in determining the dynamics of ruminal fermentation, ruminal passage and stimulating rumination so its relation to the formation of ruminal mats needs to be considered. For all these reasons peNDF is crucial for animal health and milk fat depression (Mertens, 1997).

Effective fiber is related to intrinsic rumen pH buffering capacity of fiber. Fiber eNDF refers to the characteristics and ability of feed to replace forage in the ration and to maintain a constant milk fat percentage. The eNDF can be measured as the total amount of feed that needs to be replaced with a quantity of forage NDF to maintain constant milk fat. The animal response to eNDF is seen in its milk fat percentage. This fiber eNDF takes into account the buffering capacity, fat concentration and composition, quantitative and qualitative production of acids during rumen fermentation substrate, peNDF and other characteristics of the feed or metabolic factors that influence the milk fat production. ENDF is more associated with the concept of the effectiveness of the fiber and conceptually broader than the peNDF.

The eNDF can vary from 0 for feeds that do not have the ability to maintain milk fat percentage, to values grater than 1 for feeds that stimulate chewing activity and maintain milk fat percentage.

For feeds that differ only for particle size, peNDF and eNDF should be highly correlated. The eNDF may be smaller than the peNDF for some feeds that decrease ruminal fermentation and milk fat (feeds containing sugars) without reducing chewing activity. Conversely eNDF may be greater than peNDF for some feed that has an intrinsic buffer capacity and does not stimulate chewing activity.

Because peNDF relates only to the physical characteristics of fiber, it is a more restricted term than eNDF . While the peNDF will always be less that NDF, eNDF can be greater or smaller than NDF (Figure 2).

1. Physiological aspects: role of the fiber in lactating dairy cows diets

2.1. Fermentation of carbohydrates by rumen microorganisms

The rumen environment has a vast population of microorganisms. Bacteria are the major microbial population with a concentration is about 10^{11}/ ml. There are also protozoa (about 106/ ml) and fungi (about 8% of the microbial flora), which play an important role in the digestion of fiber and in the degradation of complex carbohydrates (Bortolami et al., 2001; Dell'Orto and Savoini, 2005). Microbial cells formed as a result of rumen digestion of carbohydrates under anaerobic conditions become the major source of proteins and vitamins for ruminants (Van Soest, 1994).

In ruminants a large proportion of carbohydrates are fermented by bacterial microflora and protozoa microfauna (Beghelli, 2002). Microorganisms in the rumen ferment non structural carbohydrates such as starch, sugars and pectins, and structural carbohydrates, such as cellulose and hemicelluloses, and into volatile fatty acid (**VFA**), energy (in form of ATP), and microbial proteins.

The non fiber carbohydrates (**NFC**) are an important source of energy for ruminants. In fact, thanks to their fermentation, large amounts of propionic acid, which is a glucose precursor, are produced. There are four categories of NFC: organic acids, sugars, starch and neutral soluble fiber. These four different categories behave differently in the rumen. Sugars tend to have rapid fermentation. Starch is degraded more slowly, with large variations depending on its source, physical form and processing to which may be submitted. Neutral soluble fiber, which include pectins, fructans and others polysaccharides which are not included in NDF, generally ferments rapidly (20-40%/h).

Sugars and starch main fermentation products are propionate and butyrate, with lower amounts of acetate. When supplied in excess, they might cause lactic acid accumulation. In this they differ from soluble fiber (particularly pectins), which produces more acetate and never lactate.

The VFA composition depends obviously on the percentage of fiber in diet, the optimal relationship among VFA should be 60% acetic, 25% propionic and 13% butyric.

Non digested carbohydrates such as lignin-encrusted cellulose and hemicelluloses escape the rumen and are expelled in faeces (Succi and Hoffmann, 1997).

2.2. Feed and digesta distribution in the rumen

The ruminal content has a liquid phase component (about 85% of ruminal contents) and a solid phase (Dell'Orto and Savoini, 2005).

Sutherland (1988) described the rumen as an "effective first-stage separator. Through filtration and mechanical entanglement, the ruminal mat retains potentially escapable fiber particles, thereby increasing the time for digestion".

Ideally, in the rumen ingested particles form layers according to their size and density. Coarser particles are found at the top , while the heavier particles settle in the upper rumen. Coarse particles form a mat that filters the smallest particles, which otherwise would escape the rumen (Welch, 1982, 1986; Grant, 1997).

2.3. Effects of fiber on rumen functionality

Total mixed rations (**TMR**) techniques allow lactating dairy cows to have a standard ration all day and have limited fluctuation in ruminal pH. Thus particles in TMR should have a physical role and should promote chewing to prevent ruminal acidosis and laminitis.

The fiber content of the diet is a strong stimulus for rumination, thus long fiber is regurgitated and chewed; the rumination consists of several cycles that generally last 35-40 minutes (Swenson and Reece, 2002), producing a large amount of saliva (a colorless liquid containing bicarbonates, chlorides, phosphates, proteins, mucins and urea), which naturally buffers the acid of the rumen (Succi and Hoffmann, 1997). Without the natural buffer that comes from chewing, the ruminal pH decreases, leading to ruminal acidosis and determining a cascade of events like a reduction of cellulolytic microorganisms, a reduction of percentage of acetate and a consequent milk fat depression.

In formulating diets, nutritionists should be mindful of these recommendations, and it is

important to understand that rapidly fermentable carbohydrates may have even greater effects on variation in rumen pH than ration particle size alone (Krause and Combs, 2003).

The NRC (2001) recommends a minimum of total NDF (as % of DMI) of TMR, a percentage of which must be from forage (Table 1; NRC, 2001). Current NRC requirements outline that maximum ration NDF is a function of the NFC concentration, which effects the intake and the animal's NEL requirements (Table 1; NRC, 2001). Although rumen fermentation and rumen function may be negatively affected in cattle fed rations which are deficient in fiber, excessive levels (over 44% of NDF) may also result in negative effects, reducing intake and digestibility.

Although many studies demonstrated that fiber requirements are very important in formulating diets for dairy cows, the current NRC recommendations do not provide details about the ratio of physical form.

Fahey and Berger (1988) have shown that cows consuming sufficient quantities of NDF but insufficient quantities of long particles tend to have the same metabolic disorders as cows consuming diets which are deficient in chemical fiber.

2.4. Fermentation and rumen pH

The quantity of organic matter fermented in the rumen drives to VFA production; although this VFAs are relatively weak acids there is a high correlation between the acids concentration in ruminal content and ruminal pH (Allen, 1997).

The ratio of three major volatile fatty acids produced (acetate, propionate and butyrate) are critical in determining ruminal pH. VFAs are absorbed across the rumen wall. Lactic acid is produced only in small amounts when the ruminal pH falls below 6. During its passage across the rumen's wall, butyric acid converts into ßhydroxybutyric acid (**BHBA**). Acetic and propionic acid pass almost unchanged across the rumen wall into the portal blood and are carried, together with the BHBA, to the liver, where they are used as sources of energy and fatty acids (McDonald et al., 1988). The ruminal pH is the result of VFA produced by fermentation and buffer secretion. The proportions in which they are produced, determines fat and protein content of milk. The ratio of the various VFAs produced depends on the type of feed that is

being digested. There is great variation of ruminal degradation on DM depending on diets. The rate of digestion in the rumen is the cause of diurnal pH variation (McDonald et al., 1988).

Diets containing high non-fiber carbohydrates (such starch and sugars) are rapidly fermented in the rumen, driving to rapid production of VFA. When the rate of VFA production exceeds the rate of buffering capacity of the rumen, the result is a low ruminal pH and sub-acute acidosis may occur. (Beauchemin, 2008). This affects the amount peNDF that is necessary to maintain adequate ruminal pH.

Ruminal pH declines after meals, and more precisely, pH decline when meal size increases and when dietary NDF concentration decreases (Allen, 1997). Low pH is negative for rumen microbial population; thus rumen residence time is reduced and undigested fibre particles pass quickly to the large intestine and caecum, resulting in hindgut fermentation, where only VFA are absorbed, whereas microbial proteins are lost in faeces as undigested particles (Hall, 2002 a).

Many studies demonstrated 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 content and yield (Figure 3). Low ruminal pH decreases DMI, fiber digestibility and microbial yield and thus decreases milk production. (Mertens, 1997; Beauchemin et al., 2003a).

The optimal pH for ruminal microbes ranges from 6.5 to 6.8; below pH 6.2, NDF digestion declines for all forages. Cellulolytic bacteria indeed are more sensitive to low ruminal pH than the amylolytic ones (Grant, 1997; Mertens, 1997). The interaction of ruminally fermented carbohydrates and physically effective fiber must be considered when diets for dairy cattle are evaluated and formulated. Diets rich in carbohydrates allow an increase of ruminal fermentation, but it is essential to increase NDF of TMR or peNDF to maintain an optimal ruminal pH.

2.5. Particle size definition

The particle size is the representative dimension that describes the degree of reduction of the particle in TMR. In particles with spherical form, the diameter itself is the dimension and coincides with the size. When particles deviate from spherical symmetry, the size can be represented as any dimensional distance from two points of the external surface and the diameter

of them intersects the centre of gravity (Irani and Callis,1963, cited by Kononoff et al., 2003). Graphically, the illustration of particle size distribution attempts to determine the frequency of the proportion of individual fractions of the sample. Several mathematical distributions have been used to interpret particle size using results based on sieving techniques. These include: exponential (Smith et al., 1984), gamma (Allen et al., 1984), and weibull (Lammers et al., 1996) distributions. Finner et al., (1978) described a method of sieving based on a lognormal distribution that has been adopted by the American Society of Engineers (ASAE, 2001) for describing forage particle size. Even though the best fit of a specific mathematical distribution is likely to depend upon the methods of sieving, sample type and the nature of processing, the lognormal approach may be the most suitable over others, as others are more mathematically complex due to parameter estimation. Kolmogoroff (1941) was the first to describe a lognormal distribution with respect to ground particles. This approach is simple and has generated two useful parameters; the log mean and log standard deviation, that can be used as estimates of the sample geometric mean and standard deviation. It is possible to use a spreadsheet that performs all of these calculations and graphs, available on the Penn State Dairy Nutrition website. Normally, cows consume particles of many size, making both the mean particle and the variation in particles very important. The variation allows for a steadier rate of digestion in the rumen and passage from the rumen (Van Soest, 1994). The means (distribution of particle size) should provide a sufficient quantity of long particles for cud chewing and smaller particles for rapid fermentation, which would allow for maximal dry matter intake (Mertens et al., 1984).

2.6. Effects of particle size on rumen pH

Although many studies have shown either no effects or negative effects of peNDF on ruminal pH (Fernandez et al., 2004; Beauchemin and Yang, 2005), many others have demonstrated the relationships between the physical form of the diet, such as the forage particle size, and the ruminal pH (Krause et al. 2002a). The peNDF proposed by Mertens (1997) is strongly associated to ruminal pH; this fraction is able to stimulate chewing activity and saliva secretion, which contains high concentration of bicarbonate and phosphate, important in neutralizing acids produced in rumen fermentation. Longer particle size stimulates the rumination; the longer the cows ruminate, the greater the amount of saliva they produce, and more saliva produced means more buffering capacity (Grant et al., 1990a,b). Microbial fermentation of carbohydrates from

fiber is a relatively slow process, while NFC are easily and rapidly fermentable. Consequently, forage particle size must be sufficient to stimulate rumination, prevent the decrement in ruminal pH, and capture small feed particles (Grant, 1997).

2.7. Intake and particle size

Waldo (1986) suggested that NDF is the best single chemical predictor of dry matter intake (**DMI**) by ruminants. Although NDF has been used as sole characteristic of forage to predict the filling effects, this is inadequate (Mertens, 1987, 1994); its filling effect depends on initial particle size, particle fragility, rate of passage and NDF digestion (Allen, 1996). The effect of forage and TMR particle size on feed intake is unclear and sometimes conflicting results are experienced. Difficulty in interpreting the response of particle size on DMI might be due to digestibility and specific gravity-factors independent of the individual feed particle size. Allen (1996) cited many studies reporting the negative relationship between particles and density and retention time in the rumen.

Intake response when reducing particle size is usually positive, with the magnitude depending upon the extent of particle size reduction as well as the type and digestibility of the forage fed (Kusmartono et al., 1996). Dietary particle size influences the passage rate in the rumen and modifies the digestibility of feed. Diets containing very long fiber limit the intake due to the filling effect (Van Soest, 1994) and the animal will need more ruminating time. Forage composition and level of concentrate in diets have a variable effect on intake (Sutton, 1989). NDF has been related to the intake. According to Mertens (1985), NDF intake should be 1.2 ± 0.1 of body weigh (BW) and 70-80% of NDF should be from forages.

Diets with short particles are consumed in the greatest amounts and have the highest intake of digestible nutrients, resulting in greater rumen VFA concentrations (Kononoff and Heinrichs 2003a). Allen (2000) and Tafaj et al. (2004) found a positive correlation between increasing amounts of digestible fiber (hay or corn silage), forage intake and fiber digestibility. Other studies carried out by Nocek and Russel (1988) have shown that increasing NFC in diets increased DMI; this may be due to the high palatably of NFC.

As particle size decreased, there was an increase in DMI because of the density of particles increases (Allen, 2000). Shaver et al. (1988) and Beauchemin et al. (1997) found that when poor

quality, high fiber diets were fed, reducing the forage particle size significantly increased DMI. Forage particle size has less impact on intake when well-balanced rations are fed to lactating cows (Beauchemin et al., 1997).

Reducing particle size decreases the filling effects of forage and increases ruminal passage rate (Allen, 2000). Hence, forages that occupy larger volumes per unit of DM weight (which have lower bulk density) should have a greater ruminal filling effect than more dense forages.

2.8. Chewing activity and particle size

Chewing activity is the first mechanism to reduce particle size in feed and influence both the nature of the digestion and the passage through the gastro-intestinal tract. When cows feed on diets with increased particle size rumination time is longer (min/kg DM, and NDF intake) (Grant et al., 1990a,b). Chewing activity per kg of DM varies depending on fiber content, on the particle size of the feed, on the level of intake and on the physical state of the animal (Murphy et al., 1983; Mertens, 1986; Sauvant et al., 1987).

To avoid ruminal diseases, the time spent on chewing should be increased by adjusting either the level of particle size or by the NDF content in diets (Yang et al., 2001). As outlined and confirmed by Beauchemin (1991), the time spent chewing and ruminating is a function of animal and dietary factors. Bae et al. (1983) described the body weight as the main determinant of chewing rumination; large animals tend to be more efficient in rumination than smaller ones.

Beauchemin and Buchanan-Smith (1989) demonstrated that cows feeding on different diets with different NDF concentration spent the same time ruminating. This indicates that rumination is more efficient when the level of fiber in the diet is higher, and indicates a reduction in rumination time per unit of hay NDF. When the intake increases the time spent on ruminating per gram of feed is reduced, this may explains why at higher intakes faecal particles increase (Van Soest, 1994).

Rations with smaller forage particles size go into the rumen at a smaller size after initial chewing and swallowing; as a result, they leave the rumen more rapidly. The effect is an increase in the fractional turnover rate of ruminal DM, and increased DM (Jaster and Murphy, 1983; Weston and Kennedy, 1984; Fahey and Berger, 1988). Smaller forage particles stay in the rumen for a

shorter time, consequently they are less available for microbial digestion and the effect is decreased digestibility, particularly with regards to fiber digestion (Allen, 1996).

Grant et al. (1990b) demonstrated that cows which were fed a finely chopped ration ruminated 2.5 h less than those fed a coarse ration; this shorter rumination time decreased bicarbonate production by 258 g/d Beauchemin et al. (2003a).

Chewing activity is drastically reduced when hay is ground to 2.9 mm. Several authors (Beauchemin and Rode, 1997; De Brabander et al., 1999; Tafaj et al., 1999; Bal et al., 2000; Schwab et al., 2002; Yang et al., 2001; Kononoff and Heinrichs, 2003a,b) demonstrated that a severe reduction of forage particle size (usually less than 3 mm mean particle length) decreased chewing activity.

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., 2001).

PeNDF fiber corresponds to the NDF in ration that stimulates chewing activity and rumination. Fiber which is greater than 1.18 mm needs to be reduced and chewed before to pass out the rumen. According to critical size theory, particles longer than 1.18 mm have the greatest resistance to passage and are largely responsible for stimulating chewing and rumination (Poppi et al., 1980).

Many studies in fact have shown that the peNDF of a feed is associated to chewing activity because this fraction of fiber influence the bi-phasic mat and the rumen environment (Welch and Smith, 1969; Welch and Smith, 1970; Camell and Osbourn, 1972; Mertens, 1997). In particular, diets containing high peNDF have a higher stimulatory effect on mastication than diets with finely chopped forage (Figure 4). Particle size is negatively correlated with chewing activity per kilogram of DM (Allen, 1997; Mertens, 1997) and positively related with ruminal pH (Allen, 1997).

2.9. Milk fat and particle size

Milk fat percentage has been the focus of many recent researches and field applications of effective fiber (Beauchemin and Buchanan-Smith 1989; Grant et al. 1990a,b; Beauchemin 1991; Armentano and Pereira 1997; Krause et al. 2002a). Milk fat percentage can have significant

economic impacts, it is easy to measure, it reflects animal performance and effective NDF, which in turn accounts for factors that affect peNDF - factors that affect ruminal acid production and milk fat production. Low milk fat percentage is often associated with a reduction of particle size in diet (Mertens, 1997). Cows consuming different diets with equivalent amounts of NDF concentration, showed a milk fat depression when particle size was reduced (Grant et al., 1990a). The primary effect of small particle size may be a lower ruminal pH due to the smaller salivary production (Welch, 1982). Because fermentation is influenced by the composition (environment) of microbial rumen population, when the ruminal pH falls below 6 there is a drastic reduction of cellulolytic microorganisms (Russell et al., 1979). Ruminal cellulolysis is totally inhibited when pH< 6.0 (Mould et al., 1983). Allen (1997) in his study found that milk fat percentage is less responsive in early lactation cows than cows in later lactation, but also that fat percentage and rumen pH were positively correlated, supporting the idea that lower levels of rumen pH < 6 resulted in higher propionate levels was the cause of milk fat depression (Figure 3). Finely chopped TMR influences the metabolic answer of serum insulin, which plays an important role in milk fat depression and alters the lipid synthesis of milk fat (Grant et al., 1990b). Small particles size have a negative effect on ruminal pH and acetate- propionate concentration. The consequence is a deficiency of ß- hydroxybutyrate (precursor of milk fat) to the mammary gland and a decrement in milk fat (O'Dell et al., 1967). In order to maintain milk fat percentage, some authors suggest that TMR diet should contain adequate amounts of fiber, thus chewing activity should be stimulated for minimum 30 minutes per kg of DM (Sudweecks et al., 1979 ; Norgaard, 1986, quoted in Mertens, 1997) or 24 minutes (Woodford and Murphy, 1988).

Yansary, (2004) reported a 10% reduction in milk fat when dietary peNDF decreased from 25% to 17.2%.

Mertens (1997) proposed the use of peNDF as an indicator of fiber requirement in TMR and to assure chewing activity and milk fat level. He suggested to use peNDF to calculate some regressions based on 36 published studies:

Milk fat % = 4.32 - 0.171 x (1/peNDF) $r^2 = 0.63$; SE = 0.17

ruminal pH = 6.67 - 0.143 x (1/peNDF) $r^2 = 0.71$; SE 0.10

These regressions allowed to calculate peNDF requirements in relation to the milk fat percentage and rumen pH (Table 3). Both milk fat (%) and rumen pH are reliable physiological parameters that summarise the effectiveness of the fiber in the ration fed to cows.

2.10. Faeces and particle size

Faeces can provide indirect information about the diet fed to cows. The feed consumed by a dairy cow is digested in the rumen, whereas the majority of nutrients are absorbed in the small intestine. When animals are fed unbalanced diets, low in fiber or too rich in NSC for example, some of particles may be drastically undigested, escape the rumen, and may reach the small intestine where hindgut fermentation may be excessive. This has a negative effect on the animal's health and its milk production. All undigested particles are excreted in the faeces and urine (Kononoff et al., 2002). In the case of hindgut fermentation this can frequently be identified in the faeces where in severe cases it takes the form of diarrhea. An adequate peNDF is the key to satisfactory ruminal digestion and intestinal absorption.

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 NFC on ruminal pH (Hall, 2002a). When lactating dairy cows are fed diets low in peNDF or high in digestible NFC, ruminal residence time is shortened and proper ruminal fermentation process will be disturbed; thus long particles may be found in faeces.

Large amount of particles in faeces are 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).

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 to have better rumen fermentations and nutrient utilization than when they are fed concentrates and forages separately (NRC, 2001). Animal access to feed and sequence of feeding also have a role to play in the consistency and particle size of the faeces (NRC, 2001).

2. Methods to measure peNDF

3.1. Measuring peNDF content in total mixed ration

The use of TMR as feeding technique for lactating cows is widespread in large and highly mechanized dairy farms. Often they main component is corn silage. The inclusion of corn silage in TMR reduces the cost of feeding and improves the final characteristics of TMR (better mixing, high moisture and low dust) (Bonsembiante, 1983; Berzaghi et al., 2000).

Currently, dairy cattle are fed more silages than before. In Sardinia, in particular, long hay has been commonly replaced by corn silage in total mixed ration. This feeding strategy has a strong influence on how much time cows and heifers spend eating and ruminating. This also has different effects on animal health (Huzzey et al., 2006). The proportion of forage and concentrates may also significantly influence the ruminal stratification in the rumen and the DM intake.

The type of fiber is something that is indirectly and yet strongly linked to the fibrous layer (stratification) in the rumen, the vegetative stage of plant, the techniques of preservation, length of chopping and the moisture content of the plant (Andrighetto, 1999). TMR is generally accepted as an adequate technique to meet increased requirements of high lactating cows and animals energy supply (Heinrichs et al, 1999).

TMR plays an important role in formulating diets for lactating dairy cows, it allows animals to eat particles of many sizes, thanks to feeding frequency and ingested amount (Van Soest, 1994).

The mean particle size and the distribution in TMR are important to ensure a good relationship between rumen degradation rate and rumen passage rate, from which depends the true degradability of nutrients. Having the proper particle size distribution is as important to formulate balanced diets (Henrich and Kononoff, 2002). Thus for a correct management is important not only the mean particle size but also the particle size distribution of TMR (Mertens et al., 1984).

3.2. Particle size measurement

Nowadays many nutritional models used in dairy industry require peNDF as a key input to predict lactating response, thus measuring peNDF content of TMR has become important to nutritionists.

The concept of peNDF measurement based on cows chewing response to forage feed particle size and NDF is considered to be reliable in preventing metabolic disorders such the risk of acidosis.

Several methods mentioned by Mertens (1997) have been proposed to measure peNDF. Some of them were based on specific experiments with animals, some others were based on mathematical models developed regressing different NDF forages sources at different length-size against the minutes of chewing per day. Other methods used analytical approaches and were based on standard procedures involving laboratories sieve instruments or field sieve methods. The instrumental measurement of peNDF assumes that only the fibrous particles that are large enough to have to be chewed are associated with peNDF (Mertens, 1997). This hypothesis is based on the distinction between particles which have a size that would stimulate rumination and those that do not stimulate any rumination and pass through the rumen without being chewed. Based on these considerations Poppi et al. (1985) and Mertens (1986) suggested that particles greater than 1.18 mm would have been reduced through chewing and rumination before moving through the rumen; as a result these particles would stimulate saliva secretion, more than those lower than 1.18 mm.

All particles > 1.18 mm have a high resistance to pass out of the rumen. This measure has became a standard laboratory measurement for *pef* for feed using dry sieving techniques.

The standard method for measuring peNDF based on chemical and physical characteristics of the feed has been proposed by Mertens (1997). Originally peNDF was defined from an analytical perspective, as the proportion of sample NDF found in particles retained in 1.18 mm sieve screen, sieving dry samples TMR by a vertical shaking method. Since then, in order to be used on farm, peNDF has been simplified to the proportion of DM particles (>1.18 mm) retained after dry sieving and multiplied by the percentage of NDF sample.

This method is based on the assumptions that NDF is uniformly distributed across all particles size and that chewing activity stimulated by all the particles retained on the 1.18 mm screen is

similar. Furthermore, this method is also based on the hypothesis that fragility of particles during chewing activity is similar among the different NDF forages sources.

3.3. Evaluating peNDF by laboratory method

Ruminants require forage fiber in coarse physical form (NRC, 2001), making it effective in maintaining proper rumen health and function (Kononoff, 2002). It is well known that a sufficient amount of coarse fiber is needed to stimulate rumination and to maintain proper rumen fermentation (Yang et al., 2001). The physical and chemical characteristics of TMR are also extremely important to achieve high-quality milk and production.

The standard method (Mertens, 2002) to measure peNDF is a laboratory device to measure particle size distribution of TMR and forages. This standard procedure involves the use of a vertical sieve, the Tyler Ro-Tap Sieve Shaker RX-29 Model (278 oscillations with horizontal and vertical shaking for 150 minutes) with different screens and with different apertures. The procedure using a Tyler Ro-Tap Sieve Shaker RX-29 Model provides dry sieving samples with vigorous vertical shaking.

Hemicellulose is a complex of carbohydrate polymers composed of xylose, galactose and mannose.

Pectins are derived from the acid galacturonic and are characterized by high digestibility.

he sieve is made by different circular steel screens (diameter 20 cm), with different hole sizes: 19.0, 13.2, 9.5, 6.7, 4.75, 3.35, 2.36, 1.18, 0.6 and 0.3 mm and a bottom pan.

The screens with 4.75 mm hole sizes or greater are 5 cm high, while those < 4.75 mm are 2.5 cm high. The screens have a square grid and are arranged vertically (arranged from the grater hole size to the lower one). The vertical sieving must vigorous to separate the small particles from the greater ones. For a correct evaluation of peNDF, the number of screens used should be the maximum possible, to prevent the accumulation of material sample in some of them.

The method allows the measurement of peNDF of TMR directly determining the NDF in particles that pass through 1.18 mm aperture screen, and subtracting this amount from the total amount of NDF.

Pef = 100 - (particles that pass through 1.18 mm)

peNDF = NDF of sample – (particles that pass through 1.18 mm) x (NDF of the fraction <1.18mm)

A simple way to calculate peNDF proposed by Mertens (1997) is to multiply the fraction retained in the screen at 1.18 mm by the NDF content of the sample.

peNDF = particles fraction retained in 1.18 mm screen x total NDF of sample.

For a correct ration formulation, the optimal peNDF in TMR for lactating dairy cows was determined to be 22% (as % of DM in TMR). This allow to maintain rumen pH > 6. To maintain milk fat percentage higher than 3.4 % peNDF should be equal to 20% (as % of DM in TMR) (Mertens, 1997).

3.4. Evaluating peNDF by field methods

Various methods have been proposed in order to simplify the laboratory method. All of them were based on the challenge of measuring ration physical form with the aim to maximize productivity and reduce health problems. On-field instruments may have monolayer or multilayer screens. While the monolayer sieves are composed of only one screen and a pan, the multilayer ones are composed of different screens and a bottom pan.

These on-field sieves, contrarily to the laboratory one, are developed for use on TMR samples as found in farms. The most common on-field models are the Penn State Particle Size Separator (Penn State University) and the Z-Box (Miner Agricultural Research Institute Chazy, NY).

3.5. Evaluating peNDF with the Penn State Particle Separator

The Penn State Particle Separator (PSPS) is one of the most common on-field method to measure particle size of feeds or particles size distribution of TMR (Lammers et al., 1996). This sieve is based on the characteristics of the standard S424 of American Society of Agricultural Engineers (ASAE, 1998). The original model of PSPS consisted of two screens (Lammers et al., 1996) with circular holes sized 19 mm (Upper) and 8 mm (Middle), and a bottom pan (Bottom). The pef was measured as the percentage of particle fraction retained (greater than 8 mm), while peNDF was obtained multiplying the pef by the percentage of NDF in TMR sample. To measure the pef is important to use fresh samples and because the *pef* should be expressed as a proportion of the

total DM, analysis of DM for each screen is needed. Correction of DM is very important because the moisture content may affect the pef value results sometimes overestimating pef by up to 30%.

The PSPS now includes an additional third screen with 1.18-mm opening (Lower) to better fractionate smaller particles of TMR and also to improve the repeatability of the particle shaking technique (Kononoff et al. 2003c).

The measure of *pef* is the percentage of particles retained in 1.18 mm screen and peNDF can be measured multiplying this fraction by the NDF of the sample TMR.

peNDF = pef x NDF of the sample.

The use of PSPS is simple and consist of taking a representative sample of TMR of approximately 1.5 litres or 1.4 litres + / - 0.5 (Lammers et al., 1996) (about 500 g), which is placed on the top screen, and by sieving shaking the PSPS horizontally five times in one direction. It is then rotated 90° degree. This sequence is repeated again five times. By repeating this operation for eight sets of five shakes, for a total of 40 shakes, the stratification of feed particles on each sieve as a function of their size is guaranteed (Lammers et al., 1996). There should not be vertical shakes (Heinrichs and Kononoff, 2002) (Figure 5). Each shake is a forward and backward motion over a distance of about 20 cm. while the frequency used for each shake is approximately 1 Hertz. The force of the shakes and the direction must be enough to enable the particles to fall through the holes of the screen.

Using this methods the larger particles stay in the top screen, the middle and the lower screens retain nearly equal portions and the remaining portion falls to the bottom (Lammers et al., 1996). It is suggested to repeat this evaluation in three different points of the bunk feed, (beginning, middle and end) and calculate the mean. This is because there may be differences or granulometric anomalies along the bunk. In fact, sometimes, mixing and distributing all the feed to prepare the final TMR may reduce particle size. The material sieved kept by each screen is weighed and the percentage of each screen is then calculated. Guidelines (Table 2) are recommended to achieve a proper particle size distribution in TMR; this may help particularly in case of a need to solve nutrition problems. Ideally, the first screen (upper) should not keep more than 8% of material, whereas the middle and the lower should contain from 30% to 50%, and the bottom one no more than 20%. Although there is a wide variability in dairy cows diets, this

method provides clear information about the characteristics of the fiber in TMR rations, thus allowing to measure the distribution of feed that the cows actually consume (Heinrichs and Kononoff, 2002).

This method provides further information on how well the feed mixing wagon is working, and the ability of the operator who uses it, judging the degree of homogeneity of the product along the bunk. It is also possible to investigate if cows are selecting one of the three fraction of particles (Cozzi, 2004).

One aspect which may affect negatively the outcome of sieving process may be the moisture of the sample. In fact, if the moist content is high the PSPS may not separate the sample accurately. The optimum percentage of moisture should be around 50% (Shaver, 2002). When the DM of the TMR is higher than this, there is a reduction of DM intake. To highline sorting behaviour, nutritionist should evaluate the particle size of the original TMR and compare it to the feed remaining in the bunk at different times during the day and at the end of the 24 hr feeding period to monitor this activity (Beauchemin, 2007). Alternatively they should calculate " the selection index" comparing the percentage of the content in each screen at the beginning of the feeding time and the percentage of content in the same screens after four hours (Shaver, 2002). The PSPS continues to be used as a tool that quantitatively estimates forage and TMR particle size. Figure 5 shows the scheme of sieving proposed by Heinrichs and Kononoff (2002).

Although the effects of particle size on rumen fermentation are well known (Fischer et al., 1994; Grant et al., 1990 a, b), on-farm routine-analysis has only recently received attention. Taking fresh samples from the feed bunk before cows sort the feed is extremely important when measuring the particle size distribution in TMR (Heinrichs et al. 1999).

Even if the moisture content of the TMR may affect sieving properties it is not practical to recommend analysis at a standard moisture content (Finner et al., 1978). The role of PSPS is to describe particle size of the feed offered to the animal, thus, it is suggested that samples should not be chemically or physically altered before sieving. Even though moisture can affect the results of measurement, only small differences have been reported from collected samples. Sieving completely dried samples would lead to further size reduction of particle feed and great differences in particle size (Kononoff, 2002).

According to Allen (2000), the size and density of feed particles influence ruminal motility and the passage of the ingested material through the rumen. Forage and TMR particle size may affect feeding behavior and rumen fermentation. The speed of transit through the rumen depends on different levels of cutting of the fibrous portion in diets. Dry matter intake depends on length size of diets. In particular, diets containing alfalfa haylage as a forage source, increase the portion of particles > 19 mm in TMR, thus increasing chewing activity (Kononoff and Heinrichs, 2003a).

In fact, the proportion of material > 19.0 mm retained on the top screen is very important, because the intake of DM from this portion of the diet is known to be positively correlated with ruminating activity and has been demonstrated to be negatively correlated with the amount of time the rumen pH is below 5.8 (Kononoff and Heinrichs 2003a, b; Krause et al., 2002b).

One of the limitations of using this method may be that many ways to measure *pef* can be used and the intensity and the duration of shaking could influence the final result. This can have a considerable impact on the percentage of fiber that passes through the holes of the sieve.

Yang and Beauchemin (2006a), in their studies measured and compared peNDF (two screen sieve) peNDF(2s) and peNDF (three screens sieve) peNDF(3s), and found that peNDF(2s) were always lower than peNDF(3s) (Table 4). Additionally peNDF(3s) readings were closer to 21 % suggested by Mertens (1997). This seems to show that peNDF(2s) is not accurate compared to the dry standard method. Furthermore *pef* (*3s*) values are more closely to peNDF values used by CNCPS and CPM software.

Because peNDF (3s) contains a large pool of particles, the peNDF (3s) is always higher than peNDF (2s), Kononoff and Heinrichs (2003 a); Kononoff et al. (2003 c); Plaizer, (2004).

Conversely the main disadvantage using *pef3s* is that in case of diets with forages with varying chop lengths, *pef3s* values show only small differences (Beauchemin, 2007). In fact when *pef* is calculated with two sieves, the range of variation goes from 0.41 to 0.72, while when it is calculated with three sieves, pef 3s ranges only from 0.93 to 0.96. In addition, large quantities of grain could be trapped in the third sieve (1.18 mm) increasing *pef3s* values (Table 4).

Zebeli (2006) examined and reviewed with meta-analysis all researches in which peNDF was measured and then correlated them with cow responses. The result was that peNDF(2s) was poorly correlate to ruminal pH (R= 0.27), whereas peNDF (3s) was more correlated (R=0.67), and it is also closer associated with daily rumen activity than peNDF (2s).

On the contrary, using two sieves, there is only a small difference between using the TMR itself or the component forages to measure peNDF2s, except for the fact that TMR contains very coarse grains or large pellets. Both two and three sieves system to measure peNDF have advantages and its disadvantages.

3.6. Evaluating peNDF with the Zeta Box

The Z-box, a new instrument to measure *pef,* was recently introduced. This separates forage particles by length much like the Penn State Forage Particle Separator does. It was developed by Kurt Cotanch and Rick Grant at the W. H. Miner Agricultural Research Institute (Chazy, NY), in cooperation with Zen-Noh National Federation of Agricultural Co-operative Association of Japan. It is a box that measures 21x21x11 cm in size and has an open plastic side and 2 interchangeable sieves (Figure 6).

The Z-box is a monolayer screen and produces with a single value, the *pef*. This is based on 2 different interchangeable sieves, at 3.18 mm and at 4.76 mm (with circular hole apertures). The 3.18 mm aperture screen is used for corn silage and TMR while the 4.76 mm screen works best with hay silage. The box works in the same way as the PSPS by shaking the sample vigorously and it only takes 30 seconds to do it.

Given the small size of Z-box, the volume of sample should not be big. The volume of the sample should in fact be adequate to the small surface area sieve of the Z-box in order to ensure particle separation, and at the same time it should be representative of TMR. A 50 g (250 ml approximately) sample of feed is placed in the box and weighed. The box is then shaken vertically 50 times then rotated by 90 degrees after each 10 shakes. Short forages may fall through the screen and out of the box. The proportion of material retained on the screen after shaking is used to determine peNDF. Because the sample weighs 50 g it may not always be a representative sample of TMR and forages it is recommended to repeat 3 replicates per sample and average the results to be sure to have a representative sample of TMR. The results were validated against the Merten's reference method using a 1.18 mm screen with a Ro-Tap vertical shaker. After three replicate per sample it is possible to measure *pef* value, the number is then multiplied by the forage's lab-test NDF to get its peNDF (Cotanch and Grant, 2005).

pef = Σ of 3 weight retained on screen divided by the Σ of 3 initial sub-sample weight x 100

PeNDF = pef x NDF of TMR sample

The advantage of using the Z-box is to be a is very simple and useful instrument. While it is not intended as a replacement for the Penn State box, it may do a better job of helping to monitor rumen health. Recent research has shown that the amount of forage on the top two screens of that box is not always an accurate indication of effective fiber.

3. Evaluating TMR by NIRS techniques

Currently there is a widespread use of a quick method of analysis, based on the Near Infrared Reflectance Spectroscopy (NIRS) technology. This technology uses some physical properties of matter and in particular the interaction of this with the near-infrared radiation. This technique relies on the ability of any specific chemical compound to absorb, transmit or reflect light radiation. The combination of the absorptive properties, combined with those of dispersion of the light, determines the diffused reflectance of light, which contains information on the chemical composition of the sample. The important aspect of this method is that it allows for rapid investigation of many samples with a significant reduction of time and costs compared with traditional techniques. This is mainly due to the simplicity of the operations of preparation and variety of analysis possible, the ability to use the sample again and the lack of reagents. (Norris et al.,1976). The method has been adopted by the Association of Official Analytical Chemists (AOAC) for many official analyses of forage evaluation like CP and ADF (Barton and Windham, 1988 and Murray 1993).

4.1. Calibration

Calibration is the mathematical process that allows to relate the information of the physicochemical properties of a test samples with NIRS optical measurements. The information sought must be determined in parallel with an independent technique. The first step to start a calibration experiment is the creation of a set of reference data containing the maximum amount of samples with the maximum number of all possible variations. When there is an adequate and representative number of calibration curves, it is important to choose an appropriate statistical method for calibration (Deaville and Flinn, 1989). The most classic is linear regression. When a

greater number of variables are involved , the use of multiple linear regression, which allows to obtain more precise estimates is preferred. In both cases the high degree of inter-related absorbances at different wavelengths creates difficulties. Best results are obtained with the Principal Component Regression

or the Modified Partial Least Square System, which are based on information of the entire spectrum, building factors that can capture all the variability of experimental data. The calibration curve should be validated properly at the beginning with a set of known samples, only then it is possible to proceed with the introduction of new samples. The NIRS method is generally reliable, but this depends on the degree of mathematical-statistical update of the curves drawn on the basis of the response of each array to IR. Currently calibrations have been developed for several parameters, and they can be determined simultaneously in real time.

4.2. Analyses

Since the early seventies (Norris et al.,1976; Murray, 1986, 1993) calibration has been adopted as official method for the measurement of protein content in cereals and after, in the evaluation of forage quality, like CP and ADF. During this time calibrations have been developed for several parameters and they can be determined simultaneously in real time:

• Dry matter, Crude Protein, Ash, NDF, ADF, ADL, fat;

• soluble Nitrogen , N-NDF, N-ADF

• NDF Digestibility at 24 hours (%)

• Starch, total sugar, glucose, fructose, sucrose

• Calcium, Phosphorus, Potassium, Magnesium, Sulfur, Chlorine, Sodium, Iron.

On the contrary, the NIRS instrument itself is very expensive, and it is quite complex choosing the right algorithm to use for the interpretation of data and the careful calibration procedure of sophisticated equipment, and it is also necessary to depend on traditional chemical methods (Norris, 1989). The method is not applicable to a complete analysis of all constituents, including, for example minerals , because they do not absorb the energy in the NIRS region; but in some cases it is possible to obtain a satisfactory NIRS calibration due to the correlation between minerals and others organics components Clark et al. 1987 quoted by Deaville and Flinn (1989).

4. Evaluating the appropriateness of the nutritional management

5.1. Management of total mixed rations

There are a great number of management variables among farms (stall comfort, hygiene, ventilation, forage quality, etc.) that influence animal's productivity, and there are also many in feeding management, such as feeds, the feeder and the cows. These may be likely to alter the formulated ration from the consumed one (Stone 2004a). Nutritionists must have accurate knowledge of the feed used in formulating rations. Tabular values are often used for some feeds, such as forages, which are more variable than other feed. Consequently it is possible to reduce the variation in rations by analysing forages, and/or by using more consistent feedstuffs and by formulating a ration increasing the number of feedstuff (St. Pierre, 2001 cited by Stone, 2004a), (Table 5).

Maintaining consistent rations when these contain more than 4 or 5 ingredients is simpler. When there are not many ingredients in fact any change in moisture, fiber content or in palatability of any of the ingredients can greatly affect diet composition or feed intake (Mertens, 2000). When several ingredients compose the ration, it tends to be more constant because no one single ingredient is the major portion of the ration and it is improbable that all the ingredients would change in composition at the same time (Mertens, 2000).

Chemical analysis should be associated with visual evaluation of feedstuffs or visual analysis of conservation quality, which may reveal storage alterations. Both the ingredients sequence when loading into the mixer wagon and the mixer operation (time and speed) are extremely important to prepare a good ratio (Heinrichs et al., 1999).

Total mixed rations nowadays are largely used as a method of feeding dairy cattle. The main benefit of TMR is that cows are unable to express their preferences about feed ingredients and are more likely to consume balanced diets. Even though TMR promotes more balanced intake of forages and concentrates, if they are not correctly formulated in particle size, cows may exhibit the tendency to sort the feeds, preferring concentrate and smaller forage particles. When this happens, the cow is no longer consuming the desired amount of peNDF or even total NDF and is presumably more susceptible to health problems. Thus, monitoring the TMR quality is essential

in order to continuously evaluate the balancing of the diet with respect to the specific nutritional requirements of dairy cows. However, cows assuming sufficient amounts of NDF with finely chopped forage or feeding diets subject to large variations in the chemical composition over the day can also exhibit the same metabolic disorders as cow fed a diet deficient which is in fibre (Bailoni et al., 2006). For that reason, an adequate particle size of forages in TMR, and a limited process of diet demixing over time are necessary for proper ruminal function (Lammers et al., 1996) and consequently for animal welfare (Bailoni et al., 2006). Even if diets have been formulated with adequate amounts of fiber and peNDF, when TMR ratio is not well prepared because it is too coarse, cows may sort and the result is the consumption of variable rations. This is because dietary NDF is a very important component of rations of dairy cows, and because NDF alone has not significant relationship with ruminal pH (Allen, 1997). Many systems have been proposed to estimate the minimum amount of fiber and the correct particle size distribution in TMR. All these systems try to guide nutritionists in ration formulating and attempt to guide them in predicting the amount of chewing that different feed would produce, or their ability to maintain milk fat percentage (Mertens, 2002).

5.2. Sorting behaviour

When cow select the feed (sorting activity) may eat several types of TMR throughout the day. One of the signs of sorting is the presence of "holes" eaten into the offered TMR. In this phenomenon of feed selection, short particles may been preferred over the longer ones. As a result, some meals may have greater grain content than intended (Leonardi and Armentano 2000). This sorting behaviour may lead to an insufficient amount of buffering capacity and a subacute ruminal acidosis or displaced abomasums. Conversely, excessive mixing of the diets or excessive chopping of the coarse particles can lead to diets with low peNDF (Stone, 2004). Sorting can be minimized by avoiding excessive coarse particle in TMR. Furthermore, adding water to dry feed leads to changes in particle size distribution and could be a cost-effective management practice in dairy farms to also reduce the effect of sorting (Shaver, 2002).

Sieving TMR samples immediately and 4 hour after the distribution allows assessment and evaluation of cows sorting behaviour. Using the PSPS sieves it is possible to measure the so called "selection ratio", i.e. the percentage of particles retained on each of the screens 4 hours

after the TMR distribution in relation to the percentage of particles retained immediately after the distribution. Selection ratios >1 reveals that cows are selecting and sorting. Today more than ever, providing adequate physical form (particle size) of cows' diet is extremely important, particularly with regards to high concentrate diets for high producing cows (Cozzi, 2004).

5.3. Homogeneity of TMR

As well as chemical analysis, in order to define the correct feed composition of TMR it is also important to have information about the physical characteristics (particle size and particle size distribution) and its homogeneity along the bunk feed. Sieving TMR samples in different points of the bunk feed (initial, middle and end), it is possible to evaluate the homogeneity along the feeding area. TMR are considered homogeneous when the differences between the samples sieved along the bunk feed are less than 10% (Cozzi, 2004).

5.4. Faecal evaluation as a tool to monitor the efficiency of the diet

Many authors have shown the relationship between diet composition and faecal consistency. In other words the quantity of solids excreted is directly linked to the dry matter digestibility of the diet (Ireland-Perry and Stallings, 1993). Shellenberger and Kesler (1961) have shown that the physical characteristics of the diets effected the rate of passage and at the same time influenced manure consistency.

High-producing cows in early lactation excrete more fluid faeces and at the same time drink more water and eat more feed. High producing dairy cows have a faster rate of passage and more fluid faeces than those producing less. 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).

The ration composition influences the consistency and the colour of manure; in fact cows fed excessive protein diets or that experience high levels of rumen degradable protein, excrete liquid faeces. Conversely, cows which eat diets that are poor in proteins, or with lower water intake excrete firmer faeces (Kononoff et all. 2002). A lower faecal consistency may occur also when animals are fed excessive starch (Hall, 2002). Van Soest (1980) in his studies explained that the undigested dietary fraction in faeces is largely made by undigested parts of plants, mainly cell

wall, or structural carbohydrates (90%). The metabolic fraction (10%) secreted by the cow is made up of microbial debris (85 to 90%) and a small endogenous component (10 to 15%). The endogenous constituent may be reduced significantly by microbial fermentation in the lower digestive tract. Microbial matter in faeces is for the most part the indigestible remnants of rumen bacteria. Van Soest (1994) observed that when pelleted forages are fed, the particle size of faeces is larger than when long forages are fed. The conclusion was that large fiber particles may trap smaller ones in the ruminal mat and the consequence is a more extensive chewing of smaller particles. This logically suggests that the effectiveness of grains or kernels and other concentrates with small particle size may depend on the inclusion of some large particles in the ration.

Moreover Mertens (2000), found that cotton hulls behaved differently if cows were fed only roughage or if cows fed on chopped forage, and in the same way the effectiveness of cotton hulls was completely different depending on the length composition and on the inclusion of some larger particle.

To give the right evaluation of manure particle fraction it is necessary to walk inside the barn and observe the consistency, the colour of the entire herd, or to observe the animals within the same group. Normally there is a diurnal variation in manure, due to feed intake throughout the day. Large variations suggest the need of an adjustment of the ration.

Manure evaluation is an empirical method to understand how efficient the diet fed to cows is, describing the interaction of the herd with the ration. Although manure evaluation is not a totally scientific method, it may be helpful to identify some health problems. It should be used combined with other evaluations such ration management, sorting behaviour and cows comfort, to interpret the results and in particular those related to diet. The method is based on scoring herd faeces base on colour, consistency and content (Kononoff et al., 2002)

5.5. Colour

When animals are fed unbalanced diets, such as ones high in carbohydrates, faeces are bright, yellowish, with a sweet-sour smell (Kleen et al., 2003), foamy with gas bubbles and contain more undigested fibre or grain (Hall, 2002). Moreover, due to insufficient fiber ruminal mat, fiber is not effectively retained in the rumen and faeces contain more fiber particles with a 1.0 to

2.0 cm size instead of 0.5 cm (Hall, 2002). Manure is normally dark green when animals are fed fresh forage, and brown-olive when animals fed on more hay forage in the ration. The colour of manure is influenced by different factors such as passage rate and the type of feed. Either dark or bloody, yellow or light green manure are symptoms of diseases and are related to the passage rate (Kononoff et al., 2002). Cows feeding on unbalanced rations lacking in fiber or high in NSC or containing large amount of grain, may excrete yellow-olive manure. Dark manure may indicate the presence of hemorrhagic events in the intestinal tract. A yellow-olive colour combined with diarrhoea may be indication of bacterial infection such as salmonella.

5.6. Consistency

Faeces contain a great amount of water and this affects their consistency. Normal faeces reflect a well digested ration and appear in a well circumscribed pad. Sucking is not felt when the boot is retracted from the pad and a boot sole profile is not visible.

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 in height when dropped on the ground. It should also present the characteristic light plopping sound when it drops on the floor (Varga, 2003).

Loose faeces may be noticed either due to a period of heat stress, the fact that the animals are being fed a ration with excessive proteins, or the presence of high level of rumen degradable proteins. When dropping they splash wide out on the floor. Restricted water, or diet lacking proteins may result in stiff ball-shaped faeces, which look like horse faeces. This condition is often observed in dry cows and heifers. This points to a ration imbalance which needs to be corrected.

Normal faeces should appear uniformly digested. A considerable presence of long particle forage (more than 1.2 cm) indicates that animals are not ruminating properly and/or that the rate of passage is too fast. This may be due to inadequate amount of peNDF in ratio which is important to ensure ruminating a pH balance. It is important to underline that long particles are not available to the ruminal bacteria. Although it is normal to find some kernels or grain in fresh cows, because they have a faster passage rate, if this amount is excessive it may be cause of concern. Sometimes it is possible to find intact kernels even if the starch inside is partially or

completely digested. Finely ground grains are more difficult to be detected, but they may be present.

A white-yellow colour on the surface of manure may indicated undigested starch presence. Faecal evaluation gives information about severe, chronic inflammation of the intestinal tract when there is an excessive presence of mucin. When the feed is not properly fermented in the rumen, its proteins, fats and starch may be adsorbed in the small intestine; if there is no digestion in the small intestine, carbohydrates and proteins may be fermented in the caecum and large intestine (Hall, 2002a). Bacteria ferment all the indigested feed that passes through the intestine and the bacterial proteins produced would not be absorbed passing out as manure. The consequence is that gas and bubbles may appear (in manure), thus the faeces may have the texture of shaving cream (Hall, 2005).

5.7. Content

Different techniques can be used to evaluate particle size and the content of manure, all of them were based on the use of sieves. These instruments may be classified as monolayer and multilayer sieves, dry sieving or wet sieving methods. Among the on-field sieve instruments, the monolayer sieve is composed of only one screen with 1.18 mm pore size, while both the laboratory and the on-field multilayer ones are composed of different screens and a bottom pan. Although a dry sieving method may be used to understand the different characteristics of plant materials fed to ruminant animals (Robertson and Van Soest, 1981; Kennedy and Poppi, 1984; Ulyatt et al., 1986; Faichney and Brown, 1991; Nørgaard et al., 2004), the widely accepted method for manure is the wet sieving.

The faecal evaluation by these on-field sieve methods allow to get the general herd health and nutritional information. Although the results are subjective they help to understand the interactions between ration and animal.

5. Evaluation of feces

The correlation between the characteristics of the ration and faeces is widely accepted. Faeces are an indicator of the digestive efficiency. The physical nature of the diet has been showed to influence the rate of passage (Ewing and Smith, 1917; Balch et al., 1954; Rodriguez and Allen 1960). The first requisite for particles to flow from the reticulo-rumen is the length reduction of particles. This may be not always true, because many studies (Evans et al., 1973; Welch and Smith, 1978; Poppi et al., 1981b; Ulyatt et al., 1986) have shown that most of the particulate matter were smaller than the diameter of the reticulo-omasal orifice, which was greater than the particles length in faeces (McBride et al., 1983). Evaluation of manure can provide information to understand what is happening in the gut (Figure 7). Particles which small enough to escape the rumen will be retained only if fermentable, while when particles are no longer fermentable, they have high density and "sink" so they may be washed from the reticulum-omasal orifice. Although many differences of particle size in manure are considered due to different type of forage used in diets, the specific gravity of particle is considered the most important factor able to influence rumen retention (Welch, 1982; Kaske et al., 1992).

All particles with a critical length size (high mean particle size) are related back, except for the corn grain particles that escape from the rumen without being entrapped in the fibrous mat because of their high density (Hooper and Welch, 1985; Sekine et al., 1994; Wilson and Kennedy, 1996).

The different components of the diets are digested and fermented in different parts of the intestine. Microbial fermentation in the rumen or in the intestine influence the appearance of manure. All factors that cause changes in look, consistency and particle size, are related to ratio formulation and management (Varga, 2003). Changes in how manure looks may be caused by some diseases, most of which are related to the ration. Faecal particle size and consistency have been showed to be correlated with the peNDF content of the feed, and with the amount and the type of NSC in the diet (Hall, 2002a). Most fiber digestion and particle reduction in fact occurs in the rumen if enough peNDF has been fed. When cows are fed ratios with a low peNDF or high NSC, ruminal residence time gets shorter affecting the fermentation process. This leads to a higher hindgut fermentation and to a greater percentage of particles in faeces (Varga, 2003). When the rumen is working properly the fermentation in the hindgut is limited (Hall, 2002a). Long particles in faeces may be due to inadequate intake of peNDF (Poppi et al., 1980), or it may suggest a sorting behaviour (Hall, 2002a). Faeces evaluation associated with the particle

size evaluation of TMR may be useful tools to find out information about health herd conditions. The rumen will also be the main site that determines the size of particles that reaches the manure.

6.1. Manure evaluation by monolayer screen with apertures of 1.18 mm

To evaluate faecal particle fraction and undigested feed, it is possible the use of a screen or kitchen strainer with 1.18 mm openings. This is a qualitative, on-farm evaluation so getting very specific about mesh size is not crucial (Hall, 2002). After having collected a number of samples from individual cows that are representative of the variety of the group (they may be 3 per pen or 6 samples per pen out of 100 cows) a representative manure sample is put into a container (a large plastic cup) and gently rinsed with flowing water into the screen which is rinsed again gently but carefully until the water flows clear. The remaining material gives a clear view of large particles and undigested feed in the manure. In case of diets with insufficient peNDF, particle size in the faeces will increase. In fact with not enough fiber to make a good mat in the rumen, larger particles pass out. It is possible to find long particles of hay, pieces of corn stalk and kernels in the faeces. Whole kernels of corn in the manure often mean that the grain in the corn silage was not properly processed, or there is a problem with the ground corn. As effective NDF in the diet decreases, faecal particles become coarser. In correct TMR, we do not expect to see many fiber particles greater than 1.3 mm in faeces. When small particles and no grains are found in faeces, this suggests that the ration was retained in the rumen enough time to be chewed, fermented and digested. On the contrary, a great number of long pieces of coarse fiber in manure suggests that rumen retention time was excessively short (Hall, 2002).

6.2. Manure evaluation by Cargill Digestion Analyser (multilayer screens)

Multilayer screens are instruments used to assess particle faecal particle size by wet sieving. One of them is the Cargill Digestion Analyzer, which combines stainless-steel top, middle and bottom screens at, respectively 4.76 mm, 3.17 mm, and 1.58 mm (diameter apertures), allow to sift manure much as TMR shakers do. To use this manure sieve is very simple, it allows to assess the digestibility of rations by washing with water and subsequent filtration of a sample of faeces (Hall, 2002). A representative sample of faeces is collected from cows that eat the same diet (four or five samples per group) or about 2.3 litres (NASCO, 2005) (Figure 7). It is very important to collect a fresh sample which is not contaminated with feed or bedding. The sample

is placed on top of the sieve, and then is washed with a pistol spray nozzle, using the "shower-mode" to minimize the differences in pressure at different locations. The sample is washed until the top screen is free of all small particles, and until the entire sample is washed through the screens. With regards to the middle and bottom screen plug, we should fill the container until ¾ full with water, and then to pulsate the sieve past the level of the lowest plugged screen until water is removed. Then again we proceed with the next screen. We repeat this two steps until the water flows clear.

This process allows to evaluate the digestibility of the ratio and is a powerful tool to identify the failure of the ration digestibility of components of the diet. Manure should be seen as an important parameter to understand how cows are interacting with the unifeed ration. If everything else in the farm looks fine but the manure (consistency or the colour) does not seem right, it is important to keep watching the cows for signs of what we have not notice or checked yet.

Hall (2002) stated that "Ultimately, the cow is the only one who knows what effective fiber is and how much is enough". This kind of evaluation is not quantitative, for that reason it is paramount to find any important observable variation over time: within groups, between groups, between rations. When manure is sampled its great variability should be considered. Manure qualities may vary in fact with the same individuals over any 24-hour period, and/or even as weather changes; many outliners should be expected, because perfection does not exist in nature (Hall, 2002).

Observation and evaluation of the retained material in each screen is subjective, and as stated above, may be affected by many factors, such as DM intake, level of peNDF in diet, rumen pH etc. It is suggested to date and record the results and when possible to take pictures of each screen,. Observation of screening results over time may help to assess the impact of the ration changes on diet digestibility. Ideally the majority of retained material should be found in the bottom screen (> 50%), and few materials on the bottom screen, less than 10% or < 20% in early lactation cows.

When large fiber particles are found on the top screen, this may be due to many causes such a poor rumen mat, low quality of forage, type of NFC (inadequate fermentation), or sudden change in ratio composition. However it is extremely important to correlate this result with other

observations such foot problems, percentage of cows ruminating, etc, and we might conclude that there is acidosis.

When many grains such as whole or partial cottonseed, corn kernels or soybeans are found on the top screen, it may depend on inadequate processing of grain, or on cows sorting TMR. If the fiber content in the TMR is adequate and the ruminal pH is good, it is possible to increase the energy content, slowing the process fermentation rate of the diet by simply processing the grain or reducing the particle size of them (Cargill Animal Nutrition, 2004). The presence of whole cottonseed indicates that part of the energy of the ration is not properly used. The presence of weed seeds indicates the use of poor forage and this may be corrected improving the quality of forages. In synthesis this screener is a thorough understanding of key elements required to assess cow ability to digest and increases the chances of achieving expected enhanced animal performance. This understanding can then be translated into more accurate nutrient supply and diet reformulation.

6. Aims of the research

Based on the results of the literature review, it appears that the role of the particle size of TMR is crucial to produce high milk yield and optimal milk composition with healthy dairy cows. The quantification of the dietary particle size and peNDF requires specific equipments. They need to be evaluated and calibrated for each specific production setting. There is also a need to clearly define the different equipments and method available and to diffuse their utilization. Alternative methods to measure the dietary particles size can be envisaged with the application of NIRS technologies.

The evaluation of the effects of dietary particle size on the animals can be assessed indirectly looking at the composition of the faeces and, in particular, at the size of their particulate matter. However, field methods to estimate the faecal particle size of dairy cows have been little studied and standardized.

The appropriateness of dietary particle size can be tested studying its relationships with milk fat content. This approach has been extensively used in temperate areas but no information exists on

Mediterranean conditions. Indeed, the local climatic conditions and the feeds used can affect the feeding behaviour and the feeding pattern of dairy cows. Thus it is possible that the relationship between dietary particle size and milk fat content is not equal everywhere.

Based on these considerations, the research described in the present dissertation aimed at:

- testing the appropriateness of two field methods currently used to evaluate the particle size of TMR for dairy cows (the Penn State Particle Size Separator and the Z-Box) in respect to the reference laboratory method;
- testing the appropriateness of two field methods (a monolayer Sieve and the multilayer Cargill Digestion Analyzer) that can be used to assess the particle size of faeces in respect to a reference laboratory method;
- investigating if the particle size of TMR can be predicted by using NIRS techniques;
- investigating the relationship between dietary peNDF and milk fat content of dairy cows in Mediterranean conditions.

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TABLES AND FIGURES

Table 1. Recommended NDF and NFC concentrations in dairy cattle diets (NRC 2001), modified.

NDF from forage, % DM	Minimum NDF in diets	Maximum NFC in diets
15	33	36
16	31	38
17	29	40
18	27	42
19	25	44

Table 2. Guidelines of the Penn State Particle Separator (Heinrich and Kononoff, 2002, modified).

Screen	Particle size	Optimal distribution (% fresh weight)		
		Corn silage	Haylage	TMR
Upper	> 19 mm	3 to 8	10 to 20	2 to 8
Middle	8 to 19 mm	45 to 65	45 to 75	30 to 50
Lower	1.18 to 8 mm	30 to 40	20 to 30	30 to 50
Bottom	< 1. 18 mm	<5	<5	<20

Table 3. Milk fat, chewing activity, PeNDF and rumen pH requirements according to the equation proposed by Mertens (1997).

Milk fat	Chewing activity	peNDF	ruminal pH	peNDF	peNDF
%	min/kg DM			kg/day	% of DM
-	-	-	6.2	6.32±0.44	30.0±1.2
3.6	3.6±1.1	24.0±0.5	6.1	5.25±0.16	25.6±0.9
3.4	27.7±2.1	19.7±0.8	6.0	4.40±0.28	22.3±0.7
3.2	22.2±3.1	16.4±1.0	5.9	3.66±0.5	19.3±1.0

Table 4. Example of physically effective NDF (peNDF) values determined for some feeds using the Penn State Particle Separator with two sieves (2s) or three sieves(3s). pef2s = determined using two sieves (19 mm, 8 mm); pef-3s = determined using three sieves (19 mm, 8 mm, 1.18 mm); (peNDF2= % NDF × pef modified). Yang and Beauchemin (2006a).

	Proportion of DM retained on each sieve				Physically effectiveness factor		peNDF (% of DM)	
Feed	Top	Middle	Bottom	Pan	Pef s2	Pef s3	peNDF 2s	
peNDF 3s	19 mm	8 mm	1.18 mm					
				Corn silage				
Coarse	10.2	61.3	24.0	4.5	0.72	0.96	35.5	47.3
Medium	8.3	59.8	27.6	4.3	0.68	0.96	31.5	44.5
Fine	2.7	38.7	51.5	7.2	0.41	0.93	19.6	44.5
				TMR with corn silage				
Coarse	7.6	47.9	33.8	10.7	0.56	0.89	17.7	28.1
Medium	4.8	43.7	38.6	12.9	0.49	0.87	15.0	26.6
Fine	2.3	29.9	52.8	15.0	0.32	0.85	10.0	26.5

Table 5. Expected mean crude protein level and variance in either a simple TMR without forage analyses, a simple TMR with forage analyses, and a TMR with forage analyses and a multi-component feed prepared by a feed manufacturer (St-Pierre, 2001).

	Rations formulated with concentrate tabular values and								
	Tabular forage values			Forage analyses			Multi-comp. feed and forage		
Ingredients	Lbs DM	Lbs CP	varia nce	Lbs DM	Lbs CP	varia nce	Lbs DM	Lbs CP	varia nce
Alfalfa	16.8	3.36	2964	16.8	3.36	282	8.1	1.6	64.8
Corn silage	11.2	1.00	226	11.2	1.0	25	16.1	1.4	46.6
Alfalfa hay	-	-	-	-	-	-	2.7	0.5	6.0
Corn meal	12.9	1.26	67	19.9	1.26	67	6.5	0.6	16.7
Wheat mid	-	-	-	-	-	-	4.0	0.8	19.2
Ground	-	-	-	-	-	-	3.2	0.4	8.0
Barley	-	-	-	-	-	-	-	-	-
DDG	6.8	2.6	324	6.8	2.6	324	3.0	0.9	63.0
CGF	-	-	-	-	-	-	3.0	0.7	15.3
SBM-48	3.6	1.95	25	3.6	1.95	25	2.7	1.4	13.1
Soy hull	-	-	-	-	-	-	1.0	0.1	1.0
Canola meal	-	-	-	-	-	-	1.0	0.4	25
Min/vits	0.9	0	0	0.92	0	0	0.92	0	0
Total	52.2	9.63	3606	52.2	9.63	723	52.7	9.27	257

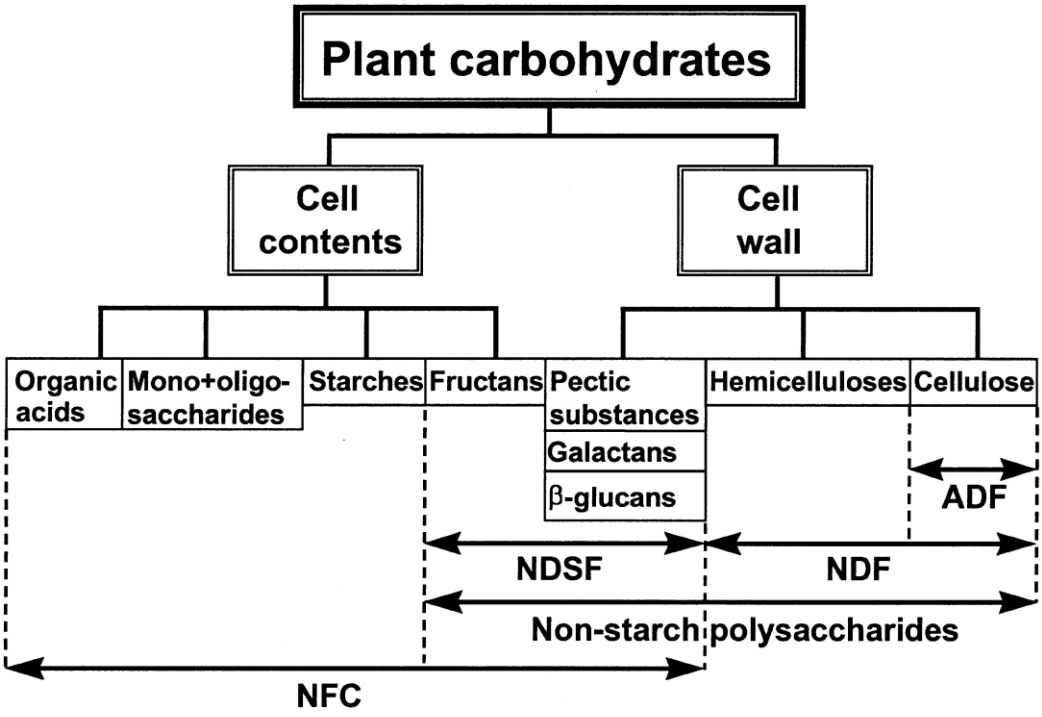

Figure 1. Plant carbohydrate fractions. ADF = acid detergent fiber, ß-glucans = (1-3) (1-4)-ß-D-glucans. NDF =neutral detergent fiber, NDSF = neutral detergent-soluble fiber (includes all nonstarch polysaccharides not present in NDF), NFC = non-NDF carbohydrates (from Hall, 2003).

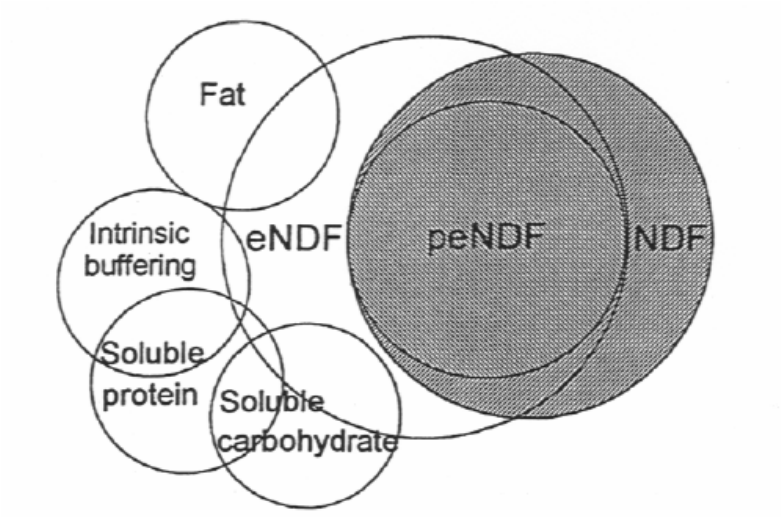

Figure 2. Relationship between chemical and phisical characteristics, NDF, peNDF and eNDF (Mertens, 2002).

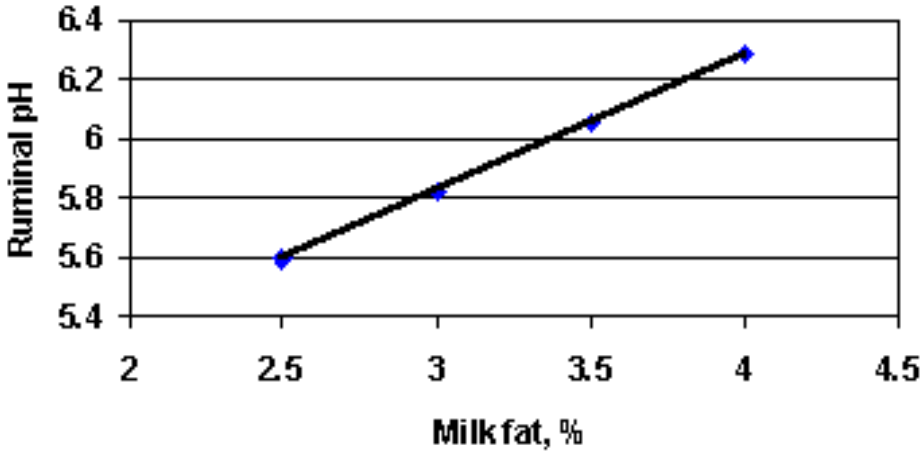

Figure 3. The relationship between ruminal pH and milk fat content. Adapted from Allen (1997).

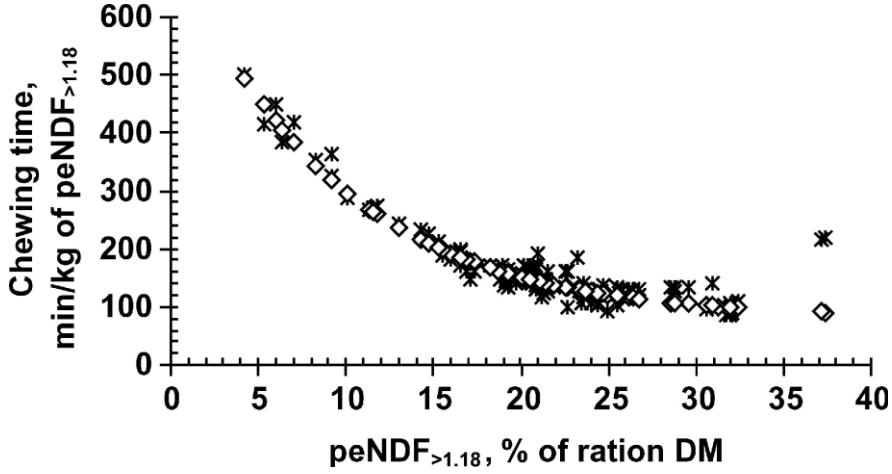

Figure 4. Relationship between time spent chewing per kg of peNDF and intake concentration of peNDF in total mixed rations. From Zebeli et al. (2006).

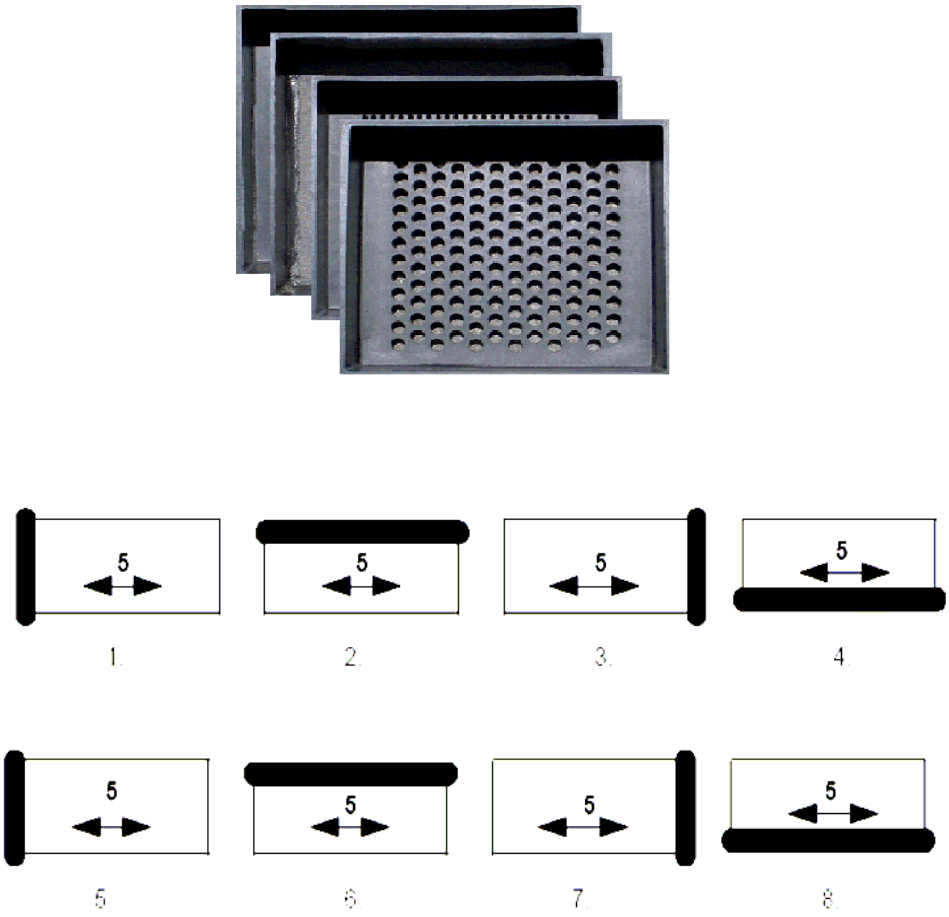

Figure 5. Penn State Particle Separator (above) and scheme of sieving proposed by Heinrichs and Kononoff, (2002).

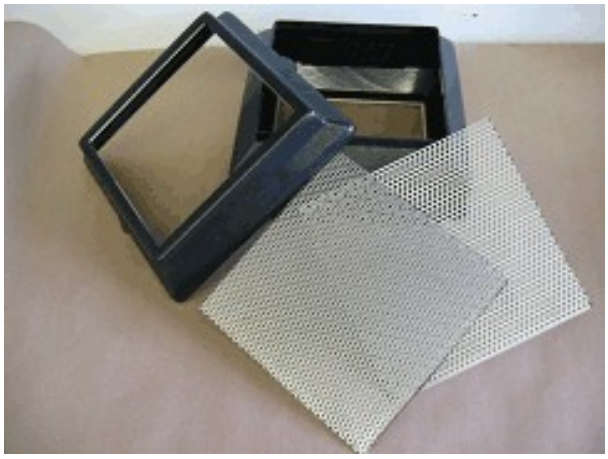

Figure 6. Zeta-box sieving equipment developed by Grant and Cotanch (2005) of the Miner Institute (Chazy NY) in cooperation with Zen-Noh National Federation of Agricultural Co-operative Association of Japan.

Figure 7. The Cargill Digestion Analyzer during a sieving session.

Chapter 1.

Comparison of laboratory and field methods to assess the physical structure of total mixed rations and manure of dairy cattle

Introduction

Total mixed rations (TMR) are commonly used to feed lactating dairy cows. When correctly designed and prepared, this type of diet preparation and supply force the animals to eat in each meal boluses balanced in terms of nutrient composition and physical structure. In particular, the physical characteristics of TMR may influence their ruminal fermentation and passage through the gastro-intestinal tract. When TMR are too coarse, intake and milk production decrease, while when too fine both animal selection is increased and rumination activity is reduced, with possible negative effects on animal's health, milk production and milk composition (Henrich and Kononoff, 2003).

To evaluate the physical characteristics of TMR, Mertens (1997) proposed the concepts of physically effective NDF (**peNDF**). The peNDF of a feed is the product of its NDF concentration and its physical effectiveness factor (***pef***), measured as the fiber (or diet) retained in a screen with mesh size of 1.18 mm. Thus, peNDF should represents the fiber that can be actually ruminated, not being too short, and it is related to rumen mat stratification and rumen pH (Mertens, 1997). The concept of peNDF has been adopted by feeding systems such as the Cornell Net Carbohydrate and Protein System (Fox et al., 2004) to balance diets in terms of physical structure.

Several methods have been proposed to measure peNDF. For laboratory assessments usually feeds are dried, a series of sieves are used and vertical shaking is applied. Field equipments are usually simpler and shaking is done manually. The most used field equipments is the Penn State Particle Separator (**PSPS**; Henrich and Kononoff, 2003), developed by Pennsylvania State University (US), namely a multilayer sieve composed of three different screens and a bottom pan. Recently, another field equipment has been proposed. It is the Z-Box, developed by the

Miner Agricultural Research Institute (Chazy, NY, US; Cotanch and Grant 2006) and based on a monolayer screen. Both methods have been developed and calibrated to sieve as fed TMR, thus without previous drying. The two field equipments have not been compared yet, so it is not clear if and how they differ in the estimation of dietary peNDF of TMR.

Field equipments are widely used and represent a useful tool for nutritionists to evaluate the physical characteristics of the diets. Since diet composition influences not only the content, but also the consistency and the colour of manure, feces can be used as a nutritional indicator of diet utilization and digestion. The same laboratory used for diet sieving can be used to sieve the feces. Regarding field equipments, one field multilayer sieve, the Cargill Digestion Analyzer (Cargill Animal Nutrition, Minneapolis, US), has been specifically developed for this use. Unfortunately, very little scientific literature exists on feces sieving and assessments of the Cargill Digestion Analyzer have not been published.

Thus, the this paper aims to:

a) compare laboratory and field methods to assess TMR physical structure;

b) compare laboratory and field methods to assess feces physical structure;

c) study the interrelationships between dietary (TMR) and fecal physical structure, as assessed by laboratory and field methods.

1.1. Materials and methods

The study was carried out in three dairy farms (one of them located near Sassari, Italy, and the others located in Arborea, Italy). In all the farms studied the TMR used were based on corn silage as main forage source Two of them had two feeding groups: early lactation group, for cows from 30 to 90 days in milk (DIM), and late lactation group, for cows from 90 to 300 DIM. The other one had only one feeding group for lactating cows from 30 to 300 DIM. During the experimental measurements, carried out from November 2008 to December 2009, the three farms were visited twice. During the first visit the dietary composition for each group of cows was recorded. In addition, samples of TMR for each feeding group and of all ingredients used to make the TMR were taken to analyze their chemical composition and their physical structure by sieving.

During the second visit, 9 animals were randomly chosen in each farm in each group of cows. One composite fecal samples of three cows from each feeding group was collected for later chemical analyses. Three composite fecal samples were collected and sieved to compare different sieve's methods. Six fecal samples were taken from each of the three farms (three from the group of cows from 0 to 90 DIM and three from the group of cows from 90 to 300 DIM).

1.2. Collection of feed and TMR samples

The sampling routine is described in detail in Table 1.

The samples of TMR from each group of cows were taken just after the TMR distribution at the beginning, in the middle and at the end of the feed bunk.

Each sample, of about 3 kg as fed, was portioned in four aliquots. Two of them were then immediately sieved in the farm to determine their particle size by using the PSPS, following the procedure described by Kononoff et al. (2003), and the Z-Box, following the procedure described by Cotanch and Grant (2006).

The remaining two aliquots of the same sample were taken to the laboratory. One was analyzed to assess the chemical composition of the diet and the second one was sieved with the laboratory sieve Octagon 200 (Endecotts Limited, Lombard Road, London, SW193TZ, England).

Sieving of TMR with the PSPS

The PSPS is made by 3 screens pile-stacked (Upper screen: 19.0 mm of mesh size; Middle screen: 8.0 mm of mesh size; Lower screen: 1.18 mm of mesh size) and a Bottom pan, that collects the particles that go through the Lower screen.

The TMR samples were collected and measured (1.5 litres; approximately 500 g as fed) with a plastic cup whose volume was 1.5 litres. The sample was later placed on the top of the PSPS screen and then sieved as described by Kononoff et al. (2003), shaking the equipment horizontally five times in one direction, then rotating it one-fourth turn and repeating the shaking other five times, until eight sets of five shakes (2 sets per side), for a total of 40 shakes, where completed. This methods assures that the stratification of feed particles on each sieve was a function of their size (Lammers et al., 1996). Particles retained in each screen were then weighted on a portable scale with 1 g of detection limit. The particle distribution was calculated and recorded, for each of the 3 screens of PSPS and for the Bottom pan, as percent of the initial sample weight. All samples retained from different screen were then placed in paper bags, identified and taken to the laboratory for dry matter determination and chemical analysis.

This evaluation was repeated in three different points of the feed bunk (beginning middle and end), to assess possible differences in the particle size distribution along the bunk.

The pef of the TMR was calculated with the PSPS as the proportion of sample retained in the three screens (upper, middle and lower) in respect to the total weight of the sample sieved. The peNDF was calculated multiplying the pef by the NDF content of the TMR.

Sieving of TMR samples with the Z-Box

The Z-Box is made by a plastic box with a screen with a mesh size of 3.18 mm. This screen has been developed to estimate the peNDF of TMR as determined by dry sieving with 1.18 mm screen.

The samples used for the Z-Box sieving were of about 250 ml (approximately 50 g). They were weighted on a scale (1 g of detection limit) and placed inside of the box, in the top of the screen. The samples were then sieved according the procedure described by Cotanch and Grant (2006), which suggest a total of 40 vertical shakes, rotating the box 90 degrees after each 10 shakes.

After the process was completed the particles retained on the screen were then weighted and recorded.

This operation was repeated three times for each sample, each time using about 250 ml.
This evaluation was repeated in three different points of the feed bunk (beginning middle and end), to assess possible differences in the particle size distribution along the bunk.
This evaluation was done in the samples repeated in three different points of the feed bunk (beginning middle and end).
All samples retained were placed in paper bags clearly identified and taken to the laboratory for chemical analyses and dry matter Determination.
The pef of the TMR was calculated with the Z-Box as the average proportion of sample retained in the feed in respect to the total weight of the sample sieved. The peNDF was calculated multiplying the NDF (% DM) of the TMR for the pef.

Sieving of TMR samples with the Laboratory sieving equipment

The laboratory equipment (model Octagon 200, Endecotts Limited, Lombard Road, London, SW193TZ, England) is made of a series of pile-stacked sieves with a vibrating engine of variable amplitude.
The samples of TMR were dried at 65 °C. The dry samples of TMR (approximately 100 g each) were dry sieved with the shaking equipment for 30 min at amplitude 7, following as close as possible the procedure of Mertens (2002). Six sieves (mesh size: 4.75 mm, 2.36 mm, 1.18 mm, 600μm, 300μm , 150μm) and a bottom pan were used.
After the sieving process was completed, the content of each sieve and of the pan was weighted, ground to a 1 mm size and analysed for ash, NDF and ADF (Van Soest et al., 1991). The peNDF was calculated as the sum of the NDF found in the fractions retained on the screens with mesh size equal or higher than 1.18mm.

1.3. Collection of manure samples

During the second visit in each farm, fecal samples were collected from 9 animals for each feeding group (early and late lactation for two farms and for the mid-late one for the other farm) chosen randomly. Fecal pH and temperature were determined immediately after sampling using a portable thermometer and a pHmeter (Hanna Instrument).
The feces of the 9 cows were pooled to make 3 composite fecal samples from 3 cows each. The pooled samples were collected in big plastic jars, and, after been well homogenized with a

spoon, from each composite sample 2 subsamples of about 500 g were taken; one of them was frozen for later analysis of contents of DM and NDF, the other one was wet sieved in the farm with the multilayer screen Cargill Digestion Analyser. Other 2 subsamples of approximately 100 grams of the same composite fecal sample were collected, one to be sieved with a monolayer sieve screen (1.18 mm apertures), the other one to be wet sieved with the laboratory equipment Octagon 200.

Wet sieving of the feces with the multi-layer sieve (Cargill Digestion Analyzer)

The Cargill Digestion Analyzer is made by three different screens (4.76 mm, 3.17 mm, 1.58 mm mesh size).

Composite samples of feces of about 500 grams weighted and put on the top of the multi-layer sieve. Each sample was then washed under running water until the rinse water was not clear. The three fractions obtained from the 4.76 mm, 3.17 mm, 1.58 mm were weighed, put in plastic jars and identified for the subsequent laboratory analysis of DM, NDF, and ash.

Wet sieving of feces with a monolayer screen

A composite sample (from 3 cows) of manure was weighted (approximately 100 g) on a scale with 1 g detection limit and then was put on the top of a monolayer sieving screen (1.18 mm mesh size), and rinsed with water until the rinsed water was not clear. As a monolayer sieve was used the Lower screen of PSPS (1.18 mm mesh size), which characteristic is to have square pores.

During the process the feces were softly dissolved in the sieve by hand and the sieve was shaken gently with two hands to ensure that all the particles were thoroughly washed.

The rinsed particles were then weighted again and placed on a plastic surface of graph paper, where all the particles longer than 1 cm were removed with a forceps and were measured, then counted, as well as the kernels of corn present in the sample. All the sieved material, the particles of fiber and of kernels of corn were then put in a container and dried at 105 °C for 24 h. The content of the sieve (particles and kernels of corn) were then weighted again.

Laboratory sieving of manure

Approximately 100 g of composite fecal samples were added with 300 ml of distilled water and were wet sieved with a laboratory equipment (model Octagon 200, Endecotts Limited, Lombard

Road, London, SW193TZ, England) by using the sieves with mesh size of 4.75 mm, 2.36 mm, 1.18 mm, 600μm, 300μm and 150μm and a top-sieve which had a spraying nozzle on its center. The sample was first put in the top screen (4.75 mm) and wet sieved for 12 min. Then the sieve was taken out from the stack and the top-sieve with the nozzle was put on the top of the next sieve (2.36 mm), which was sieved for 12 min. The procedure was repeated for each sieve. The amplitude was set at 7. The content of each sieve was then collected and dried at 105 °C.

1.4. Chemical analysis

Chemical analyses were carried out on the laboratory of Dipartimento di Scienze Zootecniche of Sassari (Italy). The DM content of the TMR sample and of all components of the diets were dried at 65°C until there was no variation in weight occurred. CP and ash were determined according the method IPRA (Martillotti et al., 1987). Feed and TMR simples were analysed for NDF, ADF, ADL according to Van Soest et al. (1991). The DM content of TMR, feed, and fecal samples was also determined at 105 °C for 24 hours.

1.5. Statistical analysis

The data were compared by using a monofactorial ANOVA with 3 levels. The differences among treatments were evaluated with the Tukey test.

Results

2.1. Diet composition

All the three farms studied used the TMR as feeding technique for all stages of lactation. The major component of the diets was corn silage in all farms, with farm 3 using also wheat silage in both stages of lactation (Table 2). Grass hay was used only in farm 1 (1.4 kg of DM) and in farm 2 (2.5 kg of DM), whereas alfalfa hay was used in all farms, with amounts supplied ranging from 1.3 kg of DM (late lactation, farm 3) to 2.4 kg of DM (early lactation, farm 3). The lowest DMI (19.6 kg/d) was found in farm 2, which used only one feeding group. DM intake was higher in early lactation than in late lactation in farm 1 (22.9 kg vs. 22.3 kg, respectively) and in farm 3 (24.3 kg vs. 20 kg), both farms using two different feeding groups.

2.2. Chemical composition of the diets

The rations fed in the three farms studied had different characteristics and chemical composition, as illustrated in Table 3. The forage: concentrate ratio (F:C) was 60:40 in farms 1 and 3 in late lactation, as well as in farm 2 throughout the lactation. Differently, in early lactation the F:C ratio was higher in farm 3 than in farm 1 (63:37 vs. 57:43). The DM of the TMR in farms 1 and 3 was higher in early than in late lactation. Similarly, the CP (as % of DM) in farms 1 and 3 was higher in early (19.9 % and 17.7%, respectively) than in late lactation (16.4% and 13.0%, respectively), with the lowest CP among the farms being that of late lactation in farm 3. The percentage of CP in farm 2 was similar to that of the late lactation stage in farm 1 (16.4%). The NDF varied substantially among the different farms and diets. NDF in farm 1 was higher in early lactation (42.2% of DM) than in the late one (41.1% of DM), whereas that in farm 3 was lower in early (34.1%) than in late lactation (43.4 % of DM).

The chemical composition showed a difference between sampling site in the bunk only in the Early lactation group of farm 1, where the DM and the CP content markedly decreased going from the beginning to the end of the feeding bunk, while NDF and ADF increased. In the other two farms there were not differences among sampling sites (Tables 5 and 6).

2.3. Particle size distribution of the TMR

The particles size distribution evaluated with the PSPS at the different farms and stages of lactation is reported in Table 7. The particle size distribution varied noticeably among the compared TMR rations for the Upper, Middle and Lower screens and for the Bottom pan. In particular, the highest percentage of Upper fraction (19 mm of sieve size) observed was that of the early lactation group of farm 1 (9.4 %, expressed as % of total sample fresh weight). This value was higher than the maximum suggested reference value (Heinrichs and Kononoff, 2003), which is 8% (expressed as % of total sample fresh weight). In the same farm, the Upper fraction for the late lactation stage was right above the maximum reference value. In farms 2 and 3, the percentage of the Upper fraction varied from 3.4 % to 7.5 %.

The Middle fraction (8 mm of sieve size) of TMR ranged from 36.6% (early lactation, farm 3) to 60.9% (late lactation, farm 3). The Middle fraction values of farms 1 and 2 were within the guidelines reported by Heinrichs and Kononoff (2003), which suggest a mean incidence of Middle fraction ranging from 30 to 50% (Table 7). The Middle screen percentage of farm 2 (46.6%) was higher than those of farm 1 (41.4% and 40.0% in early and late lactation, respectively). The fraction of particles retained in the Lower screen (1.18 mm sieve size) was the lowest in the late lactation group of farm 3, whereas the other Lower screen percentages observed ranged from 30.3% (early lactation, farm 1) to 37.6% (late lactation, farm 1). The fraction of particles retained in the Lower screen in the late lactation group of farm 3 (25.4 % of total fresh weight) was lower than that recommended values (30-50 %; Table 7). On the other hand, for all the other stages of the three farms the fractions of particles retained in the Lower screen were within the suggested range. The percentage of the fraction collected in the Bottom pan (< 1.18 mm) varied from 14.1 % in farm 2 to 19.6% in farms 3. It was in general within the suggested range (<20%), even though in farm 1 and in farm 3 (early lactation) it was very close to the limit (19% and 19.6 %).

2.4. Evaluating pef and peNDF using different sieving methods

Table 8 summarizes the amounts of pef and peNDF of the TMR estimated by the laboratory method and by the on field methods, namely PSPS and Z-Box.

The pef calculated by the PSPS ranged from 80.3% to 91.9 % of DM of TMR, while the pef calculated by the Z-box ranged from 69.4% to 80.4 % of the DM of TMR. The pef measured by

both on field methods were higher in late lactation than in early lactation in farms 1 and 3. In all the farms studied, the values of pef measured by the PSPS method were always higher than those calculated with the Z-box sieve and the laboratory sieve methods.

The highest values of pef using PSPS and Z-box were found in farm 3 in late lactation (91.9 % and 80.4 % of DM of TMR, respectively), whereas the lowest ones were found in farm 1 in early lactation (80.3 % and 69.4 % of DM of TMR, for PSPS and Z-box, respectively).

The peNDF content of TMR diets measured by the laboratory sieve ranged from 23.6 to 34.6% (expressed as % of ration DM). The peNDF values measured by PSPS ranged from 33.3 % to 40.0%, being always higher than those measured by the laboratory method.

The peNDF measured by Z-Box ranged from 23.8% to 35.0 %. These figures were closer to the laboratory peNDF than those calculated by the PSPS. The differences between PSPS peNDF and laboratory peNDF ranged from 2.0% to 6.8% of DM, with a mean of -4.63 (significant at $P< 0.01$), whereas those between Z-Box peNDF and laboratory peNDF ranged from -2.8% to 0.4% of DM, with a mean of 0.11 (not significant). For that reason, Z-box peNDF had figures more similar to the laboratory peNDF ones.

The relationship between measured laboratory peNDF and PSPS peNDF (expressed as % of DM of TMR) is shown in Figure 1 ($y = 0.780x + 2.88$, $R^2 = 0.66$), whereas that between measured laboratory peNDF and Z-box peNDF (expressed as % on DM of TMR) is shown in Figure 2 ($y = 0.841 x + 4.79$, $R^2 = 0.69$).

2.5. Evaluating the homogeneity of TMR along the feed bunk

Sieving the TMR samples at different points of the feed bunk with different field instruments allowed the assessment and evaluation of the way the feeder mixer wagon was working, and how homogenous the TMR ration was along the feed bunk. The values and differences of the TMR samples taken in the 3 farms along the feed bunk (Beginning, Middle and End) using the Z-Box and the PSPS methods are reported in Tables 9 and 10, respectively. The level of variation of Z-box ranged from 0.93 (farm 1, early lactation stage) to 1.06 (farm 3, late lactation stage), whereas that of PSPS ranged from 0.96 (farm 3 early lactation stage) to 1.03 (farm 2, mid-late lactation stage). The highest differences expressed in percentage were 6% with the Z-Box and 3% with the PSPS sieve. This operation was repeated three times for each sample.

This evaluation was repeated in three different points of the feed bunk (beginning middle and end), to assess possible differences in the particle size distribution along the bunk.
This evaluation was done in the samples repeated in three different points of the feed bunk (beginning middle and end).

2.6. Fecal samples

Temperatures and pH of fecal samples measured for each lactation stage (early and late lactation) in the three farms are reported in Table 11. There was a great variability of temperatures measured among the groups of lactation and the farms studied, which ranged from 19.3°C to 29.7°C. The highest temperatures were found in farm 3 in both stages of lactation, while the lowest ones were found in farm 1 in early lactation. Differently, farm 1 in late lactation and farm 2 in both stages of lactation had similar and intermediate temperatures. The variability of the mean temperature was greater in early lactation stage (ranging from 19.5 to 28.1) than in late lactation one (ranging from 22.2 to 28.2). The highest mean were found in late lactation stage in farm 3 (28.2) while the lowest one was found in early lactation stage farm 1 (19.5).
The variability of the mean pH measurements among farms was greater in early lactation (ranging from 6.1 to 7.7) than in late lactation (ranging from 6.5 to 6.8). The highest and the lowest mean pH were found in early lactation in farms 1 (7.7) and farm 2, respectively (7.7 vs. 6.1). Dry matter (DM) concentration (% of fresh weight) of fecal samples taken in each of the 3 farms studied is given in Table 11. The mean of DM in early lactation was slightly lower than that of late lactation (14.5% vs. 14.8%) in farm 1, whereas the opposite trend occurred in farm 2 (13.8% vs. 13.4% in early and late lactation, respectively). The mean fecal DM differed the most in farm 3, being between 13.3 % and 12.7 % in early and late lactation, respectively.

2.7. Comparison of fecal sieving methods

Table 12 shows the results of the fecal sample (expressed in % of DM) retained in the 1.18 mm screen by using three different sieving methods and expressed in percentage of the initial sample weight. The mean DM retained by the laboratory, the monolayer and the multilayer sieves differed significantly ($P<0.01$) and were 15.2 %, 26.5 % and 21.4 %, respectively.

Taking into consideration the three methods compared, in farm 1 (early and late lactation), farm 2 (late lactation) and farm 3 (early lactation) the highest percentage of retained DM was given by the monolayer sieve, whereas in farm 2 (early lactation) and farm 3 (late lactation) the highest values were found with the multilayer sieve screens (Table 12). The relationship between particle fractions > 1.18 mm retained in the monolayer and multilayer sieves and the fractions retained by the laboratory method in the whole range of measurement is shown in Figures 3 and 4. It clearly appears that both methods overestimated in the whole range measured the fecal particles above 1.18 mm. The relationship between laboratory method and field methods was very weak, in particular for the Cargill Particle Separator (multilayer screen).

The number and length of particles longer than >10 mm observed in the feces taken in the farms studied in which there were two groups of lactation are shown in Figure 5. The number of fecal particles was higher in early lactation than in late lactation in all the farms. The particles retained sieving the fecal samples with.18 mm sieves were longer in early lactation (being close to 50 mm) than in late lactation (being shorter than 30 mm).

Discussion

3.1. Chemical evaluation of TMR

The rations used in the three farms studied had different characteristics, ingredients and chemical composition (Tables 2 and 3). The F:C ratio varied little among the groups studied. Dietary CP, in contrast, was much higher in early than late lactation groups, to account for the variation in milk yield and requirements of the cows in the two stages. Dietary NDF concentration, however, did not follow the same pattern, since it was reduced from early to late lactation only in farm 3. Daily DM intake was also different among groups in farm 3, while did not differ in farm 1. Farm 2 had the lowest DMI. Overall, the farms and groups considered allowed to diets with quite diverse composition.

3.2. Estimation of Pef and peNDF in the TMR by different sieving types and methods

The PeNDF was calculated as the fraction retained in 1.18 mm sieve multiplied by the NDF of the TMR in the case of the PSPS, while it was estimated multiplying the fraction retained in the 3.18 mm sieve by the NDF of the TMR for the Z-box (Table 8).

The pef values obtained with the PSPS (85.22%) were higher ($P<0.01$) than those obtained with the laboratory (69.60%) and the Z-box (73.67) methods, which did not differ between them. The differences was quite large, were quite large. It is possible that the PSPS overestimated the pef when compared with the reference laboratory method because while both method use the same sieve size (1.18) to estimate pef, the PSPS is used on as fed rations, while for the reference method the rations is dried before sieving. Firstly, the drying process reduces the particle size, and in particular the cross dimensional section of the particles, allowing them to pass through the sieve more easily than when they are rich in humidity. Secondly, dry particles are lighter and move more during shaking, while wet particles tend to stay flat on the screen. Thirdly, wet particles tend to stick on each-other, while dry particles separate more easily. The rationale behind the development of the Z-box was to find a screen size to be used on wet particles that was highly correlated to the pef obtained with dry sieving on 1.18 mm sieve (Cotanch and Grant, 2006). Based on the results of this study it seems that the goal was achieved.

In all the diets tested the peNDF was higher than the minimum (22% of DM) suggested by Mertens (1997). While in farm 3 the values increased going from early to late lactation, as should be based on the differences in requirements among these two categories, the same did not occur in farm 1.

The PeNDF calculated using the two field methods and the laboratory method reflected the difference observed in the pef estimation. The mean differences between the peNDF estimated by the PSPS and that measured with the laboratory method was equal to 4.63 percentual points ($P<0.01$), while the Z-box differed from the reference method only by 0.1% and the difference was not significant.

The better performance of the Z-box compared to the PSPS is even more evident when the values of the two methods are plotted against the reference method. The PSPS consistently over estimated the peNDF in the whole range of the values studied (Figure 1). In contrast, the Z-box produced estimates very close to those of the reference method in the whole range of peNDF explored.

3.3. Evaluation of the appropriateness of the TMR by the PSPS

Even though often used to estimate the peNDF of the diets, the PSPS was originally developed to compare the distribution of the diet among the sieves with reference optimal values suggested by Heinrichs and Kononoff (2003), the same authors who developed the equipment. These values are reported in Table 7 together with the distribution among sieves measured by the PSPS of the TMR tested in this study. The inherent rational of the method is that the estimation of the amount of dietary particle that are above of a certain size (e.g. 1.18 mm) is not sufficient to define the ruminability of a diet, because large particles are considered proportionally (i.e. per unit of weight) more effective in stimulating the chewing activity of the animals than the particles close to the limiting size.

The proportion of material retained on the top screen (Upper, screen size 19 mm) is particularly important because the intake of DM from this fraction of the diet is considered to be positively correlated with rumination and chewing activity, and salivary buffer capacity (Kononoff and Heinrichs 2003a,b; Krause et al., 2002). When TMR is too coarse (i.e., based on the PSPS, when in the Upper screen there is more than 8% of the diet), cows tend to select the ration, discarding the longer particles and preferring the smallest ones. Rations containing a too high proportion in

Upper fraction are likely to have great differences between the offered ration and the consumed one, due to the sorting activity. This sorting behavior may lead to insufficient buffering capacity and to sub-clinical acidosis. In addition, the coarseness of the diet might limit DMI for its filling effect (Kononoff and Heinrichs, 2003a; Tafaj et al., 2001). While farm 2 and farm 3 were within the range of recommendations, which are indicated to be between 2 % and 8 %, in farm 1 the amount of particle retained in this screen was higher than 8 % in both stages of lactation (9.4% in early and 8.1% in late lactation). Because this fraction was observed to be highly correlated with dry matter intake, diets with short particle size had a higher DMI than diets with long particle size. For this reason farm 1 would probably improve its performances, especially in early lactation, if the TMR was mixed and chopped for a longer time by the feeding wagon.

The Middle (8 mm) and Lower (screen size 1.18 mm) fractions were within the suggested range for all but farm 3 during late lactation, where the Middle fraction was above (60.9%) of the suggested values (30-50%) and the Lower (25.4% of fresh weight of the diet) was below the suggested range (30-50%). This may have occurred because the TMR of this group was made by large amounts of silage and concentrates and no grass hay forage was used.

The Bottom fraction (particles that passed the 1.18 mm screen) was within the range in all farms and groups. However, the values for the early lactation groups of farms 1 and 3 were very close to the maximum acceptable value. The proportion of these particles is very relevant because they outflow the rumen quicker that those larger than 1.18 mm retained in the other 3 screens of the PSPS. For this reason they are less digested. When the percentage of these particles is too a reduction of the performance of the animals is likely.

3.4. Homogeneity of TMR along the feed bunk

Feed delivery and bunk management are important aspects that enable to avoid metabolic disorder in dairy cows. Sieving the TMR samples in different points of the feed bunk allows to evaluate the homogeneity of distribution of TMR. This is particularly important for coarse particles that are prone to being sorted by the cows along the feed bunk. The use of the Z-box and PSPS sieves allowed to calculate the pef and to verify its variation across the feed bunk (Tables 9 and 10). This variation was within 6% for the Z-box and 3% for PSPS. These results suggest that the TMR diets were fairly homogenous, in line with the suggestions of Cozzi

(2004), who advised to stay below 10 % of pef variation along the feed bunk. The higher sensitivity of the Z-box is in to its better ability to predict peNDF, as discussed before.
The variability was higher in farm 1 during early lactation and in farm 3 in both stages, with the pef being higher at the end of the feed bunk. This is likely to cause changes in the chemical composition of the ration, as clearly observed in farm 1 (Table 4), and in the forage to concentrate ratio.

3.5. Characteristics of the fecal samples

There were not large differences in the DM concentration within the groups. The feces DM content should be higher in late lactation cows than in early lactation ones. Indeed, high producing cows or early lactation cows excrete more fluid feces because they drink more water and eat more feed, with a resulting faster passage rate. However, only in farm 1 the fecal DM was lower in early than in late lactation (14.5 % vs. 14.8 %), while in farm 2 and farm 3 the fecal DM was surprisingly higher in early lactation cows than in the late ones (Table 11). This might have happened because fecal DM depends by many other factors than lactation stage, such as heat stress or ration composition. For example, excess of proteins in the diet or high level of rumen degradable proteins increase fecal fluidness.
In all farms and groups the number and the length of fecal particles (> 10 mm) measured in the farms studied were higher in early than in late lactation (Figure 5). The particles retained sieving fecal samples with 1.18 mm screens were as average close to 50 mm in early lactation and were less than 30 mm in late lactation. Similarly the total number of the long particles retained in the screen was higher in samples from early lactation stage than in the late one. This confirms the well now fact that high producing and/or early lactation dairy cows have a lower selective retention activity at rumen level and thus faster rate of passage and more fluid feces than those producing less. This might lead to reduced chewing activity, reduced bacterial activity and lower digestibility.

3.6. Feces particle size assessment by different sieves methods

The mean DM retained by the laboratory, the monolayer and the multilayer sieves differed significantly ($P<0.01$) and were 15.2 %, 26.5 % and 21.4 %, respectively.

Thus, the fractions retained by using the laboratory method were always lower than the monolayer and the multilayer sieve, in all the farms and in all the stages of lactations. In addition, with the laboratory method the range was narrower (12.7 % to 18.2 %) than by using the other two methods (monolayer sieve: from 17.8 % to 33.8 % of the total fecal weight; multilayer sieve: from 18.9 % to 29.6 %).

Based on these results it appeared that the fraction retained in the 1.18 mm screen was inversely proportional to the number of screen by which the sieving equipment was made. Indeed, the monolayer equipment gave the highest value of feces retained, the multilayer Cargill apparatus (3 screens) gave the intermediate value in fraction retained and the lowest values was that of the laboratory methods (6 screens). Thus, it seems that if the number of screens is too low, the flow of fecal particles through the 1.18 mm sieve is impaired or limited. This might be due by the fact that the use of water to separate the fecal particles tends to stratify the particles horizontally, creating a fiber mat on the screen that act as filtering system by itself (Van Soest, 1994). When more sieves are used, this phenomena does not occur with the same intensity because the sieves with the largest mesh diameter retain only the large particles and allow a better separation by size. This hypothesis is confirmed by the fact that when comparing the equipments to sieve TMR, the Z-box, made by only one screen, performed better than the PSPS, made by 3 screens. In the case of TMR no water is added to the diet and even though the feeds contain some water this does not really affect much the movement and the flow of feed particle during sieving.

Conclusions

The assessment of the particle size distribution of TMR is critical step in the formulation of diets for high producing lactating cattle. For this reason laboratory and field methods have been developed and are currently widely used.

The present studied compared the ability of two field methods, the PSPS and the Z-box, to predict the physically effective NDF (NDF particles retained in 1.18 screens) of the TMR. The results showed that the Z-box was able to accurately predict the physically effective NDF of the TMR, while the PSPS grossly over predicted it. The Z-box appeared also more sensitive in assessing differences of particle size distribution among samples taken in different points of the same feed bunk. from In addition, the Z-box is easy to carry and handle, being much lighter and smaller than the PSPS. These results do not preclude the utilization of the PSPS to assess if TMR have an appropriate particle size, as long as the reference values suggested by Heinrichs and Kononoff (2003) and not the peNDF values suggested by Mertens (1997) are considered.

The comparison of field (monolayer and Cargill multilayer) and laboratory equipments to estimate the particle size of the feces showed that the field equipments grossly over predicted the proportion fecal particles larger than 1.18 mm. It appeared that the values obtained were inversely proportional to the number of screens used, suggesting a difficulty in separating fecal particles of different size with the field methods.

Further research on this area should assess if the field methods can be improved or substituted with faster laboratory methods. In addition, there is a need to verify if the optimal particle size distribution of TMR suggested by the developers of the field and laboratory methods are valid for the diets and the feeding conditions typically used in Italy and more specifically in Sardinia.

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TABLE AND FIGURES

Table 1. Sample collection scheme, sieving procedures and peNDF evaluation with different sieving methods.

Sieve	PSPS*	Laboratory Sieve**	Z-Box*
Site of collection	B, M, E of each bunk	B, M, E of each bunk	B, M, E of each bunk
Sample collected	1.4 ± 0,5 l	100 g	250 ml
NDF measurement	From TMR analyses	Chemical analyses of each fraction	From TMR analyses
pef measurement	Weight of sample > 1.18mm	Weight of sample > 1.18mm	Weight of sample > 3.18mm
peNDF	NDF of TMR × pef	∑ of NDF > 1.18mm	NDF (%) of TMR× pef

TMR = total mixed ration; B= beginning of the feeding bunk; M = middle of the feeding bunk; E = end of the feeding bunk. *Sieved as fed; **sieved after drying at 65°C.

Table 2. Ingredients (kg of DM per day per head) used in the rations of the 3 farms studied.

	Farm 1		Farm 2	Farm 3	
Stage of lactation	Early	Late	Intermediate	Early	Late
Corn silage	7.6	8.7	8.5	11	7.7
Lolium hay	0.7	0.7	2.5	-	-
Lolium hay 2	0.7	0.7	-	-	-
Alfalfa hay	1.4	1.4	1.6	2.4	1.3
Corn meal	3.5	2.2	3.5	4.2	2.2
Soybean meal 44%	2.8	1.8	3.5	3.3	2.3
Beet pulps	2.5	2.3	-	-	-
Concentrate mix	3.7	4.5	-	0.9	4.1
Wheat silage	-	-	-	1.3	0.7
Corn flakes	-	-	-	1.3	1.8
TOTAL	22.9	22.3	19.6	24.3	20

Table 3. Characteristics and chemical composition of the diets fed to the lactating cows of the studied farms.

	Farm 1		Farm 2	Farm 3	
Stage of lactation	Early	Late	Intermediate	Early	Late
F:C	57:43	60:40	60:40	63:37	60:40
DM, % as fed	44.02	41.1	46.9	53.3	37.7
CP, % DM	19.9	16.4	16.4	17.7	13.0
NDF, % DM	42.2	41.1	39.1	34.1	43.4
ADF, % DM	23.4	25.1	24.2	19.0	27.5
ADL, % DM	4.2	4.4	3.7	2.9	3.1

F:C = forage to concentrate ratio.

Table 4. Chemical composition of the TMR samples taken in three different points (beginning, middle, end) of the feeding bunk. Farm 1.

Feeding bunk	Early lactation				Late lactation			
	B	M	E	*Mean*	B	M	E	*Mean*
DM, % as fed	48.0	44.4	39.6	*44.0*	40.7	41.9	40.6	*41.1*
CP, % DM	20.9	20.4	18.4	*19.9*	16.1	16.7	16.4	*16.4*
NDF, % DM	40.8	41.6	44.1	*42.2*	40.4	40.5	42.4	*41.1*
ADF, % DM	21.6	23.9	24.6	*23.4*	24.9	25.4	25.0	*25.0*
ADL, % DM	4.2	4.2	4.3	*4.2*	4.3	5.2	3.9	*4.5*
Ash, % DM	7.2	7.9	6.6	*7.3*	6.2	6.8	6.5	*6.5*

B= beginning of the feeding bunk; M = middle of the feeding bunk; E = end of the feeding bunk.

Table 5. Chemical composition of the TMR samples taken in three different points (beginning, middle, end) of the feed bunk. Farm 2.

Feeding bunk	Intermediate lactation			
	B	M	E	*Mean*
DM, % as fed	47.4	46.8	46.6	*46.9*
CP, % DM	16.3	16.7	16.2	*16.4*
NDF, % DM	39.2	40,0	38.1	*39.1*
ADF, % DM	24.3	25.1	23.1	*24.2*
ADL, % DM	3.2	3.4	4.4	*3.7*
Ash, % DM	7.6	7.6	7.7	*7.6*

B= beginning of the feeding bunk; M = middle of the feeding bunk; E = end of the feeding bunk.

Table 6. Chemical composition of the TMR samples taken in three different points (beginning, middle, end) of the feeding bunk. Farm 3.

Feeding bunk	Early lactation				Late lactation			
	B	M	E	*Mean*	B	M	E	*Mean*
DM, % as fed	53.5	54.2	52.3	*53.3*	37.5	37.8	37.8	*37.7*
CP, % DM	17.4	17.9	17.7	*17.7*	12.9	13.0	13.0	*13.0*
NDF, % DM	33.7	33.3	35.2	*34.9*	43.8	43.2	43.2	*43.4*
ADF, % DM	18.8	19.2	18.9	*19.0*	27.3	27.6	27.6	*27.5*
ADL, % DM	2.9	3.0	2.8	*2.9*	3.1	3.2	3.2	*3.2*
Ash, % DM	8.5	8.7	8.5	*8.6*	8.1	8.0	8.5	*8.2*

B= beginning of the feeding bunk; M = middle of the feeding bunk; E = end of the feeding bunk.

Table 7. Results of the sieving (expressed as % of total sample fresh weight; mean ± S.D.) carried out by using the Penn State Particle Separator in different farms and stages of lactation.

Sieve Screen	Optimal values * (%)	Farm 1		Farm 2	Farm 3	
		Early (%)	Late (%)	Intermediate (%)	Early (%)	Late (%)
Upper	2-8	9.4 ±1.1	8.1±0.7	3.4±1.0	7.5±0.3	5.6±0.2
Middle	30-50	41.4±1.1	40.0±1.6	46.3±2.7	36.6±2.4	60.9±1
Lower	30-50	30.3±3.0	37.6±0.9	36.1±1.1	36.3±1.0	25.4±0.8
Bottom	<20	19.0±1.1	14.3±0.7	14.1±3.6	19.6±1.3	7.0±0.7

* based on the guidelines of Heinrichs and Kononoff (2003).

Table 8. Values (mean ± S.D. of all sampling sites) of pef and peNDF in the TMR estimated with different methods of sieving in different farms and stages of lactation.

	Farm 1		Farm 2	Farm 3		Mean
	Early	Late	Intermediate	Early	Late	
NDF (% DM)	42.2±1.5	41.7±1.8	39.1±0.8	34.1±0.9	43.6±0.2	40.1
pef						
Laboratory [1]	71.1±1.1	71.0±2.4	64.1±1.4	62.9±2.4	78.8±0.4	69.60 [B]
PSPS [2]	80.3±1.1	85.7±0.7	85.2±1.2	82.2±1.9	91.9±0.4	85.22 [A]
Z-Box [3]	69.4±3.5	70.5±2.2	77.9±2.8	69.7±2.9	80.4±3.2	73.67 [B]
peNDF						
Laboratory	32.1±1.0	28.9± 1.7	29.0±0.8	23.6±1.4	34.6 ±1.2	29.62 [B]
PSPS	34.2±1.3	35.7±1.7	33.3±1.0	28.0±1.2	40.0±0.4	34.25 [A]
Z-Box	29.3±2.0	29.0±0.6	30.5±1.0	23.8±1.0	35.0±1.0	29.51 [B]
peNDF differences						
PSPS – laboratory	2.0±0.3	6.8±0.0	4.3±0.2	4.4±0.2	5.4±0.8	-4.63*
Z-Box – laboratory	-2.8±2.2	0.1±2.3	1.5±0.2	0.2±0.4	0.4±0.2	0.11[NS]

[1] total % feed above 1.18 mm screen.
[2] total % feed in the Upper, Middle and Lower screens.
[3] total % feed above a 3.18 mm screen.
A, B = P<0.01
* difference significant for P<0.01

Table 9. Evaluation of the homogeneity of the particle size distribution in different locations of the feed bunk (beginning, middle, end). Samples sieved with the Z-box.

pef (% of initial weight)	Farm 1		Farm 2	Farm 3		Mean
	Early	Late	Intermediate	Early	Late	
Beginning (a)	68.6	69.8	77.8	67.6	78.1	71.9
Middle (b)	67.3	71.8	79.0	70.6	80.0	73.5
End (c)	72.3	70.0	76.8	71.0	83.1	74.8
			Differences			
b/a	0.98	1.02	1.04	1.04	1.02	
c/a	1.05	0.99	1.05	1.05	1.06	
b/c	0.93	1.03	0.99	0.99	0.96	

Table 10. Evaluation of the homogeneity of the particle size distribution in different locations of the feed bunk (beginning, middle, end). Samples sieved with the PSPS.

pef (% of initial weight)	Farm 1		Farm 2	Farm 3		Mean
	Early	Late	Intermediate	Early	Late	
Beginning (a)	80.5	86.6	83.9	83.9	92.4	85.5
Middle (b)	82.3	85.3	86.1	80.2	91.7	85.1
End (c)	80.3	85.2	85.7	82.6	91.6	85.1
			Differences			
b/a	1.02	0.98	1.03	0.96	0.99	
c/a	1.00	0.98	1.02	0.98	0.99	
b/c	1.02	1.00	1.00	0.97	1.00	

Table 11. Temperature, pH, and dry matter concentration (% of fresh weight) of composite fecal samples taken in 3 different farms from cows in 2 stages of lactation. Each sample was made by the feces of three cows.

	Farm 1	Farm 2	Farm 3	Farm 1	Farm 2	Farm 3
	early lactation			late lactation		
Temperature						
Sample 1	19.3	27.0	26.9	25.1	21.4	28.1
Sample 2	19.6	21.4	29.7	20.3	20.4	28.2
Sample 3	19.6	21.9	27.7	23.9	24.8	28.4
Mean	19.5±0.2	23.4±3.1	28.1±1.4	23.1±2.5	22.2±2.3	28.2±0.2
pH						
Sample 1	7.4	5.8	6.3	6.3	6.4	6.4
Sample 2	8.4	6.7	6.3	6.8	7.3	6.4
Sample 3	7.4	5.8	6.5	6.8	6.7	6.7
Mean	*7.7±0.6*	*6.1±0.5*	*6.4±0.14*	*6.6±0.3*	*6.8±0.4*	*6.5±0.1*
DM						
Sample 1	14.9	14.2	13.6	14.8	13.3	12.6
Sample 2	14.0	13.6	13.2	14.8	13.3	12.6
Sample 3	14.6	13.7	13.1	14.8	13.7	12.8
Mean	*14.5±0.5*	*13.8±0.3*	*13.3±0.3*	*14.8±0.0*	*13.4±0.2*	*12.7±0.1*

Table 12. Fecal DM retained in the 1.18 mm screen (as % of total sieved sample, mean ± S.D.) by using 3 different equipments: monolayer (1 screen), Cargill multilayer (3 screens) and Laboratory multilayer (6 screens). For each equipment 3 composite samples for 2 stages of lactation (each one obtained mixing the feces of 3 cows) were sieved.

	Farm 1		Farm 2		Farm 3		Mean
Equipment	Early	Late	Early	Late	Early	Late	
Monolayer	33.8±4.0	37.2±3.2	17.8±4.4	19.2±3.8	25.3±3.5	26.1±4.3	26.5 [a]
Multilayer	19.1±06	21.2±4.9	20.2±2.3	18.9±4.1	19.3±1.3	29.6±3.1	21.4 [b]
Laboratory sieve	16.8±0.6	15.7±0.5	13.8±2.0	14.3±2.7	12.7±1.3	18.2±0.8	15.2 [c]

[a,b,c] Means followed by a different superscript differ (P <0.01)
Monolayer screen (1.18 mm mesh size)
Multilayer screen (4.76 mm, 3.17 mm, 1.58 mm mesh size)
Laboratory screen (4.75 mm, 2.36 mm, 1.18 mm, 600µm, 300µm and 150µm)

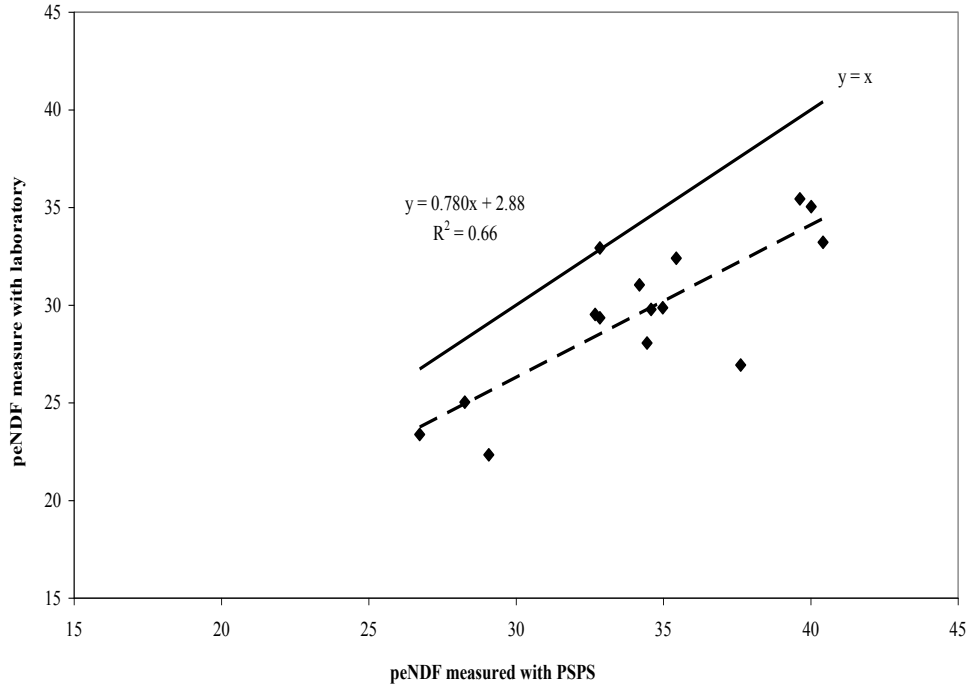

Figure 1. Relationship between measured peNDF (% of DM of TMR) by PSPS sieve and measured peNDF by laboratory sieve method. All samples were taken at the beginning in the middle and at the end the feed bunk (early and late lactation) in three farms. The full line represents the line of equality.

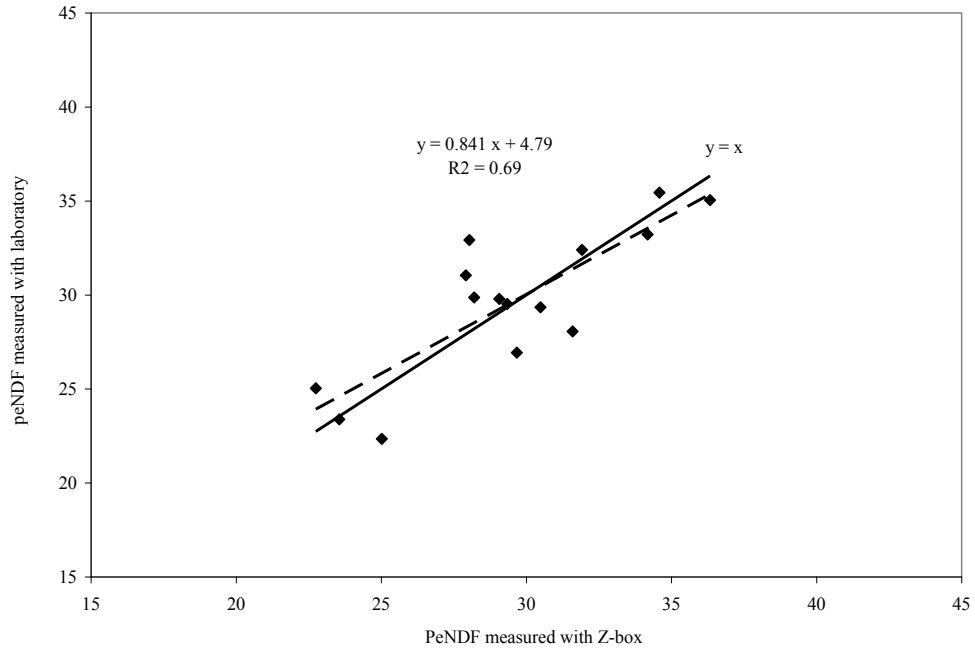

Figure 2. Relationship between measured peNDF (% of DM of TMR) by Z-box sieve and measured peNDF by laboratory sieve method. All samples were taken at the beginning in the middle and at the end the feed bunk (early and late lactation) in three farms. The full line represents the line of equality.

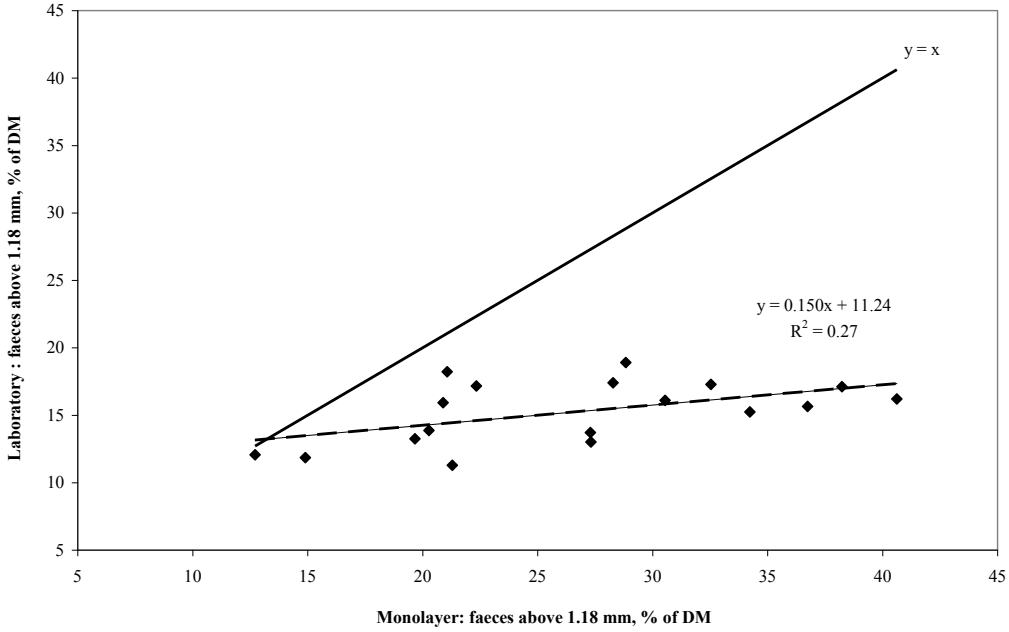

Figure 3. Relationship between particle fraction retained in monolayer sieve (screen apertures 1.18), and fraction retained by laboratory method, expressed as % of DM. The full line represents the line of equality.

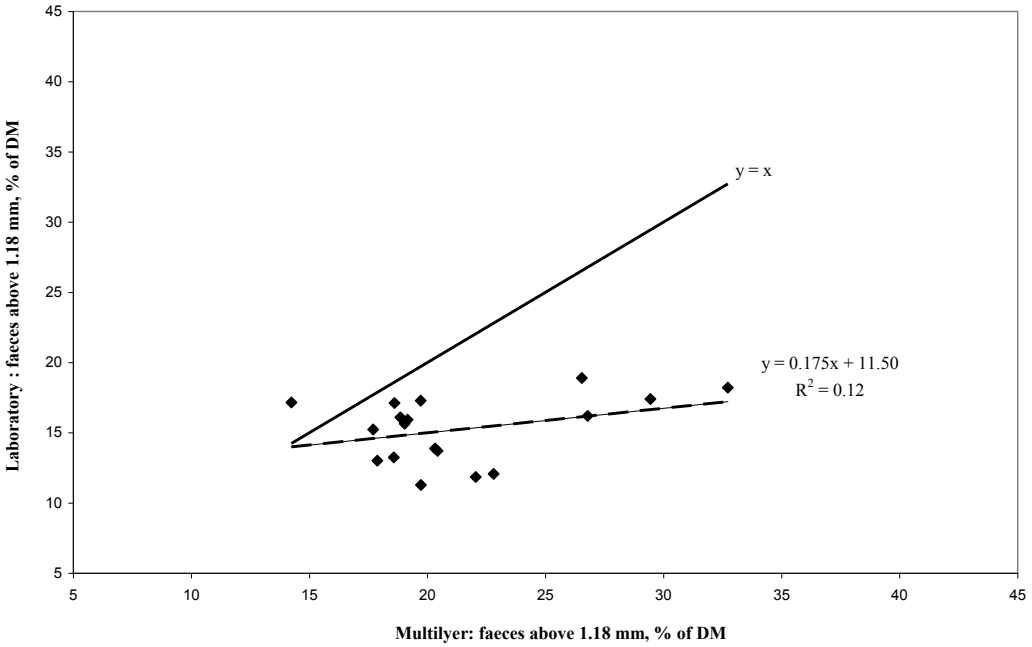

Figure 4. Relationship between particle fraction retained in multilayer sieve (4.76 mm, 3.17 mm, 1.58 mm screen apertures) and fraction retained by laboratory method, expressed as % of DM. The full line represents the line of equality.

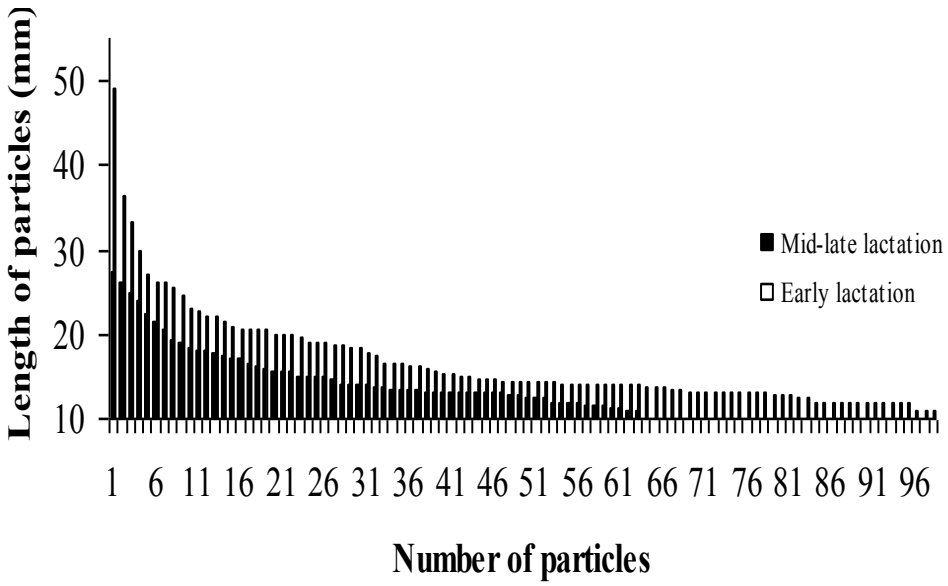

Figure 5. Number and length of particles retained in a sieve of 1.18 mm in composite samples obtained grouping the feces collected in the 3 farms: one composite sample for early lactation and one for late lactation cows. In x axis are reported the number of particles that corresponded to different sizes.

Chapter 2

Development of a calibration curve to estimate dietary particle size with near infrared reflectance spectroscopy

Introduction

Adequate physical and chemical characteristics of total mixed rations (TMR) are essential to achieve and maintain high milk yields in dairy cows. The fiber level of TMR and its forage particle size influence chewing behavior and saliva flow, rumen pH, acetate-to-propionate ratio and milk fat levels (Mertens, 2000).

For all the above reasons, it is very important to have a proper particle size distribution in TMR. Mertens (1997) stated that particles larger than 1.18 mm are reduced through chewing and rumination before moving through the rumen; as a result these particles stimulate saliva secretion more than those smaller than 1.18 mm. These findings led to the definition of the concept of physically effective neutral detergent fiber (peNDF), which is the dietary concentration of NDF included in particles larger than 1.18 mm (Mertens, 2000). These particles are able to stimulate chewing activity and saliva production, thus maintaining an adequate rumen pH (Mertens, 2000). Assuming an homogeneous distribution of NDF in feed particles, peNDF can be estimated as the dietary NDF concentration times the proportion of dietary particles retained in a 1.18 mm screen: peNDF = NDF x *pef,* where *pef* is the physical effective factor, estimated as the proportion of DM retained on a 1.18-mm sieve. The values of *pef* range from 0, when there is no stimulation of rumination activity, to 1, when there is the maximum (100 %) of stimulation (Mertens, 1997).

The measurement of peNDF requires the use of sieving equipments with vertical shaking when laboratory assessments are needed (Mertens, 2000). For field estimates of peNDF of TMR diets, specific equipments have been developed, such as the Penn State Particle Separator (Kononoff et al., 2003) and the Z-Box (Cotanch and Grant 2006). The application of field methods to estimate peNDF is time consuming, thus limiting the frequency and the number of assessments usually made on farms. For this reason, there is a necessity to estimate peNDF with faster systems. Estimates

based on near infrared reflectance spectroscopy technology (NIRS) could be a good option, since very often feed and TMR samples are routinely analyzed by this method to estimate their chemical composition. If good calibration curves for TMR particle size were available, it would be very easy to add to the estimates of the chemical composition that of particle size distribution.

Thus, the objective of this study was to determine whether NIRS could be used to predict the particle size distribution of TMR samples, using as reference for field estimates those given by the Penn State Particle Separator (PSPS).

1.1. Materials and methods

A study was carried out to evaluate the physical characteristics of TMR samples using NIRS technology. To develop a calibration curve for this type of analyses, TMR samples were analyzed to estimate their *pef* and calculate their peNDF with a field sieving method, the PSPS.

1.2. Collection of TMR samples

A total of 118 samples of TMR were collected from 24 dairy farms located in Arborea (Sardinia, Italy), and 1 farm located near Sassari (Sardinia, Italy), from December 2009 to June 2010. Samples of TMR were taken just after their distribution to lactating dairy cows. They were collected at the beginning and in the final part of the feed bunk and then identified separately as different samples. In the same day, the samples were brought to the laboratory and a sub-sample was frozen (to be later sieved and scanned after being defrosted), while the other sub-samples were immediately analyzed. Each sample of about 3 litres was portioned into 2 aliquots. One aliquot of about 1.5 litres(measured with a cup) was sieved to determine its particle size distribution by using PSPS, as described by Kononoff et al. (2003). The other aliquot was scanned by NIRS.

1.3. Sieving with PSPS

Each TMR sample of about 1.5 litres was placed on the top of the PSPS apparatus, made of 3 screens (upper, middle, lower) and a bottom pan. The samples were sieved as described by Kononoff et al. (2003), i.e. five times in one direction, then rotating the PSPS by one-fourth turn and repeating the horizontal shaking. This sequence (5 shakings and a rotation) was repeated eight times, for a total of 40 shakes, to make sure that feed particles were appropriately distributed according to their size (Lammers et al., 1996). Particles retained in each screen were then weighted on a kitchen type scale, with a tolerance of about 4% (+/-). The particle size distribution was calculated and recorded, for each of the 4 screens of the PSPS, as percent of the initial sample weight.

1.4. Measurements with NIRS and development of calibration curves

Before a quantitative analysis with NIR spectrophotometer can be carried out, it is necessary to develop a specific matrix to be analyzed using multivariate methods.

The calibration process involved the following steps: 1. selection of a representative number of samples; 2. acquisition of spectra and determination of reference values; 3. multivariate analysis on the spectral changes of the reference values of the analytical properties of the parameter of interest; and 4. cross validation of the model.

The calibration equations were computed using the following steps: 1) modified partial least squares regression method; 2) de-trending scatter correction (Barnes et al., 1989); 3) transformation of spectral data into first derivative terms; 4) calculations and smoothing over four data points; and 5) validation.

Selection of representative samples

A set of near infrared spectra (log 1/R) was obtained and saved from 118 samples of TMR. Each sample was packed into a rectangular box to be scanned (as fed) twice on a NIRS system instrument (Model 6500, Foss NIRSystems Inc., Silver Spring, MD, USA) between 400 and 1098 nm and between 1100 and 2498 nm regions. Then the samples were scanned and their spectra were recorded to create a data set of reference.

At the same time, all the information determined in parallel with the PSPS was recorded to build a calibration curve. The mathematical treatment applied was of the type 1, 4, 4, 1, where the first number indicates the order of the derivative (of log 1/R), being the derivate method the most often used in NIRS for resolution enhancement, the second number is the gap in nm over which the derivative is calculated, the third number is the number of nm used in the first smoothing, and the fourth number refers to the number of nm over which the second smoothing is applied (Infrasoft International, 2002).

Acquisition of spectra, calibration and validation

Data acquisition, calibration, and analysis were performed by using WinIsi II version 1.5 software (InfraSoft International, Port Matilda, PA, USA). Near-infrared reflectance spectra were obtained by scanning only fresh samples (as fed).

Calibrations were attempted from each spectral origin to predict particle size distribution for >19 mm, from 19 to 8 mm, from 8 to 1.18 mm and <1.18 mm. The calibration was calculated by using 118 TMR samples (scanned twice, for a total of 236 scannings). The statistical analyses were performed using a modified partial least square (PLS) regression (Shenk and Westerhaus, 1993) by using the WinIsi II software. To improve the statistics of the calibration, some samples were discarded from the data set, namely six samples from the upper sieve, nine from the middle, seven from lower and six from bottom.

Validation was performed by using a cross validation of the samples in which the prediction error was evaluated by dividing the calibration samples into four subsets, with one subset reserved for validation and the remaining subsets used for calibration. For this reason, a subset of samples was repeatedly and randomly removed from the calibration samples.. A new model was then calculated on the remaining samples, while residuals were calculated on the validation subset. This process was repeated a number of times and it was carried out by comparing the NIRS with the PSPS results. The performance of the calibration was evaluated on the basis of the standard error of calibration (SEC), while performance of validation was based on the coefficient of determination (RSQ), standard error of cross validation (SECV) and 1 minus variance rate (1-VR) (Shenk and Westerhaus, 1993). SEC is an expression of the average differences between predicted and references values, it refers to the root of the sum squared residuals values divided by the number of values corrected for degrees of freedom. RSQ represents, if expressed in percentage, the proportion of the variance explained by the regression model. SECV is an expression of the bias corrected average difference between predicted and references of the values for the subset of samples selected as prediction samples during the cross validation (ISO, 2008). The coefficient of determination (1-

VR) represents the portion of total variance explained during the cross validation and could be comparable to the coefficient of determination R^2.

1.5. Estimation of the nutrient content of the TMR samples

After being scanned for particle size distribution, all TMR samples were dried in a oven at 60°C for 48 hours. In order to estimate their DM content, they were dried until no more variation in weight occurred. The samples were then ground in hammer mill by using a 1-mm screen, enclosed in stationary metal ring cups (36-mm inside diameter), and scanned using the FOSS 5000 instrument and the WinIsi II software previously described, to estimate their content in CP, ash, EE, NDF, ADF, ADL and starch. Non fiber carbohydrates (NFC) were calculated as 100-(CP + ash + EE + NDF). All the analyses were carried out in the laboratory of the Associazione Regionale Allevatori Sardegna, located in Oristano (Italy), where TMR rations from the same area are routinely analysed for nutrient composition on the basis of the calibration curves locally developed.

Results

2.1. Chemical composition of TMR

The nutrient composition of the TMR fed to dairy cattle in the farms studied is presented in Table 1. Specifically, the DM content had the greatest variation, ranging from 40.6% to 70.7% of the as fed TMR. Likewise, starch ranged from 12.7% to 30.0% of DM, NFC from 29.7% to 45.3% of DM, NDF from 29.9% to 44.7% of DM and ADF from 15.5% to 38.8% of DM. The CP ranged from 9.9% to 18.7% of DM and ash from 4.4% to 10.0% of DM. The EE and the ADL content had the smallest standard deviation (0.5%). These values indicated the TMR diets sampled covered a wide range of chemical and nutritional composition.

2.2. Particle size of TMR measured by the Penn State Particle Separator

The particle size distribution of TMR as evaluated by the PSPS is reported in Table 2. The dietary proportion of the Upper fraction (> 19.0 mm) had the greatest variability (S.D. 8.0%, range 1.3%-50.0% of initial weight). The Middle fraction (from 19 to 8 mm) was the second most variable (S.D. 7.3%, range 12.3%-56.5% of initial weight), while the values for the Lower (from 8 to 1.18 mm) and the Bottom (< 1.18 mm) fractions were the least variable (S.D. 4.8, range 24.5%-48.7% and 3.8, range 9.5%-28%, for the Lower and Bottom fractions, respectively).

2.3. Calibration of the NIRS for particle size distribution

The statistics of the calibration of the NIRS on the PSPS particle fractions are showed in Table 3, whereas the relationship between measured and predicted values for each of the four screens is reported in Figures 1-4. The standard error of prediction (SEP) ranged from 2.310 for the Middle to 1.687 for the Bottom. The SEP for the Upper and Lower fractions were, respectively, 2.219 and 2.133. The SEP corrected for bias (SEP(C)) was only slightly higher than the uncorrected SEP for all fractions. The bias was small for all screens considered. The highest bias was observed for the

Middle fraction (+0.094), whereas the smallest was for the Lower fraction (-0.032). The slope was slightly lower than 1 for the Upper, Middle and Bottom screens (0.989, 0.997 and 0.990, respectively), whereas it was slightly higher than 1 (1.002) for the Lower screen.

2.4. Particle size of TMR by using NIRS

The particle size distribution of TMR samples estimated by the NIRS system is reported in Table 4. Similarly to what observed for the Upper fraction measured with the PSPS, the Upper fraction measured by NIRS had the greatest variability (S.D. 7.9%; range 2.1%-50.2% of initial weight), followed by the Middle fraction (S.D. 7.3%; range 12.4%-56.9% of initial weight). The lowest variability was observed for the Lower and Bottom fractions (S.D. 4.7 and 3.8%, respectively; range 24.2%-47.9% and 9.9%-28.5% of initial weight, respectively).

2.5. Refinement of calibration and Cross Validation Data Set

The statistics for the calibration after discarding the outliers and validation tests are reported in Table 5. The number of samples used ranged from 227 (Middle screen) to 230 (Bottom screen). The mean of the calibration was 10.53% of the TMR initial weight for the Upper fraction, 35.93% for the Middle, 35.56% for the Lower, and 17.68% for the Bottom fractions. The highest S.D. found was for the Upper screen (8.09%), closely followed by the Middle screen (7.57). Smaller S.D. were observed for the Lower (2.00%) and the Bottom (1.61%) screens.

The SEC ranged from a maximum of 2.07 (Upper fraction) to a minimum of 1.61 (Bottom fraction). Upper and Middle fractions had very high and similar RSQ values (0.93 and 0.94, respectively), whereas the Lower and Bottom screens had lower values (0.85 and 0.84, respectively).

The 1-VR values were higher for the Upper and Middle screens (0.91 and 0.92, respectively) than for the Lower and Bottom ones (0.79 and 0.77, respectively). The lowest SECV was that of the Bottom screen (1.96), whereas the highest values were those of Upper and Lower screens (2.42 and 2.37, respectively). The Middle screen had an intermediate SECV value (2.17).

2.6. Prediction of peNDF by NIRS

The relationship between the peNDF measured by the PSPS in the TMR samples and that predicted by the NIRS instrument is reported in Figure 5. The regression line (Y = 1.025 X - 0.70, where Y is the peNDF measured by the PSPS and X the peNDF estimated by NIRS) was close to the equivalence line and the coefficient of determination was close to unity ($R^2 = 0.95$).

Discussion

3.1. Chemical composition of TMR

The nutrient composition of the TMR sampled in this study (Table 2.1) indicated that a wide range of nutrients contents was included. The TMR with low percentages of DM (as low as 40%) were those in which large amounts of corn silage were used, while those with high DM (up to 70%) were mostly based on hay and had very little silage. The presence of TMR with excessive or too low DM contents can lead to an incorrect evaluation of the particle size fractions of the diets by the PSPS, because of an increase in the fraction < 1.18 mm (Kononoff et al., 2003). The minimum NDF concentration of 29.9% recorded in the sampled diets is within the adequate range of NDF concentration of NDF for proper ruminal function, according to the National Research Council (NRC, 1989). Some of the diets which had very low values of CP (9.9% of DM) and starch (12.7% of DM), likely had an undersupply of these nutrients. The NFC was quite variable, with the minimum and the maximum values measured beyond the minimum and maximum values suggested for lactating dairy cattle (NRC, 2001).

3.2. Particle size distribution: Penn State Particle Separator vs. Near Infrared Spectroscopy Reflectance

Based on the PSPS references data (Table 2.2) and the NIRS predicted results (Table 2.4) for each screen (Upper, Middle, Lower and Bottom), the NIRS predicted with high precision and accuracy the proportion of particles in each screen. In fact, Upper PSPS (reference) and Upper NIR had the same mean (10.6 %) and a similar S.D. (8.0% and 7.9%, respectively). The mean of Middle fraction was 36.2% for PSPS and 36.0% for NIRS, and the S.D. was 7.3% for both. Similarly, the mean of the Lower fraction calculated by PSPS was 35.5% and that by NIRS was 35.6 %, with a S.D. of 4.8 % for PSPS and 4.7 % for NIRS. Likewise, the mean of Bottom for PSPS and NIRS were 17.5% and 17.7 %, respectively, with a similar S.D. of 3.8 %.

The fact that NIRS predicted with high precision and accuracy the proportion of particles in each screen compared to the reference method PSPS (Table 2.3) is better appreciated by examining the plots of predicted vs. observed values for each screen (Figures 2.1-2.4).

3.3. Calibration and Validation statistics of particle size

The performance of the calibrations was evaluated on the basis of the coefficient of multiple determination (RSQ) and the standard error of calibration (SEC). The elimination of some samples from the initial data set, to improve statistically the data set results, had a positive outcome for most screens (Tables 2.3 and 2.5). The best RSQ improvements were found for Middle screen and Upper screen, which changed from 0.93% to 0.94%, and from 0.91% to 0.93%, respectively. Thus, the most outstanding ability to predict particle size were found for these two screen sizes. In addition, Bottom RSQ improved from 0.83% to 0.84%. On the contrary, Lower RSQ was worsened by sample removal, changing from 0.91% to 0.85%. To sum up, the ranking of accuracy of screen fractions predicted by NIRS, based on RSQ values, is: Middle (RSQ = 0.94) > Upper (RSQ = 0.93) > Lower (RSQ = 0.85) > Bottom (RSQ = 0.84), (Table 2.5).

The validation test performed using a cross validation was evaluated on the basis of the coefficients Standard Error of Cross Validation (SECV) and 1 minus Variance Rate (1-VR), as summarised in Table 2.5. According to the guidelines utilized by Deaville et al. (2009), calibrations with ratio performance deviation (RPD) > 3 are acceptable for quantitative prediction, those with 2.5 < RPD < 3 indicate equations that may be useful for screening, whereas those with an RPD below the threshold of 2.5 (RPD < 2.5) correspond to useless equations. Only Upper RPD (3.34) and Middle RPD (3.48) indicated equations with an acceptable performance, which also had the highest 1-VR values (closest to 1) of 0.91 for Upper and 0.92 for Middle. On the other hand, RPD values were too low for Lower (2.16) and for Bottom(2.09), with 1-VR values of 0.79 and 0.77, respectively. However, even if Lower and Bottom equations were unsuccessful, *pef* predicted NIRS (*pef* = Σ (%Upper + %Middle + %Lower), and peNDF NIRS (peNDF = NIRS *pef* * NIRS, NDF) could be anyway classified as successful. Figure 2.5 shows that the relationship between the peNDF measured by the PSPS and that predicted by NIRS is y = 1.0232x -0.658, R^2 = 0.95. Given that the intercept was not significant, this equation was not different from the y = x equation (P>0.1) and became y = 1.0022x. The mean bias was 0.20% of the mean measured value (P>0.1). The total mean square error was 0.577; 0.689 % was due to the mean bias, 0.978 % was due to regressing bias while 98.336 % was due to random error.

Conclusions

This study demonstrated that NIRS can be used to predict particle size distribution and to determine *pef* and peNDF in TMR samples.

This technique can be suggested as a rapid, accurate and efficient method to evaluate particle size distribution of TMR samples. Indeed, the NIRS allows a quick and cheap prediction of the particle size distribution of TMR, in contrast to the time consuming and tiring field application of PSPS. This would allow more frequent measurement of particle size distribution in TMR and a better control of the risks of acidosis or of reduced performances. Moreover, since the NIRS is already currently used to predict the nutrient composition of TMR, the inclusion of particle size determination would not disrupt the routing of nutritionists, while greatly increasing the value of TMR analyses.

However, the NIRS calibration for particle size measurement needs to be further improved by increasing its accuracy and repeatability, especially when applied to TMR with characteristics different from those of the farms in which the sampling occurred. This because all the samples were taken from the farms of a production area in which the feeds available and the feeding techniques are very homogenous. Thus, the calibration developed in this study might not be able to accurately predict the particle size of TMR used in other areas or regions.

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TABLE AND FIGURES

Table 2.1. Chemical composition of the TMR samples estimated by NIRS.

	DM	CP	EE	Ash	NDF	ADF	ADL	Starch	NFC*
	% as fed	% DM	% DM	% DM	% DM	% DM	% DM	% DM	% DM
Mean ±	54.3	15.0	3.1	7.0	37.7	22.9	4.0	20.6	37.7
S.D.	5.5	1.5	0.5	1.0	2.8	2.5	0.5	3.6	3.0
Max	70.7	18.7	4.6	10.0	44.6	38.8	5.1	30.0	45.3
Min	40.6	9.9	1.8	4.4	29.9	15.5	2.6	12.7	29.7

S.D.= Standard deviation.
* Non-fiber carbohydrates = [100 - (NDF + CP + EE + ash)].

Table 2.2. Particle size distribution of TMR measured with the Penn State Particle Separator, expressed as % of initial weight.

Screen	Upper	Middle	Lower	Bottom
Mean ± S.D.	10.6 ± 8.0	36.2 ± 7.3	35.5 ± 4.8	17.5 ± 3.8
Min	1.3	12.3	24.5	9.5
Max	50.0	56.5	48.7	28.0

S.D. = standard deviation.
Upper = screen apertures > 19.0 mm; Middle = screen apertures from 19.0 to < 8.0 mm; Lower = screen apertures from 8.0 to < 1.18 mm; Bottom = screen apertures < 1.18 mm.

Table 2.3. Statistics of the calibration of NIRS for the particle size measurements based on the measurements made by the Penn State Particle Separator on TMR samples.

	SEP	SEP(C)	Bias	Slope	RSQ
Upper	2.219	2.223	0.078	0.989	0.91
Middle	2.310	2.313	0.094	0.997	0.93
Lower	2.133	2.137	-0.032	1.002	0.91
Bottom	1.687	1.689	-0.077	0.990	0.83

SEP = standard error of prediction; SEP(C) = standard error of prediction corrected for bias; Bias = observed value - predicted value; Slope = the amount of increase in Y for unit increase in X , $\Delta y/\Delta x$; RSQ = coefficient of multiple determination.

Upper = screen apertures > 19.0 mm; Middle = screen apertures from 19.0 to < 8.0 mm; Lower = screen apertures from 8.0 to < 1.18 mm; Bottom = screen apertures < 1.18 mm.

Table 2.4. Particle size distribution of TMR measured with NIRS, expressed as % of initial weight.

Screen	Upper	Middle	Lower	Bottom
Mean ± S.D.	10.6 ± 7.9	36.0 ± 7.3	35.6 ± 4.7	17.7 ± 3.8
Min	2.1	12.4	24.2	9.9
Max	50.2	56.9	47.9	28.5

S.D. = standard deviation
Upper = screen apertures > 19.0 mm; Middle = screen apertures from 19.0 to < 8.0 mm; Lower = screen apertures from 8.0 to < 1.18 mm; Bottom = screen apertures < 1.18 mm.

Table 2.5. Calibration statistics, after discarding the outliers, and validation results for TMR samples, comparing the particle size measured by PSPS with that predicted by NIRS.

		Calibration				Cross validation		
	n	**Mean**	**SD**	**SEC**	**RSQ**	**SECV**	**1-VR**	**RPD**
Upper	228	10.53	8.09	2.07	0.93	2.42	0.91	3.34
Middle	227	35.93	7.57	1.88	0.94	2.17	0.92	3.48
Lower	229	35.56	5.13	2.00	0.85	2.37	0.79	2.16
Bottom	230	17.68	4.10	1.61	0.84	1.96	0.77	2.09

S.D. = standard deviation; SEC = standard error of calibration; RSQ = coefficient of determination; SECV = standard error of cross validation; 1-VR = 1 minus variance rate; RPD = SD/SECV ratio performance deviation.
Upper = screen apertures > 19.0 mm; Middle = screen apertures from 19.0 to < 8.0 mm; Lower = screen apertures from 8.0 to < 1.18 mm; Bottom = screen apertures < 1.18 mm.

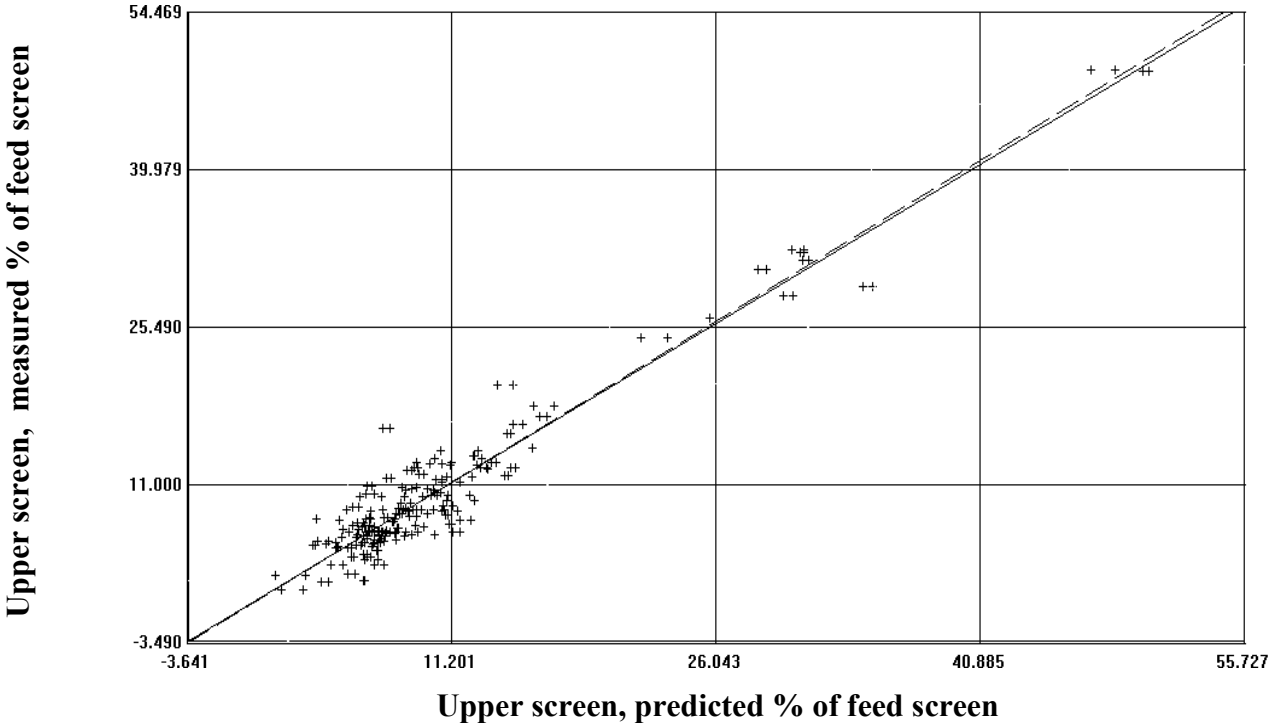

Figure 2.1. Relationship between measured (by the PSPS) and predicted (by the NIRS) particle size distribution of the Upper screen (> 19 mm). Slope = 0.989, RSQ = 0.926, SEP = 2.219, Bias = 0.078, SEP(C) =2.223.

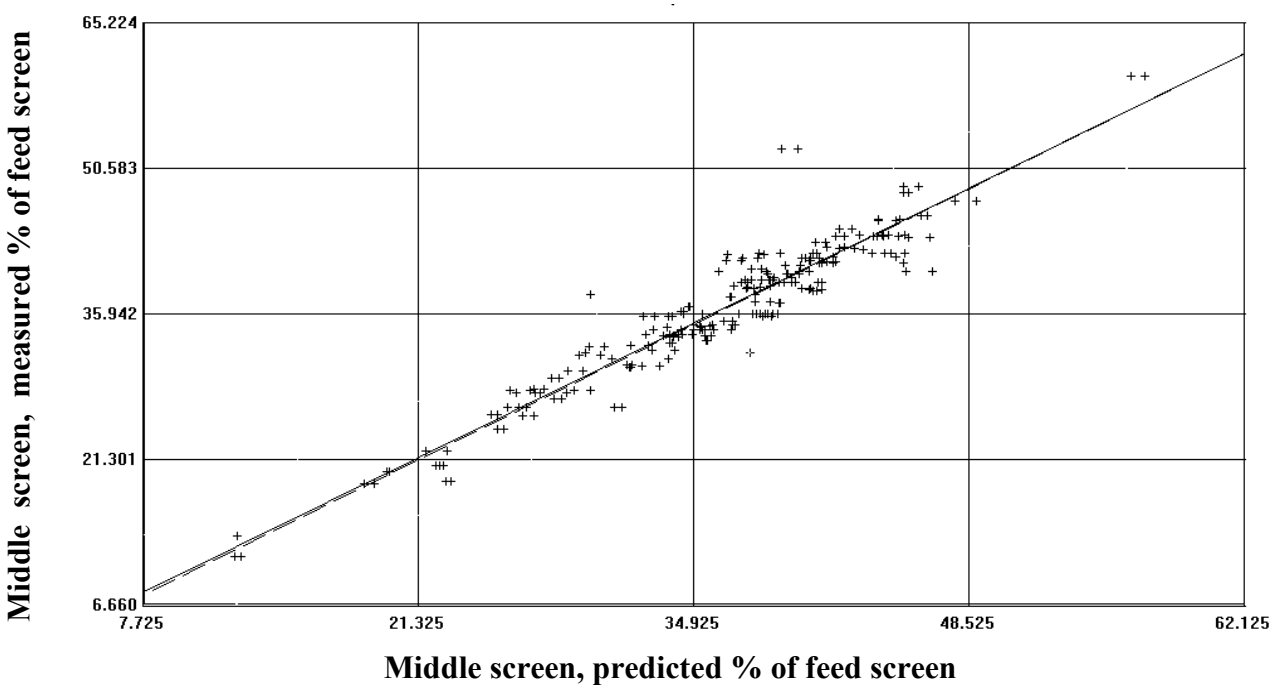

Figure 2.2. Relationship between measured (by the PSPS) and predicted (by the NIRS) particle size distribution of the Middle screen (from 19.0 to 8.0 mm). Slope = 0.997, RSQ = 0.909, SEP = 2.310, Bias = 0.078, SEP(C)= 2.313.

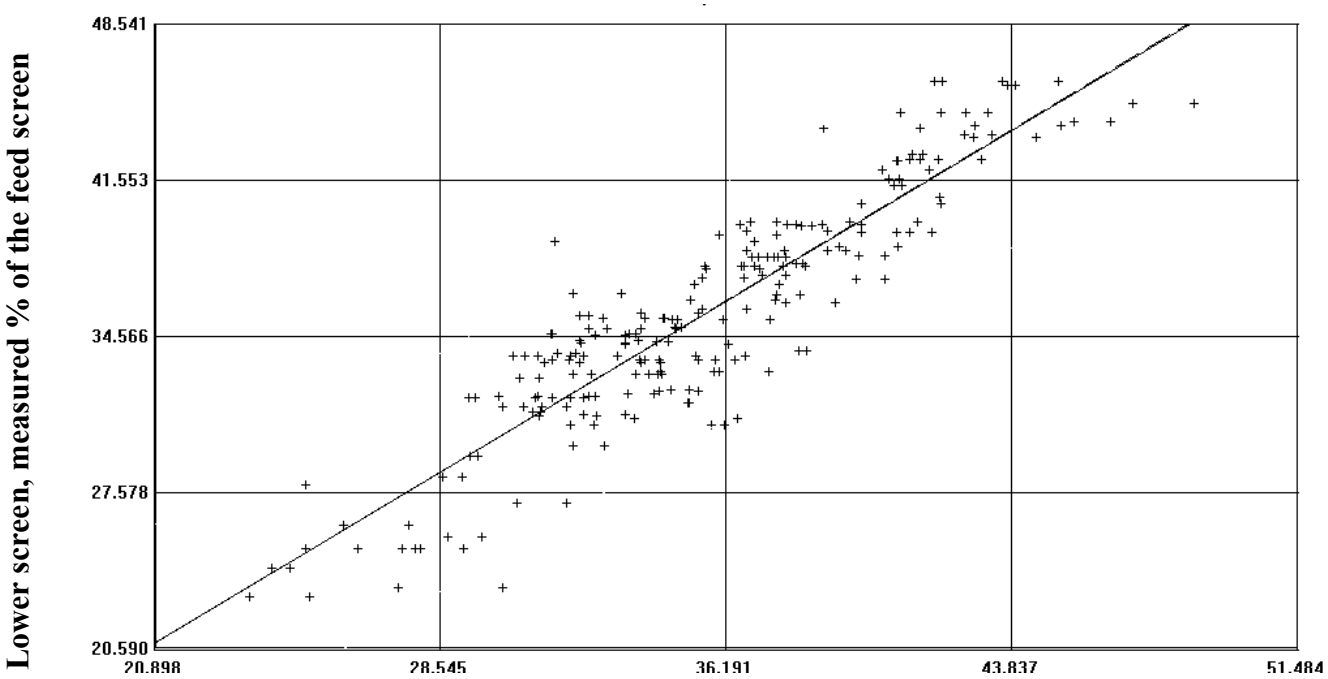

Figure 2.3. Relationship between measured (by the PSPS) and predicted (by the NIRS) particle size distribution of the Lower screen (from 8.0 to 1.18 mm). Slope = 1.002, RSQ =0.831, SEP = 2.133, Bias = -0.032, SEP(C) = 2.137.

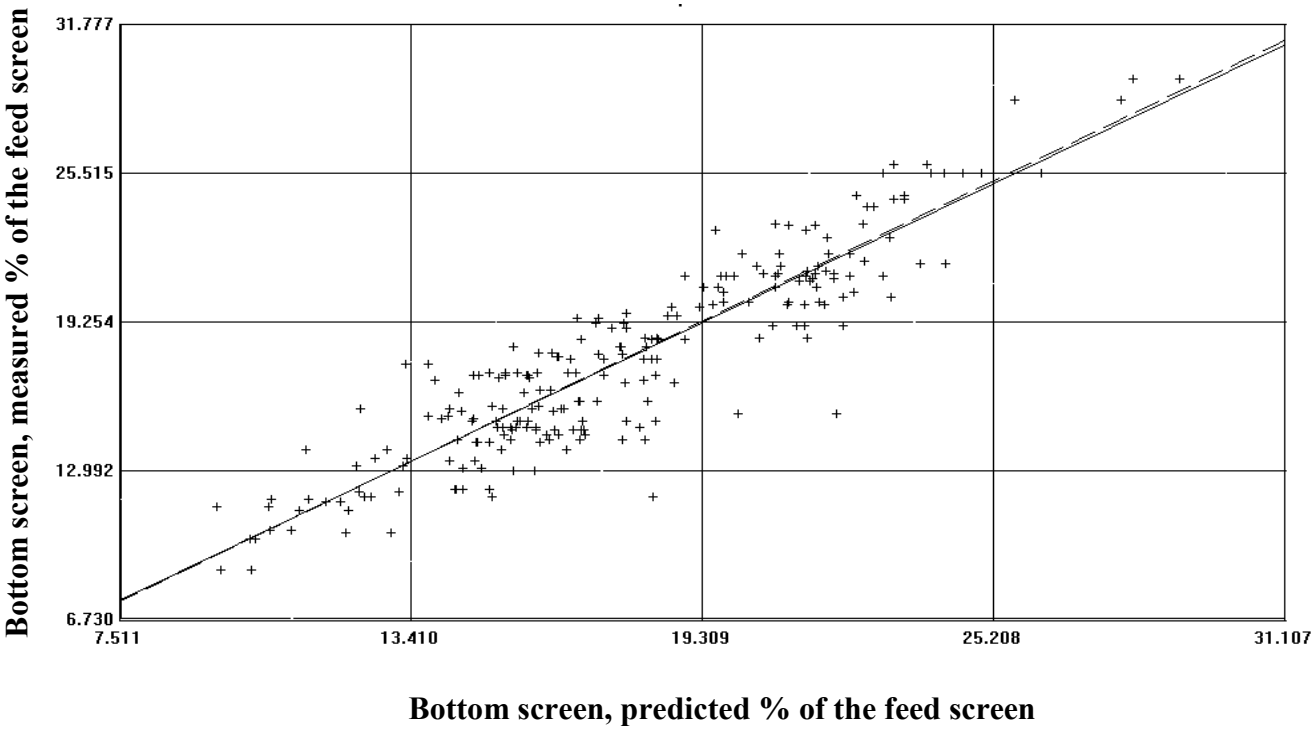

Figure 2.4. Relationship between measured (by the PSPS) and predicted (by the NIRS) particle size distribution of the Bottom screen (< 1.18 mm). Slope = 0.990, RSQ = 0.831, SEP = 1.687, Bias = -0.077, SEP(C) = 1.689

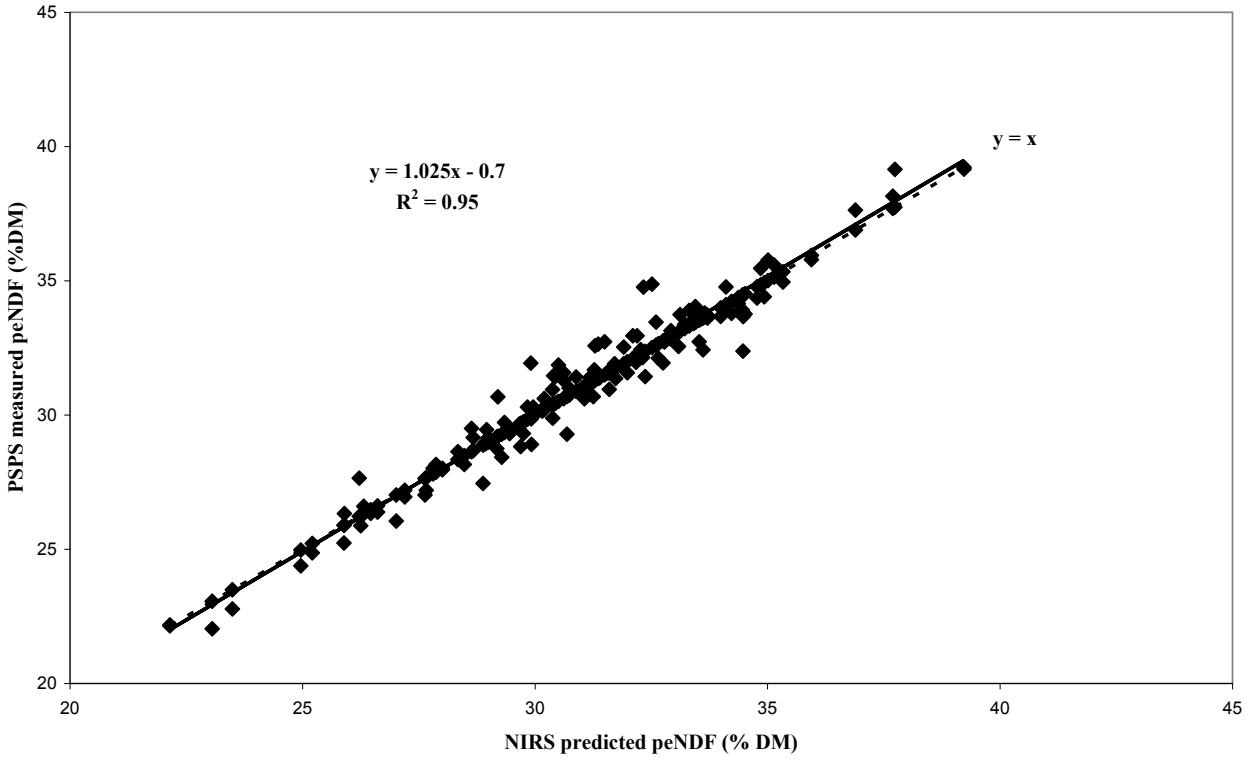

Figure 2.5. Relationship between the peNDF (% of DM) measured by the PSPS and that predicted by NIRS. The regression equation (dashed line) of measured on predicted peNDF was: y = 1.025 (0.0230) x – 0.70 (0.7173), R2 = 0.95, RSD = 0.760. The regression equation was not different from the Y = X line (P > 0.1). Since the intercept was not significant, the equation became y = 1.0022 x. The mean bias (Predicted – Observed) was -0.063 (0.20% of the mean measured value) and it did not differ from zero (P > 0.1). The root of the mean squared prediction error was of 0.76. The total mean squared prediction error was due to 0.69% by the mean bias, for 0.98% by the regression bias and for 98.34 by the random error.

Chapter 3

Milk fat variability due to dietary, animal, and seasonal factors in dairy cattle farms of a Mediterranean production area

Abstract

Genetic, nutritional and management strategies are often applied at farm level for tuning the factors which have major influence on milk fat content. The objective of this work was to investigate the effect of peNDF and of the other most important variables that affect milk fat content (MilkFat) in a Mediterranean production area (Arborea, Italy).

With this purpose, from December 2009 to July 2010a total of 54 samples of TMR and bulk tank milk fat content were collected from 26 Sardinian dairy farms. No significant correlations between MilkFat and fiber related variables were found, probably because the range of variation of peNDF was not sufficient to determine large variations in milk fat content. MilkFat was negatively associated with the DM of TMR ($r = -0.55$; $P<0.001$) and positively associated with the proportion of silage in the rations ($r = 0.30$; $P<0.05$). A significant effect of season ($P = 0.03$) was also observed, with MilkFat being lower in the hot than in the cold periods. A Factor Analysis, carried out excluding MilkFat, was also used to find related variables with observed MilkFat variation. Six factors were extracted with this analysis- They were respectively considered, in order of communality explained, an indication of energy content of diet, forage to concentrate ratio, silage effects, animal production level, ration protein balance and season effects. The Factor 3, indicating silage effect, and the Factor 6, indicating season effects, were significantly correlated with MilkFat ($r= 0.40$ $P< 0.01$; $r = -0.33$, $P<0.05$, respectively for the factors 3 and 6). Factor 3 was positively correlated to the middle fraction of PSPS and to the percentage of silage in the diet and negatively correlated to the lower fraction of PSPS and the DM content of TMR. It showed a similar effect on MilkFat in both seasons.

The most important variable related with milk fat content was identified in the DM content of TMR, which in turn was inversely associated to its silage content. These results suggest that high moisture diets were more thoroughly mixed by the wagons and less subjected to sorting and selection by the cows, reducing the risks of milk fat reduction or depression.

Introduction

Fat is the most variable component of milk (Vines et al., 1986) and it is the most relevant component in a energetic and economic way. In fact both cow energy requirements (NRC, 2001) and scales of milk payment (Banga et al., 2009; Pieters et al., 1997) are often based on algorithms that directly refers to milk fat content. Genetic, nutritional and management strategies were often applied at farm level tuning the factors which have major influence on milk fat content. The most important factor that affect milk fat is the dietary NDF, in particular the variation in milk fat is defined as the animal response to eNDF (Mertens, 1997). The eNDF concept, applied to a general feed, takes into account the buffering capacity in the rumen, dietary fat concentration and composition, quantitative and qualitative production of acids during rumen fermentation of substrates, the peNDF and other characteristics of the same feed or metabolic factors that affect milk fat production. The eNDF is more associated with the concept of the effectiveness of the fiber and conceptually broader than the peNDF. The eNDF can be measured as the total amount of food that needs to be replaced with a quantity of forage NDF to maintain a constant milk fat. The actual measurement of eNDF is not easy and not absolute; for a determinate feed is possible to obtain estimates of eNDF only in comparison to the effect of other feeds and in specific and very well defined nutritional conditions (Mertens, 1997). For this reason the physical effective fiber or peNDF remain the reference term to test the adequacy in fiber content in cow ration. PeNDF is a measurable characteristics of a feed, or diet, equal to the percent of NDF retained above a sieve of 1.18 mm after sieving following a specific laboratory protocol. Field equipment have been also developed to estimate the peNDF on farms (Kononoff et al., 2003; Cotanch and Grant, 2006).

Mertens (1997) found a curvilinear relationship to predict milk fat content from the dietary peNDF content of dairy cow rations (milk fat , %) = 4.32 – 0.171(1/peNDF, %; SE = 0.17; R^2 = 0.63) in a range that varied from about 10% to 55% of peNDF and from 2.8% to 4.3% of milk fat. This equation explains very well the influence of fiber on milk fat. In this relationship when milk fat is above 3.6% (corresponding to about 24% of peNDF on a DM basis) little variation in milk fat content occur when peNDF is further increased. This suggests that when the 24% threshold of peNDF is reached other factors control milk fat content. It is then worthwhile to investigate which

are these other factors. They can be related to territorial, management, environmental, dietary, and farm conditions.

Thus, since most of the studies conducted to investigate the influence of dietary particle size and of other factors that affect milk fat content were carried out in cold or temperate regions, the objective of this work was to investigate the most important variables that affect milk fat variability in a specific production area located in the Mediterranean region, which has a much warmer and dry climate than the above mentioned regions.

Material and methods

1.1. Studied area and farms characteristics

The study regarded 26 dairy cattle farms, 25 of them located in the area of Arborea (central West Sardinia, Italy, at sea level, within 10 km from the sea), and one farm located in Sassari (North West Sardinia, Italy, 50 m above sea level, within 15 km from the sea), characterized by homogeneous environmental and economical conditions. The 26 studied farms sell the milk produced to the same cooperative processing plant and the milk price is calculated using the same quality scale for all farms involved in the study. All farms generally housed lactating cows in a single group, but rations varied between farms mainly in terms of allowable feeds and mixer type and use.

1.2. Collection of samples

From December 2009 to July 2010 a total of 54 samples of TMR were collected from the 26 dairy farms. Each sample was obtained by taking two sub-samples, one at the beginning and one at the final part of the feed bunk, just after their distribution to lactating dairy cows. In the same day, each sub-sample was identified separately, brought to the laboratory and divided into two portions. One portion was sieved to determine its particle size distribution, whereas the other one was scanned by NIRS for chemical composition. Detailed information on fat type and percentage of silage used in the ration, average milk yield and DMI at herd level were also collected. Bulk milk samples were collected from the farm tank and analyzed by the milk plant 3 times per month per each farm, as a

routine for the milk quality payment. The milk fat analysis of the first milk sample collected after the TMR was recorded for this study.

1.3. TMR sieving with Penn State Particle Size Separator

Each TMR sample of about 1.5 litres (measured with a cup) was placed on the top of the Penn State Particle Size Separator (**PSPS**) apparatus, made of 3 screens (upper, middle, lower) and a bottom pan. The samples were sieved as described by Kononoff et al. (2003), i.e. five times in one direction, then rotating the PSPS by one-fourth turn and repeating the horizontal shaking. This sequence (5 shakings and a rotation) was repeated eight times, for a total of 40 shakes, to make sure that feed particles were appropriately distributed according to their size (Lammers et al., 1996). Particles retained in each screen were then weighted on a kitchen type scale, with a tolerance of about 4% (+/-). The particle size distribution was calculated and recorded, for each of the 4 screens of the PSPS, as percent of the initial fresh sample weight. Assuming an homogeneous distribution of NDF in feed particles, peNDF was estimated as the dietary NDF concentration times the proportion of dietary particles retained in the 1.18 mm screen (Lower screen): peNDF = NDF x pef, where pef is the physical effective factor, estimated as the proportion of DM retained above the 1.18-mm sieve. The values of pef range from 0, when there is no stimulation of rumination activity, to 1, when there is the maximum (100 %) of stimulation (Mertens, 1997).

1.4. Chemical analysis

The analysis of the TMR and of the milk were conducted in the laboratory of Sardinian regional association of farmers (ARAS, Oristano, Italy). The TMR analyses were performed with a NIRS equipment to determine the content of DM, CP, NDF, ADF, ADL, Ether Extract, Ash and starch, while NFC was determined as difference of 100- (CP, NDF, ADF, ADL, EE, ASH). Chemical analysis of fat content in milk was determined with the infrared spectrophotometric method (Combifoss 4000 FOSS, Hillerød, Denmark).

1.5. Estimation of the energy value of the diets

The NEL values of the diets was predicted with the method of the discounts system of Van Soest and Fox (1992), originally developed for cattle. With this method the energy content of the diets is estimated assuming a level of nutrition of 3 times maintenance:

$$NEL_{3m}\ (kcal/kg\ DM) = 10 \times TDN_m\ \%\ [2.86 - (35.5/(100 - NDF))]$$

where TDN_m is the diet total digestible nutrients (% of DM), estimated at maintenance feeding level, and NDF is the diet NDF concentration (% of DM). The TDN_{1m} was predicted by using the summative equations of Weiss as reported in the Table 25.7 of Van Soest (1994):

$$TDN_m = DM_d - ash + (1.25 * EE) + 1.9$$

$$DM_d\ (\%) = 147.3 - 78.9\ log_{10}\ [(ADL/ADF)*100] - 15$$

Where DM_d is the dietary apparent digestibility of DM.

1.6. Statistical analysis

The data of the chemical and physical characteristics of the rations were calculated as mean of the two sub-samples collected in the feed bunk. The samples collected in the period from December to March were attributed to the cold season, whereas those from April to July were attributed to hot season. Thus, the final dataset analysed included animal, nutritional and milk fat information of all studied farms. It was composed of 54 records, of which 30 collected in cold season (from 20 farms) and 24 collected in hot season (from 18 farms) (Table 1). Most farms were sampled one time per season, few farms were sampled more than once per season, and 12 farms were sampled both in cold and hot season (Table 1). The effects of different nutrition, management and diets' chemical and physical characteristics on milk fat content variability were analysed without considering the farm effect.

To test in this dataset the relationship between peNDF and milk fat developed by Mertens (1997), milk fat was estimated using the his equation: milk fat, % = 4.32 – 0.171(1/peNDF, % DM). Predicted milk fat was then compared with the observed values of milk fat, using the Model Evaluation System 3.1.12 developed by Luis. O. Tedeschi at Texas A & M, USA (http://nutritionmodels.tamu.edu/mes.htm).

Descriptive statistics and correlations were used to describe variability ranges and relationships between selected studied variables.

A general linear model was fitted to study the fixed effects of season, dry matter content of TMR and other selected variables (milk yield level, fat type and chemical and physical characteristics of ration) on milk fat content (**MilkFat**), using the PROC GLM of SAS (SAS Institute, 1996).

Selected variables were further analyzed using a multivariate approach with the Factor Analysis. MilkFat, fat type, pef and peNDF were excluded from the dataset before performing the Factorial Analysis. This the highlight a possible latent structure of the data.

In the factorial model, the value of the variable X_i for the *i-th* observation can be decomposed as follow:

$$X_i = \Sigma_j b_{ij} * F_j + e_i \quad (\text{for } j=1,m)$$

where F_j is the *j*-th common factor (or latent variable), b_{ij} is called factor loading and weighs the *i*-th original variable in the composition of the *j*-th factor, *m* number of factor extracted, e_i is the uniqueness of the i-th variable (Krzanowsky, 2000).

Measures of sample adequacy (**MSA**) were used to evaluate the suitability of dataset to Factor Analysis. The part of variance of original variable explained by the common factor is the communality for that variable; the sum of the communalities of all original variables is the total amount of variance explained by the factors. The communality were used as criteria to choose the number of factor to retain (~at least 80% of the variance of the original variances). Principal factor was the method used to extract the common factor implemented in the PROC FACTOR of SAS. A Factor loadings matrix (**B**) was rotated using the EQUAMAX procedure to enhance the interpretation of factors extracted. Thus, the correlations between MilkFat and estimated factor scores were discussed.

Results

2.1. Description of selected variables

The characteristics of studied variables are described in Tables 2, 3 and 4. The variables related with management and animal performances are reported in Table 2. Silage used in the rations ranged from 0% to 58% of DM supplied to the cows, with a mean value of 36% of DM. Dry matter intake and milk yield varied largely within the dataset (with a difference between maximum and

minimum observed values of about 6 kg of DM and 13 liters of milk yield), but their S.D. were not high, being equal to 1.1 kg of DM and 2.8 liters of milk yield (Table 2). The mean values of the various parameters did not vary considerably between seasons. Milk fat content (as % weight/volume) varied from 2.88% (minimum in the hot season) to 4.29% (maximum in cold season), with a mean of 3.87% in the cold season and 3.70% in the hot season. The rations had a large range of variation in the macronutrient parameters, without major differences between seasons (Table 3). Considering all data, the DM of the TMR was the chemical characteristic of the rations which varied the most (S.D. 5.4), ranging from 46% to 70%, with a mean value of 54% (Table 3). The NDF content ranged between 32% and 44% of DM (mean value 37.7% of DM), whereas lipid (EE) content ranged from 2.2% to 4.4% of DM (mean value of 3.1%). Starch ranged from 13% to 28% (mean of 21%), protein (CP) content ranged from 11.3 to 17.7 % (mean of 15%), non fibrous carbohydrates (**NFC**) content from 31.4% to 42.6% (mean of 37.3%). The mean values for TDN_m, DMd and NEL_{3m} were 65%, 65% and 1.49 Mcal/kg of DM, respectively, with maximum values of 70%, 70% and 1.64 Mcal/kg of DM. The sieving with the PSPS showed mean values of 10%, 36%, 35% and 18% for the upper, middle, lower and bottom sieves and the pan, respectively, which resulted in a mean dietary peNDF of 31% (Table 4). The maximum values of upper, middle, lower and bottom sieves and the pan, and the peNDF were 49%, 51%, 46% and 38%, respectively.

2.2. Observed vs. predicted milk fat content

The milk fat content predicted using the peNDF of TMR, as suggested by Mertens (1997), showed a smaller range of variation (from 3.61% to 3.87% for 24.2% and 38.4% of peNDF, respectively) relatively to the observed data (from 2.88% to 4.29%; Table 4 and Figure 1). The regression of observed values against predicted values calculated with the software MES 3.1.3 showed a low accuracy ($R^2 = 0.02$), a low precision ($r_c = 0.026$) and a high RMSPE of 0.26, which was composed for the 96.8% by random errors, 1.1% by systematic bias and 2.1% of mean bias.

2.3. Correlations among selected variables

Correlations reported in Table 5 showed the most important associations among the studied variables. No significant correlations between MilkFat and fiber related variables were found in the dataset (Table 5).

MilkFat was negatively associated with the DM of TMR (-0.55; P<0.001) and positively associated with the proportion of silage in the ration DM (0.30; P<0.05); the two correlation were confirmed by the negative relationship between DM of TMR and percent of silage in the ration (-0.55; P<0.001).

The PSPS fractions were also correlated with the percentage of silage in the ration; silage in ration positively affected the middle (0.70; P<0.001) and the peNDF fraction (0.27; P < 0.05), while it affected negatively the upper (-0.58; P < 0.001), the bottom (-0.24; P < 0.08) and the apparent digestibility of DM (-0.27; P < 0.05).

The DM of TMR was also associated with the middle (-0.65; P < 0.001), the lower (0.36; P < 0.01, the bottom (0.42; P < 0.001), the pef (-0.43; P < 0.001) fractions of PSPS, as well as with NDF (-0.27; P < 0.05), starch (0.24; P = 0.08) and apparent digestibility (0.28; P = 0.04) of the TMR.

The peNDF content of TMR was negatively associated with DM (-0.40; P 0.001), starch (-0.52; P < 0.001), NFC (-0.62; P < 0.001), CP (-0.39; P < 0.001) and the energy density of the ration (-0.54; P < 0.001), whereas it was positively associated with fiber NDF content (0.89; P < 0.001).

Animal performances were associated with chemical and physical composition of TMR. In particular, DMI was negatively associated with the upper fraction of TMR (-0.32; P = 0.02), and positively associated with middle fraction (0.24; P = 0.08) and milk yield (0.32; P = 0.02). Milk yield was negatively associated with the upper fraction of PSPS (-0.37; P < 0.01) and positively associated with the middle fraction of PSPS (0.30; P < 0.01) and the starch content of TMR (0.29; P < 0.05).

2.4. Dry matter of TMR and milk fat content

The variable associated the most with MilkFat was the DM of the TMR, which caused a negative variation of MilkFat equal to -0.026% per point of increase of DM (R^2 = 0.30; P < 0.001) (Figure 2). The DM of TMR and MilkFat observed in the various farms showed that farms with high DM had low MilkFat and vice-versa (Figure 3).

Based on GLM analysis, lower values of TMR DM were associated with higher MilkFat (Table 6). However, only with DM higher than 60% was MilkFat significantly reduced (P<0.05). In addition, MilkFat was higher in cold season than in hot season (P<0.05; Table 6).

According to GLM analysis, the only variables which were significantly associated with MilkFat were the season and the class of DM (Table 6). Silage in ration, NDF, milk yield, fat and fat type (classified as hydrogenated, protected, cottonseed fat and others sources) included in ration, starch, NFC, and fractions of PSPS were non significantly associated with MIlkFat when included in the model together with the variables season and DM.

2.5. Factorial analysis

Table 7 reports the results of Factorial Analysis on the dataset, including measure of sample adequacy (MSA), rotate factorial scheme of the factor loadings, i.e. the correlations among common factors and original variables, communality, and correlations between factor scores and measured milk fat.

Six factors were retained according to the assumptions. The communality accounted for a 82% of total variance.

The MSA and the total communality was 0.50. and 15.6, respectively. The first 3 factors extracted explained 65% of communality, and factor 6 for about 10%. The criterion used to explain the meaning of each factor was to focus on loading factors greater than 0.50 (signed in bold in Table 7).

The first factor was associated positively with TDN_m, NEL_{3m}, apparent digestibility of DM and EE and negatively associated with ADL; the first factor can be considered as the indicator of the *energy content of the ration*.

The second factor was positively associated with starch and NFC and negatively with NDF and ADF; this factor can be considered an indicator of *forage to concentrate ratio*.

The third factor was positively correlated to middle fraction of PSPS and the percentage of silage in diet and negatively to lower fraction of PSPS and the DM content of TMR; this factor can be identified as an indicator of the *silage effect.*

The fourth factor was positively related to milk yield and DMI and negatively to the upper fraction of PSPS, and can be denominated as indicator of the level of *animal performance;* thus the upper fraction of PSPS means the limiting effect of TMR to reach target performance.

The fifth factor was associated positively with CP content of TMR and the bottom fraction of PSPS, and can be considered an indicator of *protein balance in the rumen.*

The sixth factor was strongly and positively associated with seasons, classified as 1 for cold period

and 2 for hot period in the dataset; the sixth factor can be interpreted as an indicator of the *season.*
Correlation between extracted factors and MilkFat were calculated to find information on milk fat variability in studied farms.
A negative correlation with MilkFat was found for the Factor 2 (-0.27; P = 0.051) and the Factor 6 (-0.33; P < 0.02), while a positive correlation was found for Factor 3 (0.40; P < 0.01). The sign of correlations can be multiplied by the sign of loading factors to find the actual relationships between selected variables and MilkFat, i.e. the Factor 2 indicated that NDF should be related with higher values of MilkFat in respect with starch, negatively related to MilkFat.
The relationship between MilkFat and Factors 2, 3 and 6 are showed in Figures 4, 5 and 6, respectively; in each plot cold season is indicated by circles and hot season is indicated by triangles.
A statistical analysis with a fixed linear model was applied to MilkFat to study the effect of fat type, Factors 2, 3 and 6:

$$Y_{ijkl} = \text{FAT TYPE}_i + \text{FACTOR } 2_j + \text{FACTOR } 3_k + \text{FACTOR } 6_l + e_{ijkl}$$

were Y is MilkFat %
Fat type classes were formed separating fat type in hydrogenated, saponified, cotton and other sources and Factors were classified as negative and positive values
Based on the result of PROC GLM, Factor 3 (*silage effect*) and Factor 6 (*season*) were significantly associated with MilkFat in the studied farms (Table 8), whereas fat type (*Integration of fat in ration from different sources*) and Factor 2 (*forage to concentrate ratio*) were not.
To better explain the relationship between silage effect and season on MilkFat, a 3 dimensional plot was created (Figure 7), where data of two seasons were identified by circles (cold season) and triangles (hot season). It is possible to identify a similar trend in the effect of silage both for cold and hot season; however the range of variation of Factor 6 was smaller in cold than in hot season.

Discussion

The studied farms represent 16% of the dairy farms in the Arborea area. A large range in the use of silage in ration was observed; this variability was probably more related to land availability of each farm than to nutritional choices. On the other hand, animal performances in terms of DMI and milk yield were in agreement with the average performances in the same area. Similar values were

observed between hot and cold season and a small standard deviation was found (Table 2). MilkFat showed a high variability (from 2.88% to 4.29%) and was higher in the cold season with respect to hot season, probably due to the high temperatures that occurs during the hot season in the Mediterranean area. Mean dietary chemical characteristics can be considered adequate for dairy cows rations, considering some of the standards suggested by the NRC (2001) or the CPM-Dairy (http://www.cpmdairy.net/Index.php) software used to formulate the diets in most of the farms considered (NDF > 30%, fat < 5%; NFC 38-40%); however maximum and minimum values of many nutrients showed some limiting conditions i.e. protein levels < 13%, NEL_{3m} < 1.4 Mcal/kg DM, starch content < 20%, TDN_m and digestibility < 60%). In some nutrients, like NEL_{3m} or starch, the observed values were close to optimum target suggested by NRC (2001) to improve milk production in lactating dairy cows .

Based on the sieving results, dietary particle size did not differ between season for mean, maximum, and minimum values. PeNDF was around 30%, being much higher than 22% reported by Mertens (1997) as lowest threshold to avoid the acidosis risk. Considering and comparing the single fractions measured with the PSPS (Table 4) with the target values (upper 2-8%, middle and lower 20-40%, bottom < 20% of fresh weight) suggested by Kononoff et al. (2003), the studied farms had too high upper fractions (mean of 10.3%) and adequate smaller proportions. However the minimum and maximum values observed for each fraction reported in Table 4 showed some very critical situations, i.e a maximum value for the upper sieve equal to 49% and of bottom equal to 27%.

The milk fat content was not associated to peNDF, in contrast to what expected based on the Mertens (1997) research. The reason of this discrepancy was probably due to the fact that: a) the range of peNDF considered in this work (24% to 38 % of peNDF) was smaller than that studied by Mertens (1997) (10% to 55%); b) the minimum peNDF (24% of DM) in the studied dataset was close to the value above which as peNDF increased Mertens (1997) observed that milk fat content tended to plateau. This suggests that other variables than peNDF where affecting MilkFat variation.

MilkFat was negatively correlated to the DM of TMR and positively with the percentage of silage in the diets (Table 5). This is an indication of the positive effect of silage on MilkFat. In fact the DM of TMR was strictly associated to the percentage of silage in diet, and decreased as silage increased.

The percentage of silage in diet was also related with the PSPS fractions. The highest positive correlations were found between the ration retained in the middle fraction and the silage, starch and ADL dietary content and the highest negative correlations were found between the middle fraction and the DM of TMR, as already observed by the PSPS developers (Kononoff and Heinrichs, 2003), and estimated digestibility (Tables 5 and 6).

According with Kononoff et al. (2004) the moisture of TMR affects the results of sieving with the PSPS: high moisture increases the largest fractions of sieved TMR and reduces the lowest fractions. The fact that in this dataset as the percentage of silage in the diet increased there was an increase in the humidity of TMR caused an artifact increase in the dietary peNDF values, altering the relationship with milk fat content. Thus might explain, at least in part, the lack of relationship between MilkFat and dietary peNDF.

It is not clear, however, what caused the increase of milk fat as dietary DM decreased and dietary silage concentration increased. Since none of the nutritional variables was associated to MilkFat, one possibility is that when dietary humidity was high there was a better mixing of the dietary ingredients in the TMR wagon than when dietary DM was high. In addition, with humid diets less sorting among dietary ingredient by the animals was likely

The analysis of variance confirmed that dietary DM, together with the season, was the most important variable that affected MilkFat. Thus MilkFat tended to decrease from cold to hot season and from wet to dry rations, although significant differences were found only between the two seasons and between the lowest and the highest classes of DM. The effect of season is in agreement with the fact that MilkFat is usually lower in summer than in winter mostly for physiological reasons related to heat stress (Collier et al., 2006). This pattern was observed also in Sardinian farms (Atzori, 2008).

The factor analysis allowed to explain the latent structure of dataset and to identify the principal variables related to MilkFat. The extracted factors labeled very well the different classes of variables. The Factor 1 was related to factors associated positively (TDN_m, DM_d, NEL_{3m}) or negatively (ADL) to the energy content of diet and alone explained 27.1% of the communality of the dataset (Table 7), highlighting the importance of energy related variables in ration formulation. (TDNm, Digestibility, NEL3m, ADL). This factor was not significantly correlated to MilkFat. Indeed, dietary energy does not have a direct effect on MilkFat, except when energy is given as fat

source (Scroheder et al., 2004). Factor 2, negatively related to fiber and positively related to starch and NFC, summarized the forage to concentrate ratio of diet and its opposite effects on the nutritional system identified by this dataset. Factor 2 showed a clear relation with MilkFat ($r = -0.27$, $P < 0.051$), that was probably associated to the fiber, as demonstrated by the sign the correlation coefficient (Table 7; Figure 4). However the effect on MilkFat was not significant when considering negative and positive values of the score (Table 8).

Factor 3 was an indicator of silage effect. As previously discussed the middle fraction and the dietary DM were the most important variables associated with the quantity of silage in diet, that is also positively associated with this factor. The lower fraction of PSPS represented the complementary part of middle sieved fraction and was associated with a negative sign to the Factor 3. It was the most linked factor with MilkFat ($r = 0.43$, $P < 0.003$; Table 7 and 8, Figure 5), confirming the need to study the properties of silage to try to manage MilkFat.

The Factor 4 represented the herd production level, in terms of milk yield and of DMI needed to support production; the upper fraction of PSPS indicated the diet characteristic able to limit intake and consequently milk production (Kononoff et al., 2003). Information on mean days in milk of studied herds was not available, although the stage of lactation, as well know, is a important variable that affects milk production level but also MilkFat, (Ragsdale and Turner, 1922). In the Arborea region mean days in milk of the herds Are usually related to seasons and generally increase from winter to summer due to seasonality in reproduction. Silage in diet and middle and lower fraction of PSPS were also positively associated with the Factor 4 but in a lower extent than with Factor 3 and below the threshold fixed in 0.5 for the loading factors. However, this pattern confirms the importance of silage on affecting milk yield and milk fat content in the dataset, probably for the widespread and massive utilization of this feed in the area studied (Table 2).

Factor 5 was highly correlated to dietary CP and to the bottom fraction of PSPS, where grain meals were deposited. This factor indicated the protein balance of the ration or probably the protein to energy ratio of the diet. This factor was not associated with milk fat and explained a little part of communality, probably because the range of protein content of the studied rations was very small and CP was limiting in only few samples. The Factor 6 was strictly correlated to season and was also correlated with MilkFat. Factor six explained the smallest part of the communality (Table 7)

but was highly associated to MilkFat, confirming the seasonality of milk production in the region studied.

The Factor 3 and the Factor 6 were the only significant variables that affected MilkFat in the mixed model analysis (Table 8). Figure 7 summarized the relationship between the 3 variables; milk fat content increased, with similar trends both in cold and hot season, positively with the Factor 3, i.e. with proportion of silage in diet, with the middle fraction of PSPS and with the moisture of TMR.

This results confirm the importance of silage in controlling the nutrient supply and the milk composition of the diets studied and highlights the need to better understand what are the most important silage characteristics able to improve milk production and milk fat content.

Based on this analysis, it appears that in the conditions studied the assessment of dietary particle size and peNDF is not particularly useful in terms of predicting milk fat content. However, the effects of dietary particle size go much more beyond the control of milk fat content, since they also influence the health status of the animals and their long term performances.

Conclusions

Neither the fiber content of the diets nor their peNDF affected milk fat content, probably because the fiber effect was confounded with other factors, like season, different levels of silage in the rations and of milk production level. In addition, a smaller range of variation in the milk fat content and dietary peNDF content was observed in the studied farms in comparison with the values used by Mertens (1997) to develop his relationships.

The most important variable related with milk fat content was identified in the DM content of TMR, which in turn was inversely associated to its silage content. The mechanism underlying these relationships could not be identified with the variables studied. It is likely, however, that high moisture diets were more thoroughly mixed by the wagons and less subjected to sorting and selection by the cows. This would have allowed the utilization of more balanced diets by the cows and reduced risks of milk fat depression.

Other variables not considered in this study, such as dietary buffers, characteristics of the mixing wagons, time of mixing and number of daily distribution of the TMR, feeding patterns and dietary

sorting of the cows could have affected milk fat content. Further studies are necessary to assess their importance.

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TABLE AND FIGURES

Table 1. Sampling plan of TMR in the studied farms.

	Cold season	Hot season
Collected samples	30	24
Sampled farms	20	18
with 1 sample	*11*	*13*
with 2 samples	*8*	*4*
with 3 samples	*1*	*1*
Farms in both seasons	12	

Table 2. Characteristics of the diets and of the herds separated by season and pooled.

Season	Statistics	Silage in ration	DM intake	Herd milk yield	Milk fat content
		% of DM	kg/d per cow	lt/d per cow	%, w/v
Cold Season (Dec., Jan., Feb., March)	Number	30	30	30	30
	Mean	35.5	21.2	27.6	3.87
	Max.	50.8	24.3	33.3	4.29
	Min.	0.0	18.4	20.0	3.15
	St. dev.	9.9	1.3	3.0	0.25
Hot Season (April, May, June, July)	Number	24	24	24	24
	Mean	35.6	21.1	27.0	3.70
	Max.	58.0	22.5	32.0	4.11
	Min.	16.4	18.5	22.6	2.88
	St. dev.	11.7	0.8	2.5	0.26
All data	Number	54	54	54	54
	Mean	35.6	21.2	27.3	3.79
	Max.	58.0	24.3	33.3	4.29
	Min.	0.0	18.4	20.0	2.88
	St. dev.	10.7	1.1	2.8	0.26

Table 3. Descriptive statistics of the chemical composition and of the nutritive value of the rations sampled.

		DM	NDF	ADF	ADL	Starch	CP	EE	NFC	TDN_m[1]	DMd[2]	NEL_{3m}[3]
Season	**Statistics**	% of DM										Mcal/kg of DM
	n	30	30	30	30	30	30	30	30	30	30	30
Cold Season	Mean	53.9	37.1	22.6	4.1	21.2	14.9	3.1	38.4	65.0	64.4	1.50
(Dec., Jan.,	Max.	70.5	42.6	26.1	5.1	28.0	17.7	4.4	42.6	70.3	69.9	1.64
Feb., Mar.)	Min.	47.3	32.2	18.1	2.6	13.5	11.3	2.2	34.1	61.6	61.9	1.39
	St. dev.	5.6	2.6	1.8	0.5	3.3	1.7	0.5	2.2	2.1	1.8	0.06
	n	24	24	24	24	24	24	24	24	24	24	24
Hot Season	Mean	54.7	38.5	23.1	4.0	19.8	15.1	3.2	35.9	65.0	64.5	1.49
(April, May,	Max.	64.6	43.9	28.0	4.7	27.5	17.5	4.1	41.3	68.5	69.0	1.59
June, July)	Min.	46.0	33.1	16.2	3.2	12.9	13.5	2.3	31.4	59.7	58.1	1.36
	St. dev.	5.1	2.4	2.4	0.4	3.1	1.2	0.5	2.8	2.4	2.1	0.06
	n	54	54	54	54	54	54	54	54	54	54	54
	Mean	54.2	37.7	22.8	4.1	20.6	15.0	3.1	37.3	65.0	64.5	1.49
All data	Max.	70.5	43.9	28.0	5.1	28.0	17.7	4.4	42.6	70.3	69.9	1.64
	Min.	46.0	32.2	16.2	2.6	12.9	11.3	2.2	31.4	59.7	58.1	1.36
	St. dev.	5.4	2.6	2.1	0.5	3.3	1.5	0.5	2.8	2.2	1.9	0.06

[1] = Total digestible nutrient at maintenance; [2] = Apparent digestibility of DM; [3] = Net energy of lactation calculated at 3 times maintenance feeding level (Van Soest and Fox, 1992).

Table 4. Particle size of TMR measured using the Penn State Particle Separator.

			Sieves					
Season	**Statistics**		**Upper**	**Middle**	**Lower**	**Bottom**	**pef**	**peNDF**
			% of as fed ration					
Cold Season (Dec., Jan., Feb., March)	n		30	30	30	30	30	30
	Mean		8.7	37.5	35.2	18.3	81.4	30.2
	Max.		49.1	50.7	46.0	25.4	88.7	36.5
	Min.		2.0	12.5	24.1	9.7	74.4	24.9
	St. dev.		8.2	7.9	6.2	4.0	3.7	2.9
Hot Season (April, May, June, July)	n		24	24	24	24	24	24
	Mean		12.3	35.1	35.9	16.6	83.8	32.3
	Max.		30.9	44.9	44.1	27.0	89.3	38.4
	Min.		6.2	19.5	29.3	11.0	73.0	24.2
	St. dev.		7.3	6.8	4.1	3.6	3.9	2.7
All data	n		54	54	54	54	54	54
	Mean		10.3	36.5	35.5	17.6	82.4	31.1
	Max.		49.1	50.7	46.0	27.0	89.3	38.4
	Min.		2.0	12.5	24.1	9.7	73.0	24.2
	St. dev.		7.9	7.5	5.3	3.9	3.9	3.0

Table 5. Correlations between the studied variables.

	Milk fat %	Season	DMI	Silage in diet	Milk yield	Upper PSPS	Middle PSPS	Lower PSPS	Bottom PSPS	pef PSPS	peNDF PSPS
Season	**-0.33**										
	0.01										
DMI	**-0.02**	**-0.07**									
	0.88	*0.61*									
Silage in diet	**0.30**	**<0.01**	**0.14**								
	0.03	*0.98*	*0.32*								
Milk yield	**-0.08**	**-0.11**	**0.32**	**0.33**							
	0.56	*0.41*	*0.02*	*0.02*							
Upper PSPS	**-0.02**	**0.23**	**-0.32**	**-0.58**	**-0.37**						
	0.91	*0.10*	*0.02*	*<0.001*	*0.01*						
Middle PSPS	**0.19**	**-0.16**	**0.24**	**0.70**	**0.30**	**-0.61**					
	0.16	*0.25*	*0.08*	*<0.001*	*0.03*	*<0.001*					
Lower PSPS	**-0.23**	**0.07**	**0.05**	**0.04**	**0.11**	**-0.40**	**-0.33**				
	0.09	*0.61*	*0.72*	*0.76*	*0.44*	*<0.001*	*0.01*				
Bottom PSPS	**-0.01**	**-0.22**	**0.04**	**-0.24**	**-0.03**	**-0.29**	**-0.29**	**0.17**			
	0.96	*0.12*	*0.76*	*0.08*	*0.83*	*0.03*	*0.03*	*0.23*			
pef PSPS	**0.04**	**0.30**	**-0.11**	**0.23**	**-0.09**	**0.30**	**0.25**	**-0.12**	**-0.92**		
	0.75	*0.03*	*0.42*	*0.10*	*0.54*	*0.03*	*0.07*	*0.41*	*<0.001*		
peNDF PSPS	**0.05**	**0.34**	**-0.03**	**0.27**	**-0.01**	**0.19**	**0.12**	**0.09**	**-0.78**	**0.75**	
	0.72	*0.01*	*0.81*	*0.05*	*0.94*	*0.17*	*0.37*	*0.50*	*<0.001*	*<0.001*	
DM	**-0.55**	**0.07**	**0.03**	**-0.55**	**0.01**	**0.17**	**-0.65**	**0.36**	**0.42**	**-0.43**	**-0.40**
	<0.001	*0.60*	*0.85*	*<0.001*	*0.97*	*0.22*	*<0.001*	*0.01*	*<0.001*	*<0.001*	*<0.001*
NDF	**0.05**	**0.27**	**0.04**	**0.22**	**0.04**	**0.06**	**0.01**	**0.22**	**-0.48**	**0.37**	**0.89**
	0.75	*0.05*	*0.80*	*0.11*	*0.76*	*0.66*	*1.00*	*0.11*	*<0.001*	*0.01*	*<0.001*
ADF	**0.17**	**0.13**	**0.01**	**0.12**	**-0.01**	**0.14**	**-0.05**	**0.06**	**-0.32**	**0.26**	**0.62**
	0.22	*0.35*	*0.95*	*0.40*	*0.97*	*0.30*	*0.72*	*0.67*	*0.02*	*0.06*	*<0.001*
ADL	**0.05**	**-0.18**	**0.21**	**0.23**	**0.11**	**-0.44**	**0.43**	**-0.02**	**0.07**	**-0.11**	**0.14**
	0.71	*0.18*	*0.14*	*0.10*	*0.43*	*<0.001*	*<0.001*	*0.92*	*0.62*	*0.45*	*0.33*
Starch	**-0.18**	**-0.20**	**0.16**	**0.13**	**0.29**	**-0.51**	**0.31**	**0.02**	**0.41**	**-0.39**	**-0.52**
	0.19	*0.14*	*0.24*	*0.37*	*0.04*	*<0.001*	*0.02*	*0.89*	*<0.001*	*<0.001*	*<0.001*
NFC	**-0.08**	**-0.46**	**0.03**	**-0.14**	**0.09**	**-0.16**	**0.15**	**-0.17**	**0.28**	**-0.24**	**-0.62**
	0.56	*<0.001*	*0.83*	*0.33*	*0.53*	*0.24*	*0.28*	*0.23*	*0.04*	*0.08*	*<0.001*
EE	**0.01**	**0.20**	**0.05**	**0.11**	**0.09**	**0.26**	**0.10**	**-0.37**	**-0.20**	**0.24**	**-0.12**
	0.93	*0.16*	*0.75*	*0.44*	*0.51*	*0.06*	*0.46*	*0.01*	*0.15*	*0.08*	*0.40*
CP	**0.16**	**0.08**	**0.03**	**0.11**	**0.02**	**-0.31**	**0.07**	**0.10**	**0.41**	**-0.34**	**-0.39**
	0.26	*0.57*	*0.85*	*0.42*	*0.88*	*0.02*	*0.63*	*0.47*	*<0.001*	*0.01*	*<0.001*
TDN_m	**0.07**	**-0.01**	**-0.03**	**0.02**	**0.14**	**0.20**	**-0.05**	**-0.22**	**0.01**	**0.05**	**-0.39**
	0.60	0.93	0.83	0.87	0.32	0.14	0.75	0.11	0.92	0.74	<0.001

D.appDM	**0.03**	**0.03**	**-0.19**	**-0.27**	**-0.13**	**0.42**	**-0.38**	**-0.12**	**0.09**	**-0.02**	**-0.35**
	0.82	*0.83*	*0.17*	*0.05*	*0.35*	*<0.001*	*<0.001*	*0.38*	*0.52*	*0.88*	*0.01*
NEL_{3m}	**0.05**	**-0.08**	**-0.04**	**-0.04**	**0.11**	**0.16**	**-0.04**	**-0.24**	**0.13**	**-0.05**	**-0.54**
	0.72	*0.58*	*0.80*	*0.79*	*0.45*	*0.26*	*0.78*	*0.08*	*0.35*	*0.70*	*<0.001*

Table 5. Continuation.

	DM	**NDF**	**ADF**	**ADL**	**Starch**	**NFC**	**EE**	**CP**	**TDN_m**	**DMd**
DM										
NDF	**-0.27**									
	0.05									
ADF	**-0.28**	**0.70**								
	0.04	*<0.001*								
ADL	**-0.34**	**0.25**	**0.26**							
	0.01	*0.06*	*0.06*							
Starch	**0.24**	**-0.47**	**-0.61**	**0.18**						
	0.08	*<0.001*	*<0.001*	*0.20*						
NFC	**0.14**	**-0.70**	**-0.66**	**-0.01**	**0.69**					
	0.33	*<0.001*	*<0.001*	*0.94*	*<0.001*					
EE	**-0.09**	**-0.34**	**-0.24**	**-0.36**	**-0.10**	**0.01**				
	0.50	*0.01*	*0.08*	*0.01*	*0.49*	*0.93*				
CP	**0.02**	**-0.31**	**-0.07**	**-0.01**	**-0.11**	**-0.32**	**0.08**			
	0.88	*0.02*	*0.59*	*0.95*	*0.45*	*0.02*	*0.55*			
TDN_m	**0.07**	**-0.57**	**-0.29**	**-0.70**	**0.00**	**0.29**	**0.74**	**0.12**		
	0.64	<0.001	0.03	<0.001	1.00	0.03	<0.001	0.38		
DMd	**0.28**	**-0.47**	**-0.04**	**-0.82**	**-0.22**	**0.08**	**0.38**	**0.18**	**0.80**	
	0.04	*<0.001*	*0.77*	*<0.001*	*0.12*	*0.55*	*<0.001*	*0.18*	*<0.001*	
NEL_{3m}	**0.12**	**-0.73**	**-0.42**	**-0.65**	**0.12**	**0.42**	**0.70**	**0.18**	**0.98**	**0.79**
	0.39	*<0.001*	*<0.001*	*<0.001*	*0.40*	*<0.001*	*<0.001*	*0.19*	*<0.001*	*<0.001*

Table 6. Effect of the dietary DM of TMR on the milk fat content in the two seasons studied. DM reported as classes.

Classes of DM	Cold season	Hot season	Mean
50	4.01±0.1[a]	3.81±0.1	3.91±0.1[A]
52	3.99±0.1[a]	3.78±0.1	3.88±0.1[A]
56	3.81±0.1[a]	3.78±0.1	3.79±0.1[A]
60	3.77±0.2[a]	3.61±0.1	3.69±0.1[AB]
65	3.57±0.1[b]	3.45±0.1	3.51±0.1[B]
Mean	*3.83±0.1**	*3.69±0.1***	*3.79±0.3*

[A,B]=within columns values with different superscripts differ for P<0.05
[a,b]=within columns values with different superscripts differ for P<0.10
[* **] =within rows values with different superscripts differ for P<0.05

Table 7. Summary of results of factor analysis applied to data obtained on studied farms*.Loading coefficients.

	Commu nality	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	*Kaiser MSA*
TDNm, % of DM	0.97	**0.97**	0.16	0.04	0.05	0.02	-0.03	*0.57*
NEL_{3m}, Mcal per kg of DM	0.98	**0.93**	0.30	0.02	0.00	0.11	-0.10	*0.60*
DM_d, % of DM	0.91	**0.86**	-0.11	-0.29	-0.20	0.11	-0.11	*0.51*
EE, % of DM	0.76	**0.73**	0.15	0.30	-0.04	-0.09	0.32	*0.46*
ADL, % of DM	0.78	**-0.77**	0.01	0.38	0.11	0.08	-0.17	*0.53*
Starch, % of DM	0.87	-0.17	**0.84**	0.01	0.33	0.06	-0.14	*0.77*
NFC, % of DM	0.93	0.13	**0.79**	-0.01	0.03	-0.18	-0.50	*0.48*
NDF, % of DM	0.93	-0.48	**-0.68**	0.04	0.17	-0.34	0.29	*0.50*
ADF, % of DM	0.81	-0.18	**-0.87**	0.07	0.04	-0.11	-0.03	*0.48*
Middle PSPS, % of DM	0.92	-0.12	0.18	**0.87**	0.32	0.03	-0.06	*0.42*
Silage in diet, % of DM	0.76	-0.03	-0.10	**0.67**	0.50	0.14	0.14	*0.79*
Lower _screen_PSPS, % of DM	0.69	-0.24	-0.16	**-0.54**	0.48	0.27	0.12	*0.21*
DM, % of as feed	0.83	0.08	0.27	**-0.86**	0.06	0.08	0.07	*0.76*
Milk yield	0.65	0.12	0.05	0.09	**0.78**	-0.07	-0.07	*0.60*
DMI, kg/d of DM per cow	0.34	-0.04	0.04	0.05	**0.58**	-0.01	-0.05	*0.67*
Upper screen_PSPS, , % of DM	0.91	0.33	-0.23	-0.30	**-0.64**	-0.49	0.13	*0.43*
PG, % of DM	0.90	0.16	-0.11	0.10	-0.03	**0.92**	0.12	*0.21*
Bottom screen_PSPS, % of DM	0.78	-0.08	0.36	-0.38	-0.03	**0.65**	-0.29	*0.31*
Season	0.87	0.02	-0.08	-0.08	-0.09	-0.02	**0.92**	*0.66*
Communality**	15.59	27.1%	19.6%	18.2%	13.9%	11.6%	9.7%	
Correlation with milk fat% (r)		0.087	**-0.267**	**0.403**	-0.12	0.135	**-0.326**	
P of correlation		*0.53*	***0.051***	***0.003***	*0.388*	*0.329*	***0.016***	

*estimated Kaiser measure sample adequacy (MSA) was = 0.50; **sum of total communality and explained as % per each factor.

Table 8. Results of the fixed model applied to milk fat.

Variable	Mean of variable		P
Fat type	*Integration of fat in ration from different sources*	1.	NS
Factor 2	*Forage to concentrate ratio*	2.	NS
Factor 3	*Silage effect*	Factor 3 <0 MilkFat = 3.70±0.05 Factor 3 >0 MilkFat = 3.85±0.04	0.037
Factor 6	*Season*	Factor 3 <0 MilkFat = 3.87±0.04 Factor 3 >0 MilkFat = 3.68±0.05	0.006

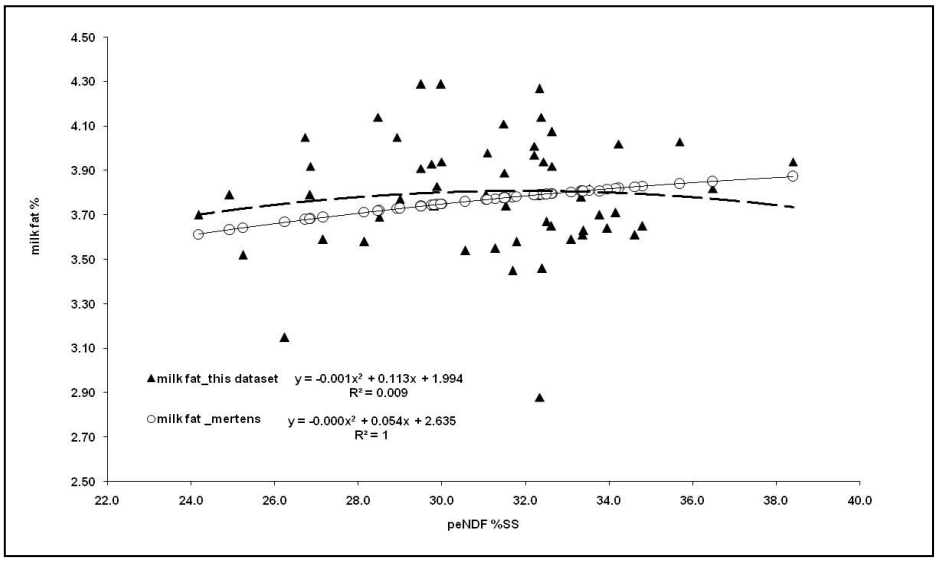

Figure 1. Milk fat versus ration peNDF as observed in the dataset and predicted with the equation of Mertens (1997); predicted range of milk fat: from 3.61 to 3.87 %, observed range of milk fat from 2.88 to 4.29.

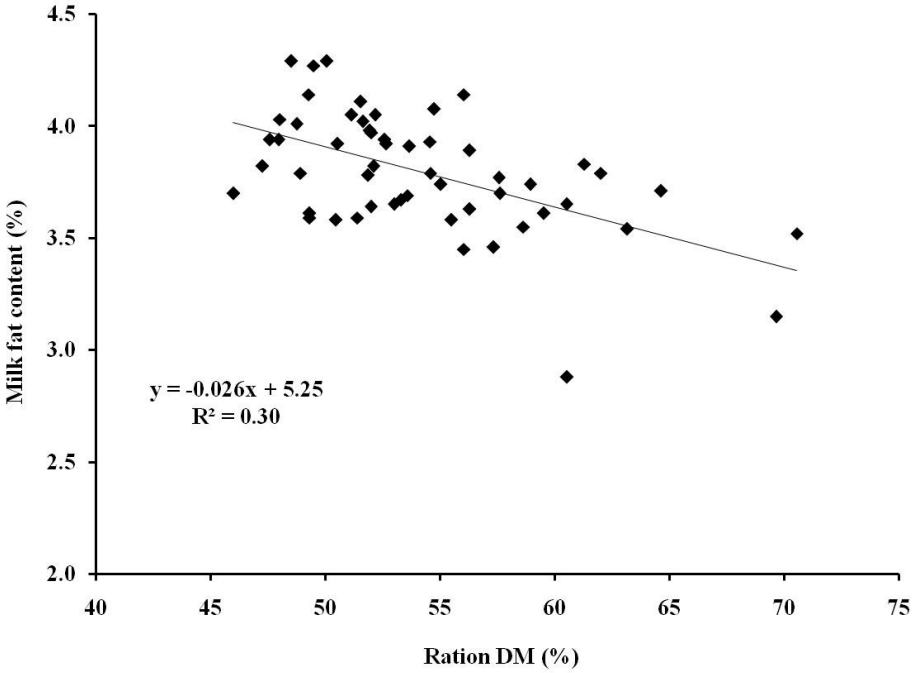

Figure 2. Milk fat content versus ration DM using all data of studied farms**.**

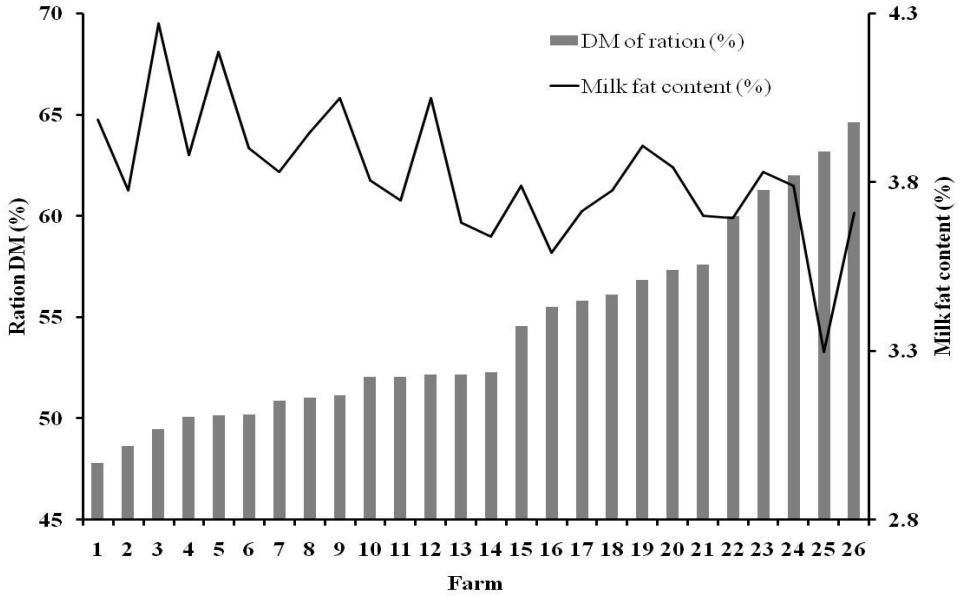

Figure 3. Mean of milk fat and ration DM per studied farm. The axis scale was set on the maximum and minimum values of the dataset.

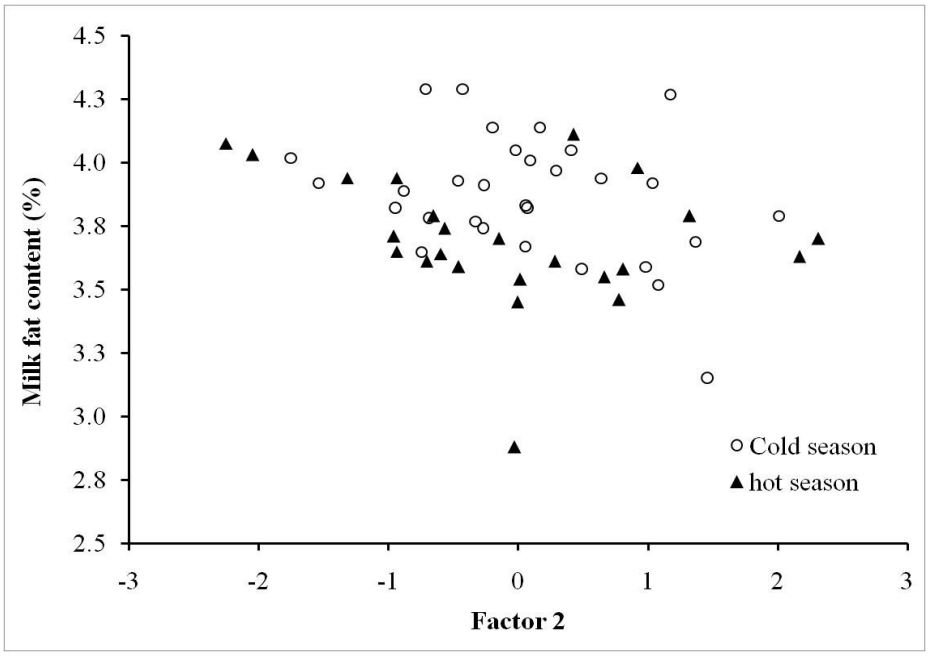

Figure 4. Factor Analysis. Milk fat content against Factor 2.

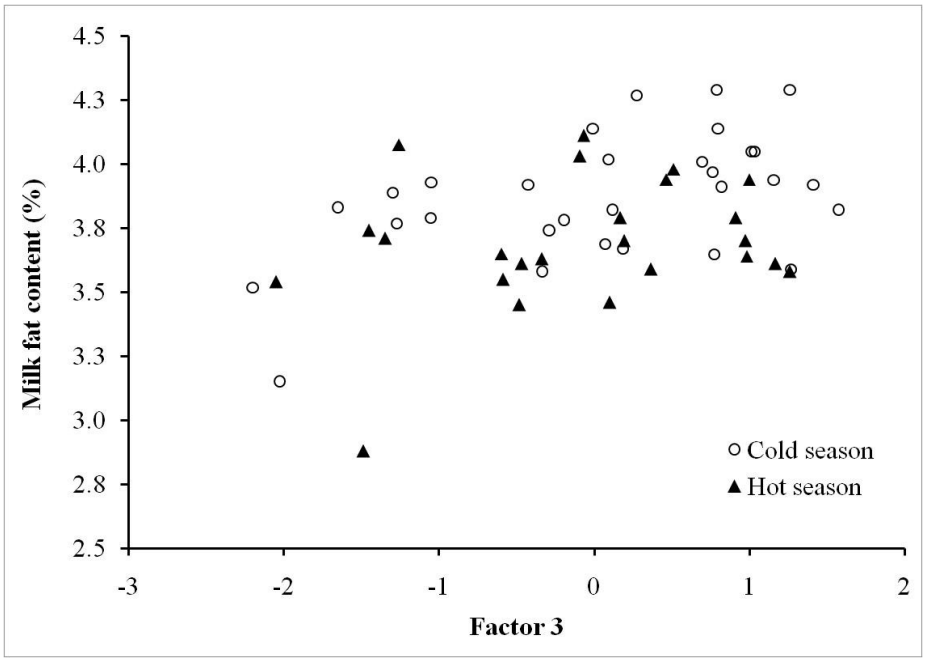

Figure 5. Factor Analysis. Milk fat content against Factor 3.

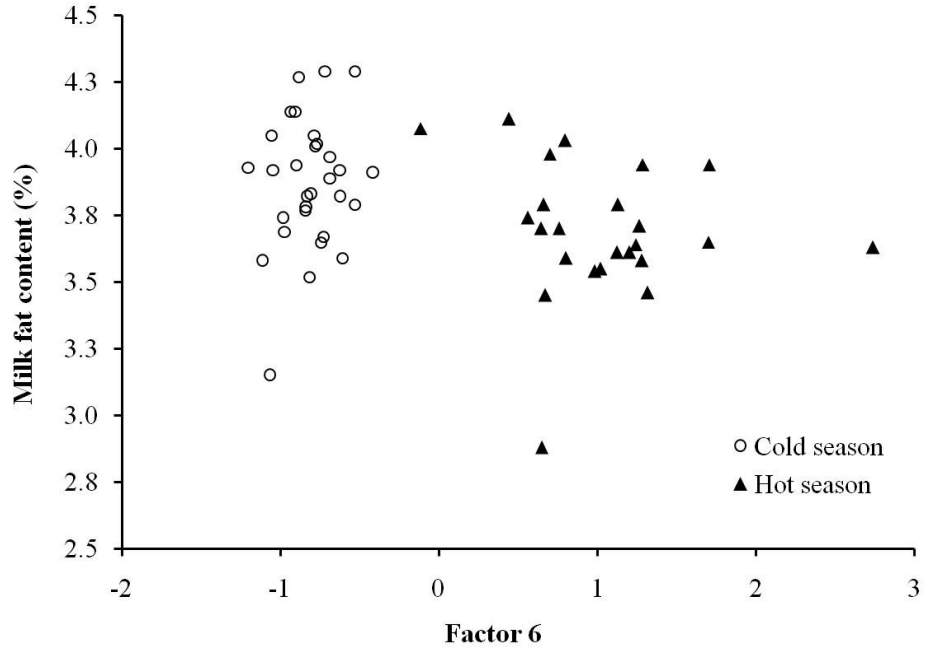

Figure 6. Factor Analysis. Milk fat content against Factor 6.

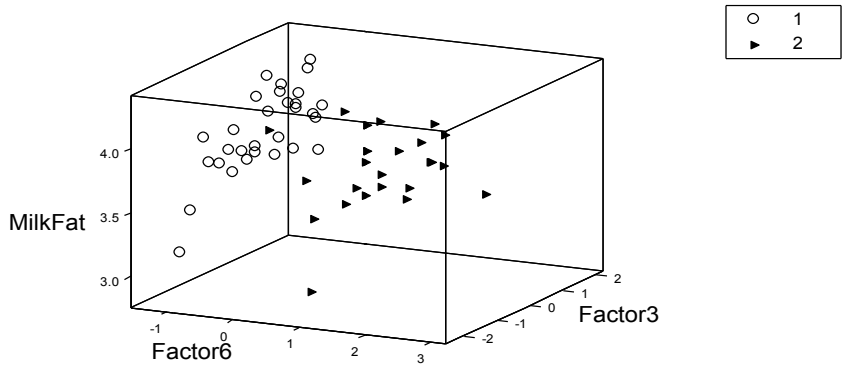

Figure 7. Factor Analysis. Milk fat content against Factor 3 and Factor 6 in a 3D plot where white circle (O) indicate cold season and black triangle (▲) indicate hot season.