**REVIEW: Methods to measure forage and diet particle size in the dairy cow**

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**ABSTRACT**

Dairy cows require adequate fiber for proper rumen function and production of milk, fat, and protein. However, cattle not only have a chemical fiber requirement but also a physical fiber requirement. An important consideration regarding forage particle size is the method used to measure particle size distribution. Many systems currently exist to measure particle size, and even more methods exist to use particle size data to calculate physically effective fiber in rations. However, because there is not a standard method for the dairy industry or dairy researchers, several different systems are currently being used, and their data are sometimes used interchangeably though their results may not be comparable. Many of the systems attempting to estimate physically effective fiber are based on the theory that there is a critical size threshold for particles leaving the rumen, and that particles above this threshold are effective because they stimulate chewing to promote particle size reduction and rumen escape. Various methods used to measure and interpret forage and diet particle size in dairy diets will be discussed.

**Key words:** particle size, dairy cow, particle separation method

**INTRODUCTION**

Forage and TMR particle size has many implications for the health and production of the dairy cow. Adequate forage particle size is necessary to maintain cow and rumen health by buffering rumen pH. In addition, varying forage particle size can influence DMI and DM digestibility (Stone, 2004). It is generally accepted that increasing forage particle size decreases DMI due to gut fill (Kononoff and Heinrichs, 2003b; Leonardi et al., 2005; Maulfair et al., 2010); however, some studies have observed no effect of forage particle size on DMI (Krause et al., 2002; Kononoff and Heinrichs, 2003a; Beauchemin and Yang, 2005; Yang and Beauchemin, 2007). There is also inconsistency in the literature regarding effect of forage particle size on DM digestibility. Increased DM digestibility (Kononoff and Heinrichs, 2003a; Yang and Beauchemin, 2005), decreased DM digestibility (Kononoff and Heinrichs, 2003b; Maulfair et al., 2011), and no effect on DM digestibility (Krause et al., 2002; Yang and Beauchemin, 2006a; Yang and Beauchemin, 2007) have all been reported. These discrepancies can be attributed to complex interactions between forage particle size, forage type, diet composition, and digestibility of individual ration components.

In addition, method of measuring forage and TMR particle size is often confounded (Maulfair et al., 2010), and differing systems of measuring and analyzing forage and diet particle size are used by different researchers (Lammers et al., 1996; Mertens, 1997; Kononoff et al., 2003; Yang and Beauchemin, 2006b). This makes for confusion in interpreting data and making conclusions when results from various researchers, analyzed with different systems, are combined in one analysis (Zebeli et al., 2006, 2010). Nutritionists who use particle size analysis with on-farm ration evaluation as well as those interpreting research publications may benefit from better understanding the different methods of sieving and the results produced by each. This review will focus on defining the different methods currently being used for diet and forage particle analysis and will note differences and similarities between methods. The objectives are to familiarize readers with the different systems and to improve understanding of the recommendations made using each system.
REVIEW AND DISCUSSION

Mertens (1997) described physically effective NDF (peNDF) as the ability of a feed to stimulate chewing and maintain the rumen mat. Although this measure is commonly used in the field, the particle separation method that best measures ration peNDF is not well defined. There currently seems to be no universal standard TMR and forage particle separating technique for determining peNDF. Problems can be created by using peNDF values determined with different sieving methods interchangeably. Several of the most popular particle separation methods in the United States (as well as many other countries) will be discussed in more detail.

Penn State Particle Separator

The Penn State Particle Separator (PSPS) has become a standard particle separation technique used in the dairy cattle nutrition industry. The PSPS is a manually operated particle separator that separates as-fed forage and TMR samples via horizontal shaking. Lammers et al. (1996) first developed the PSPS as an easy to use, practical, on-farm tool to mimic Standard S424.1 of the American Society of Agricultural and Biological Engineers (ASABE), which is the standard method of determining particle size distribution of chopped forages. The first PSPS consisted of 3 particle fractions: >19.0, >8.0, and <8.0 mm. The PSPS was later improved upon by Kononoff et al. (2003) by adding a 1.18-mm screen to allow for more accurate characterization of TMR and forages that have a large portion of particles <8.0 mm. The top 2 screens have circular holes, and the screen depth is varied (12.2 and 6.4 mm for the top and middle screens, respectively) to provide a 3-dimensional barrier to prevent particles larger than the hole sizes from falling through (Lammers et al., 1996). The bottom sieve is composed of a stainless steel wire cloth that has a nominal screen size of 1.18 mm and a diagonal screen size of 1.67 mm (Kononoff et al., 2003). Recommended sample size for the PSPS is 1.4 L or 25% of the ASABE standard sample size because the PSPS has approximately one-fourth of the surface area of the ASABE separator (Lammers et al., 1996). The recommended shaking procedure includes placing the PSPS on a flat surface, shaking the separator horizontally 5 times at 1.1 Hz with a stroke length of 17 cm (Kononoff et al., 2003), then rotating the separator a quarter turn and repeating these steps. A total of 8 sets of 5 shakes should be completed for a total of 40 shakes in 2 full turns (Lammers et al., 1996). Lammers et al. (1996) determined that there was no difference in the results of the PSPS and the ASABE separator in predicting fractions of particles <19.0 and <8.0 mm in 21 of 36 statistical tests. Several studies have used particles retained on the 1.18-mm sieve of the PSPS to determine peNDF of TMR (Yang and Beauchemin, 2006b; Yang and Beauchemin, 2007; Bhandari et al., 2008). Also, studies have been conducted that used the 8-mm screen of the PSPS to determine peNDF (Calberry et al., 2003; Plaizier, 2004; Yang and Beauchemin, 2005; DeVries et al., 2007). However, the PSPS uses a different particle separation technique from the one specified by Mertens’ (1997) peNDF procedure. In addition, it should be noted that when using the 1.18-mm sieve in the PSPS to measure peNDF there may be no significant differences in peNDF of TMR found, even though there are significant differences in particle size distribution (Yang and Beauchemin, 2006b; Yang and Beauchemin, 2007; Bhandari et al., 2008) and even cow response (Yang and Beauchemin, 2006b). This shows a lack of sensitivity when using the PSPS technique to measure peNDF, likely because when forage chop lengths are varied, most of the differences in particle distribution of the TMR are in particles above this sieve.

Advantages of the PSPS are its portability, low cost (approximately $300; Nasco, Fort Atkinson, WI), ease of use, quick results, use of as-fed samples, and good repeatability. For these reasons it has become popular with dairy farmers and nutritionists worldwide. The PSPS can easily be used in a field or barn whenever it is needed without the need for time-consuming drying of samples. Some disadvantages of the PSPS are that it determines fewer particle fractions than other methods and requires manual operation. Anytime a procedure requires manual manipulation it induces a certain amount of human error; however, the ability to rest the PSPS on a smooth, steady surface effectively limits human error (Kononoff et al., 2003). In addition, Kononoff et al. (2003) determined that moisture content of samples and shaking frequency affected particle size distribution and mean particle size measured with the PSPS. Small losses of moisture caused only minor changes in particle size distributions, whereas complete drying caused large differences by increasing the amount of particles passing through each sieve (Kononoff et al., 2003). Therefore, it is important to standardize the shaking procedure and consider the effects on moisture when utilizing the PSPS.

ASABE Particle Separator

The ASABE or “Wisconsin” separator is the standard method for determination of particle size distribution of chopped forages (S424.1; ASABE, 2007). It is a very large (>225 kg) particle separator that is mechanically operated and uses a horizontal shaking motion. The ASABE separator consists of a pan and 5 square-hole screens with sizes of 19.0, 12.7, 6.3, 3.96, and 1.17 mm when measured nominally or 26.9, 18.0, 8.98, 5.61, and 1.65 mm when measured diagonally; each screen has a frame of 565 × 406 × 63.5 mm (length × width × depth; ASABE, 2007). All of the screens are made of aluminum of varying thickness, increasing with increasing screen size, except the smallest screen, which is wire mesh. The thicknesses of the screens from top to bottom are 12.7, 9.6, 4.8, 3.1, and 0.64 mm (ASABE, 2007).
The recommended procedure for the ASABE separator is to use a sample size of 9 to 10 L of uncompressed forage, but samples as small as 2 to 3 L can be used if extra care is taken to recover particles from the screens and operate the shaker for 2 min (ASABE, 2007). Several advantages of this separator are that it is mechanically operated, has a moderate number of particle fractions, uses as-fed samples, and has screens with more surface area (longer and wider) than the PSPS. These advantages help to reduce human error, more accurately describe particle distribution, eliminate the need for sample drying, and allow for better separation of extremely long particles, respectively. Maulfair et al. (2010) found that when using rations of extremely long particle size, the PSPS did not adequately separate the particles. Extremely long (>5 cm) hay particles bound together and did not allow particles to fall through the top screen when shaken with the PSPS. The larger screens and more vigorous shaking of the ASABE separator allowed enough movement of the longest particles for the smaller particles to fall through the screens (Maulfair et al., 2010). This situation would not be realized very often in a field setting because these diets were very extreme.

The disadvantage of the ASABE separator is that it is the least portable of all separators; it is very heavy, large (102 × 64 × 145 cm; length × width × height), and requires electricity to operate. Plans can be purchased from ASABE and systems must be custom manufactured. The results of the ASABE particle separator are also susceptible to variation with sample moisture content (ASABE, 2007). The disadvantages of this particle separator strictly limit its use to laboratory use.

**Ro-Tap Particle Separator**

The Ro-Tap particle separator (RTPS; W.S. Tyler, Mentor, OH) uses a dried sample that is placed on a series of stacked sieves (same sieves used in wet sieving) and shaken horizontally while simultaneously a metal arm repeatedly taps the top of the sieve stack (holds 8 to 16 depending on sieve height) to incorporate a vertical shaking element as well. This shaking system could probably be considered obsolete, except it was used for much of Mertens’ research. Mertens (1997) developed the concept of peNDF and used the RTPS to develop the laboratory assessment of peNDF, where the particles retained on a 1.18-mm sieve after shaking are multiplied by the sample NDF content. The Mertens (2005) RTPS procedure specifies a sample size of 0.6 L, sieve sizes of 19.0, 13.2, 9.5, 6.7, 4.75, 3.35, 2.36, 1.18, 0.60, and 0.30 mm, and a 10-min operation time. A major factor that creates a difference between the RTPS and many other methods is that vertical shaking tends to separate particles by their minimum cross-sectional dimension (usually width in forage particles), whereas horizontal shaking tends to separate particles by their length (Mertens, 1997, 2005). This difference is amplified by the fact that the RTPS uses wire screens that have a minimum screen thickness versus the large thickness of the PSPS and ASABE separator screens.

Because the RTPS uses vertical shaking and dried samples, it produces results that can be very different from techniques (PSPS and ASABE separator) that use horizontal shaking and as-fed samples. Which shaking technique is optimal may depend on the samples being separated and the hypothesis being tested. For instance, separating particles based on their smallest diameter may be more similar to how particles attempt to leave the rumen. The other divergence of the RTPS from most other techniques is that samples are dried before they are separated. Drying forage samples makes particles smaller and more fragile, making them more likely to break during the separating process; both of these factors can artificially decrease the resulting particle size distributions (Kononoff et al., 2003). Drying samples also makes this technique more time consuming because samples are usually dried for at least 24 h (Mertens, 2005). Other disadvantages of the RTPS are that it is not very portable, is expensive ($2,300 to $2,500 plus the cost of sieves; Thermo Fisher Scientific, Waltham, MA), requires electricity, and is extremely loud to operate. Some advantages of the RTPS are that it is mechanically operated, many screens can be used (up to 8 or 16 depending on sieve height), and the screen sizes can be customized for the intended use. The RTPS is used for research and by commercial forage testing labs.

**Z-Box Particle Separator**

The Z-Box particle separator was recently developed at the William H. Miner Agricultural Research Institute (Chazy, NY) and was specifically designed to measure the physical effectiveness factor of as-fed forage and TMR samples. The Z-Box was also designed to be highly correlated with the proportion of particles retained above a 1.18-mm sieve when separated via the RTPS. The research and development of this separator involved testing various screen sizes (1.14, 2.38, 3.18, 4.76, and 9.53 mm), shaking motions (horizontal and vertical), and sample sizes (50 and 100 g; Cotanch and Grant, 2006). Samples of corn silage, hay crop silage, and TMR that varied in physical effectiveness factor were separated using the various combinations, and the results were compared with the RTPS. Cotanch and Grant (2006) determined that vertical shaking of 50-g samples correlated best with the RTPS particle fraction >1.18 mm and that the best screen size varied with the type of samples sieved. They suggested that a 3.18-mm screen should be used for corn silage and TMR and a 4.76-mm screen should be used for hay crop silage.

The Z-Box is a handheld plastic box (21 × 21 × 11 cm, length × width × height) that has a removable screen. Cotanch and Grant (2006) recommended the following procedure for Z-Box use: place 50-g sample in box and record weight, insert appropriate
Wet sieving for having to change screens). Chazy, NY), and ease of use (except for having to change screens).

**Wet Sieving**

There are 2 types of wet sieving reported in the literature. The first type consisted of a series of stacked sieves being completely submerged in a vat of water and moving vertically in the water for a period of time. This type of wet sieving was used by Poppi et al. (1980, 1981, 1985) when 1.18 mm was first suggested as the critical particle size for particles leaving the rumen of cattle and sheep. This type of sieving seemingly has not been used for several decades and would likely be considered obsolete. The other method of wet sieving is the type of procedure used by Beauchemin et al. (1997) and improved upon by Maulfair and Heinrichs (2010). In this procedure a series of stacked sieves of decreasing size have water sprayed onto the top screen and in the middle of the sieve stack. While the water is being sprayed onto the samples in the sieve stack, the entire stack is vibrated via vertical oscillation. The bottom pan in the sieve stack is drained to allow water and soluble matter to flow out. Soluble DM (DM that passes through the smallest sieve) can be determined by calculating the DM lost during the sieving process (Maulfair and Heinrichs, 2010). Six different sieve sizes of the many available sizes can be used at once (up to 12 if half-size sieves are used) and the sizes can be customized to suit the intended uses of the separation.

This technique lends itself very well to separating samples that have high moisture contents (rumen digesta and fecal samples) because these samples will not separate well using other techniques without drying, and drying can change the physical properties of samples. Wet sieving is valuable for research because it probably most accurately mimics conditions in the rumen as particles pass through the omasal canal. Particles in the rumen are completely water saturated and suspended in fluid when they pass though the omasal canal, and this is the only particle separating method that closely resembles this action. However, there are many disadvantages to using this method. The procedure is very time consuming; even with the modifications to increase processing time made by Maulfair and Heinrichs (2010), at least 30 min is required to process a single sample. Wet sieving equipment is expensive ($2,900 to $3,500 plus the cost of sieves; Thermo Fisher Scientific, Waltham, MA), not easily portable, and needs running water and electricity to operate. The characteristics of this method make it very valuable for research but impractical for field use.

Because peNDF is described as the ability of a feed to stimulate chewing and maintain the rumen mat (Mertens, 1997), the best separator should be the one that best correlates to chewing activity. An as-fed sample may correlate better to chewing because that is the form feed is in when presented to the cow. Horizontal separation may correlate better to chewing because it separates on longest diameter (Mertens, 1997, 2005), and the cow likely chews until the longest diameter of forage particles is below a certain size. Additionally, repeatability of the separator is extremely important, and portability, ease of use, and cost must also be considered if the separator is to be accepted for field use.

**Implications**

There is no single forage and TMR separator that is best for all uses. The type of sample being used and the hypothesis being questioned influence which particle separator to use. Wet sieving is most likely the best technique when studying particles passing out of the rumen. Rumen digesta and fecal samples can be separated without changing their physical conformation. The separating action of wet sieving also more closely mimics actions that occur in the rumen: separating on smallest diameter while suspended in fluid. The particle separator that best measures ration peNDF is not as easy to define. A reasonable particle separator for estimating peNDF may be the PSPS because of its widespread use at this point (Zebeli et al., 2006, 2010), but more research is needed to find the sieve size or combination of sieve sizes that will best correlate to animal parameters of importance.

**Literature Cited**


