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Evaluation of an on-farm fecal particle size separator

Diplomarbeit

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1. Abstract

This study aims to validate an on-farm fecal particle size separator by comparing its performance against a laboratory method as the reference standard. Additionally, the device's applicability across different age groups (calves and adult cows) is evaluated.

Fecal samples of 150 g each from calves and cows are dissolved in water and sieved through the on-farm device under water flow. Similarly, 15 g of the same samples are analyzed in the laboratory using an automatic sieve shaker. In both methods, the retained material is assessed.

The analysis revealed a higher average percentage of retained material with the on-farm device. This is likely due to human error during operation: the on-farm method lacks a shaking component in order to remove access water, and the sieving duration is subjectively determined. Beyond human factors, the composition of the feces appears to influence the results. The overall better alignment between both methods in calves may be attributed to the underdeveloped gastrointestinal tract and differences in diet, which include milk feeding. In adult animals with developed rumination behavior and ingestion of larger particles, the increased number of microorganisms and soluble particles contained in the access water seem to cluster, thereby increasing the retained percentage in the on-farm method.

Despite the observed differences, the development of standardized guidelines for implementation could make the device a cost-effective on-site tool for evaluating and optimizing calf feeding.

Das Ziel dieser Studie ist einerseits die Validierung dreier Siebe zur Bestimmung der Kotpartikelgrößenverteilung. Die Ergebnisse werden mit einer Labormethode als Referenzstandard verglichen. Andererseits wird die Anwendbarkeit dieses On-farm-Geräts bei verschiedenen Altersgruppen (Kälber und adulte Kühe) bewertet.

Kotproben zu je 150 g von Kälbern und Kühen wurden in Wasser gelöst und unter Wasserfluss durch das On-farm-Gerät gesiebt. Im Labor wurden 15 g derselben Proben mithilfe eines automatischen Siebschüttlers analysiert. In beiden Methoden wurde das in den Sieben zurückgehaltene Material bewertet.

Die Analyse ergab einen höheren durchschnittlichen Prozentsatz an zurückgehaltenem Material bei der On-farm-Methode. Dies ist wahrscheinlich auf Fehler in der Durchführung zurückzuführen: Das überschüssige Wasser wird bei der On-farm-Methode nach der Spülung nicht durch Schütteln entfernt und die Siebdauer wird subjektiv bestimmt. Neben menschlichen Faktoren scheint die Zusammensetzung des Kotes die Ergebnisse zu beeinflussen. Die insgesamt bessere Übereinstimmung zwischen beiden Methoden bei Kälbern könnte auf den Entwicklungsstand des Verdauungssystem sowie Unterschiede in der Fütterung zurückzuführen sein. Bei adulten Tieren mit vollständig entwickeltem Wiederkauverhalten und der Aufnahme größerer Futterpartikel scheint sich die Menge an Mikroorganismen und löslichen Partikeln im Kot zu erhöhen. Diese löslichen Teilchen aggregieren möglicherweise im überschüssigen Wasser und können bei der On-farm-Methode nicht effektiv entfernt werden.

Trotz der Unterschiede zwischen beiden Methoden könnte die Entwicklung standardisierter Richtlinien für die Durchführung und Interpretation der Ergebnisse das Gerät zu einem kostengünstigen, vor Ort einsetzbaren Werkzeug zur Bewertung und Optimierung der Kälberfütterung machen.

2. List of abbreviations

ADG – average daily gain

AFR – age of first rumination

ASABE – American Society of Agricultural and Biological Engineers

BW – bodyweight

DIM – days in milk

DM – dry matter

DMI – dry matter intake

FPT – failure of passive transfer

peNDF – physically effective neutral detergent fiber

PL – particle length

PSPS – Penn State Particle Separator

PW – particle width

RTPS – Ro Tap Particle Separator

SARA – subacute rumen acidosis

SCFA – short chain fatty acids

STE – steviol glycosides

TMR – total mixed ration

TPI – transfer of passive immunity

VFA – volatile fatty acids

3. Introduction

3.1. Rumen development

3.1.1. Structure and function of the rumen

In adult animals, the rumen fills the entire left side of the abdominal cavity, extending from the diaphragm to the pelvis. The *sulcus longitudinalis dexter* and *sulcus longitudinalis sinister* segment the rumen into a *dorsal* sac and a *ventral* sac. The less pronounced *sulcus coronarius dorsalis* and *sulcus coronarius ventralis* separate the *caudal* blind sac from the *dorsal* and *ventral* sacs, respectively. The rumen mucosa forms papillae (*papillae ruminis*), which increase the surface area of the rumen approximately sevenfold (1).

This epithelium consists of four layers: *stratum basale*, *stratum spinosum*, *stratum granulosum*, and *stratum corneum*. The innermost layer, the *stratum basale*, is crucial for metabolic functions such as ketogenesis due to its large number of functional mitochondria, where the ketogenic enzymes are located (2). The *stratum spinosum* and *granulosum* merge without a distinct boundary and form tight gap junctions or desmosomes. This barrier preserves the integrity of metabolite concentration gradients (3). The *stratum corneum* is in direct contact with the rumen contents. It is cornified and acts as a protective barrier against the ruminal environment. Consequently, the number of cell layers is related to the diet. With a high proportion of concentrates and a lower pH, the *stratum corneum* thickens, whereas it becomes thinner with a higher proportion of roughage (4). The rumen microbes ferment ingested carbohydrates into short-chain fatty acids (SCFAs), which are then absorbed through the mucosal papillae.

3.1.2. Development of the rumen from birth to weaning

At the time of birth, the rumen is not fully developed in terms of its mass, papillae, or ketogenic capacity (5). As a result of solid feed intake and rumen fermentation, both the rumen muscle mass and the epithelium in the form of papillae grow.

Attempts have been made to identify the specific stimuli responsible for this anatomical development. To simulate the physical stimulation of feed, nylon bristles (6) and plastic sponges (7) were inserted into the rumen, but this did not result in significant papillae growth (8). Instead, the development appears to be influenced by a range of factors, such as the type and processing of solid feed and the resulting volatile fatty acids (VFA) ratio, the rumen microbiome, hormonal influences, and the timing and duration of weaning (5). The VFAs

propionate and butyrate promote the growth of the rumen epithelium and the development of papillae. These VFAs are produced in greater quantities when feeding easily fermentable carbohydrates in the form of a concentrate diet (i.e. “calf starter”) (5). However, the lack of fiber, along with reduced chewing activity and buffering, can lower rumen pH and negatively affect the bacterial composition. Therefore, feeding for example a total mixed ration (TMR) with hay or corn silage appears to have positive effects on rumen development. The growth of cellulolytic bacteria is promoted, and the inclusion of long fibers increases chewing activity, thereby stabilizing rumen pH (9). But also with exclusive starter feeding, the effects on rumen development may vary depending on the composition. An experiment involving a grain-based starter compares different processing methods of corn. The more easily digestible steam-flaked corn starter supported the development of the epithelium and papillae despite a slight reduction in pH, compared to the slower fermenting ground corn. The improved growth parameters may also be linked to better phosphorus utilization with the steam-flaked corn starter (10).

To promote the development of a healthy microbiome during weaning, additives can be incorporated. The addition of probiotics (e.g. *Megasphaera elsdenii*) during the preweaning period resulted in greater empty rumen weight and enhanced papillae growth. This improved rumen development is likely attributed to the higher solid feed dry matter intake (DMI) observed in the supplemented calves (11). To enhance feed intake in calves during the weaning period, the inclusion of steviol glycosides (STE) could also be considered. This zero-calorie sweetener derived from the stevia plant can increase feed intake as well as fermentation efficiency by influencing the composition of the microbiome (12).

The onset of rumination is closely associated with the anatomical and functional development of the rumen. Wang et al. (2022) reported that the earliest age of first rumination was nine days, while the latest was 28 days. The 56 Holstein calves were fed up to 9 L of milk per day and ad libitum concentrate starter feed. They reported that the age of first rumination (AFR) was positively correlated with both, the age at which the calves first consumed straw and the total intake of starter feed before the onset of rumination (13). The onset of rumination can vary with different feeding regimens. Xiao et al. (2024) demonstrated an earlier onset in calves fed hay and starter (13.1 days) compared to calves receiving only starter (14.4 days). Post-weaning, the rumination time was significantly higher in the hay-fed group (275.5 min/d) compared to the starter-only group (230.3 min/d) (14). The timing of forage inclusion also impacts overall development and calf growth: Research showed early hay consumers (5 days)

demonstrated a lower average daily gain (ADG) and final body weight (BW), despite an earlier onset of rumination, compared to late hay consumers (12 days) (15). In addition to the timing of initial hay intake, the quality of the hay also influences rumination duration. Calves fed high-quality hay consumed more physically effective neutral detergent fiber (peNDF) and exhibited a longer rumination duration (591 min/d) compared to those fed medium-quality hay (539 min/d) or concentrate-rich diets (430 min/d) (16).

3.1.3. Calf diet from birth to weaning

Feeding practices in calves and their management are critical factors for optimizing digestive tract development, thereby influencing growth rates, immune function, and performance as adult animals (17).

Since there is no in utero transfer of immunoglobulins across the placenta, calves must receive colostrum to acquire passive immunity (18). In cases of failure of passive transfer (FPT) of immunity and insufficient serum IgG levels, there is an increased susceptibility to infections (19). Fischer et al. (2018) stated that early colostrum feeding within the first hour significantly increased peak serum IgG concentrations and absorption efficiency compared to feeding at 6 or 12 hours. Calves fed immediately after birth also showed enhanced colonization of beneficial gut bacteria (*Bifidobacterium* and *Lactobacillus*), supporting immunity and gut health (20). In addition to timing, both the quantity and quality of colostrum are critical factors influencing the transfer of passive immunity (TPI). Morin et al. (2021) showed feeding ≥2.5 L of colostrum at the first meal increased the odds of adequate TPI by 2.6 times. In terms of quality, a Brix score of ≥24.5% was identified as the optimal threshold for adequate TPI, surpassing the traditional ≥22% Brix threshold used to identify high IgG colostrum (21). In contrast to temperature-treated colostrum, dam-sourced colostrum allows for the absorption of leukocytes, thereby promoting a faster and more effective development of the immune system (22,23).

Subsequently, feeding is often transitioned directly to whole milk or milk replacer either ad libitum or restricted. However, Van Soest et al. (2022) recommend administering transition milk. In their study, the milk from the first milkings after colostrum contained higher levels of fat, protein, and IgG compared to milk replacer. The intestinal section revealed increased villus growth, thicker submucosa in the jejunum, and a larger mucosal surface area in the calves fed

transition milk compared to the control group receiving milk replacer (24). Commonly, waste milk from cows undergoing antibiotic treatment is fed to calves, contributing to the development of antibiotic resistance. Diniz Neto et al. (2024) observed a higher prevalence of antibiotic-resistant *E. coli* in calves fed waste milk or pasteurized waste milk (resistance to 5 out of 7 and 4 out of 7 tested antibiotics, respectively).

In contrast to exclusive milk feeding, alternative strategies include solid feed. The literature shows inconsistencies regarding the inclusion of forage, starter concentrate, or a combination of both (25). Several studies reported better weight gains with forage inclusion, which some authors attribute to slower digestion and increased gut fill (26,27). Regarding rumen development, offering concentrates to calves is often described as a more effective stimulus (28). Forage fermentation in the rumen primarily produces acetate, whereas fermentation of starter concentrate tends to produce butyrate and propionate, which more effectively induces papillae growth (29).

To properly assess the effects of forage feeding, factors such as the type, level, particle size and physical form of the forage, whether it is provided in combination with starter feed, and the amount of milk fed must also be considered. Xiao et al. (2023) determined the optimal age for the incorporation of forage to maximize rumen development and average daily weight gain. While calves in one group received only milk and calf starter, those in the other group were additionally offered oat hay and were categorized into early, middle, or late groups based on the timing of their first hay consumption. There was a trend toward significant differences BW and ADG, within the first group (receiving only milk and calf starter) and the late hay consumers showing higher values than the early hay consumers. However, all groups demonstrated similar rumination times and rumen fermentation (15). Zhang et al. (2024) compared three feeding regimens: on one hand, milk and pelleted starter concentrates, and on the other hand, either additional oat hay or alfalfa hay. No significant differences in BW and ADG were found between the feeding regimes. However, concentrate feeding was associated with a higher total VFA concentration and a lower rumen pH compared to oat and alfalfa hay administration. Rumen acidosis resulting from excessively low pH levels could potentially be mitigated through alternative feeding strategies (30). The study also aimed to identify the long-term effects of different feeding regimes, revealing that the daily milk production from 6 to 200 days in milk (DIM) was highest in the group fed alfalfa hay (31).

3.2. Feed sieving

Feed sieving is conducted in various studies to evaluate the effects of different rations on feed intake, digestive efficiency and performance (32–39). The aim is often to determine peNDF, which stimulates chewing activity and contributes to the maintenance of the rumen mat (40). Methods used in scientific literature can be grouped into laboratory and on-farm procedures.

3.2.1. Laboratory methods

The standard laboratory method for determining forage particle size is the ASABE (American Society of Agricultural and Biological Engineers; PA, USA) separator, that divides the particles through horizontal shaking. It consists of five square-hole screens with a specific thickness to create a three-dimensional barrier that prevents particles larger than the hole sizes from passing through. Ideally, 9 to 10 L of as-fed uncompressed forage are used per sieving. An advantage is that the sample requires no pretreatment, and the device is operated mechanically. However, the apparatus is large and heavy, and the results may vary with the moisture content of the forage (41).

In addition, there is the option of wet sieving. A stack of sieves is mechanically shaken using vertical oscillation, while water is sprayed onto the sample from above. The amount and mesh size of the sieves can be varied according to the needs. The water and soluble dry matter (DM) can escape through the drain from the bottom pan. Despite its broad range of applications, there are disadvantages such as the cost, the duration of the procedure, and the need for water and electricity (32,41).

The Ro-Tap Particle Separator (RTPS; RO-TAP® RX-29 Mechanical Sieve Shaker, W.S. Tyler, Mentor, OH) represents another laboratory device, which combines horizontal and vertical shaking through a metal arm that repeatedly taps on the top. In contrast to the other two methods, Mertens (2005) used dried samples in the RTPS. The drying process is time consuming and can distort particle size distribution by making the pieces smaller and more fragile (42). Differences may also occur due to vertical shaking, where particles are sorted by width rather than length (43).

3.2.2. On-farm methods

For on-farm feed sieving, the Penn State Particle Separator (PSPS; PA, USA) is most commonly used (37–39,44). Lammers et al. (1996) compared the PSPS with the ASABE's laboratory method. The aim was to find a practical on-site alternative to the complex laboratory device. The PSPS consists of two sieves of varying thickness with mesh sizes of 19 and 8 mm and a bottom pan, while the ASABE method uses a laboratory-scale separator with five square-hole screens with sizes of 19.0, 12.7, 6.3, 3.96, and 1.17 mm (measured nominally) and a bottom pan. The amount of feedstuff (haycrop silage, corn silage and TMR) used is based on the ASABE procedure, and detailed instructions for manual sieving with the PSPS ensure the method is to the highest degree of standardization. The comparison between the laboratory and on-farm method using a paired t-test revealed no difference in determining particle fractions less than 19 mm or 8 mm in 21 out of 36 tests (45).

Kononoff et al. (2003) modified the on-farm procedure and assessed the effect of an additional sieve with a 1.18 mm mesh size, different shaking frequency, and sample moisture content. The 1.18 mm pore size is based on the premise that this is the critical length influencing the retention time in the reticulorumen (46). Hence, it may be valuable to analyze smaller particles more thoroughly. The aim of this study was to more precisely define the operational guidelines for the PSPS, as it was observed that the rate of shaking, and thus the effectiveness of separation, often varies between users. After comparison of three frequencies, the authors recommend a medium speed of 1.1 Hz or greater (66 cycles/min). The different moisture content of the samples, dried for varying durations, only showed significant effects in samples that were completely dried (42).

While the execution follows standardized procedures, the interpretation of results varies across studies. Some authors use the content retained on the 1.18 mm sieve to determine peNDF (47), while others use the 8 mm sieve (48).

Another on-farm tool is the “Z box”, developed at the William H. Miner Agricultural Research Institute (Chazy, NY). The user instructions are based on comparisons with the RTPS. It was reported that vertical shaking of 50 g of feed samples revealed the strongest correlation with the RTPS particle fraction greater than 1.18 mm, and that the optimal screen size depended on the type of feed (3.18 mm screen for corn silage and TMR and a 4.76 mm screen for hay crop silage) (49). One source of error with this device is the vertical hand-held shaking. In comparison, the PSPS is shaken on a flat surface, which provides more stability (41).

3.3. Digestive tract content sieving

There are numerous studies in which rumen contents as well as feces of adult cows are analyzed using sieving tools to draw conclusions about feed utilization efficiency. The methods employed can be broadly categorized into wet and dry sieving. The procedures and equipment used in the various studies are generally similar. However, there is no established standard for conducting fecal sieving. The number and pore size of the sieves, the amount of material used, and the duration of the sieving process vary. Thus, a direct comparison of the results is often not feasible.

3.3.1. Wet sieving

Sieving gastrointestinal content is often carried out under laboratory conditions using a similar vibratory sieve shaker as for feed sieving (33–36,38,39,50–54), to determine the influence of different feed types on the fecal particle size distribution.

Yang et al. (2001) compared diets with different barley processing, forage-to-concentrate ratios, and varying particle sizes in the forage. The particle size distributions of the TMR, forage, and gastrointestinal tract contents were evaluated using a vertical oscillating sieve shaker (Analysette 3; Fritsch, Oberstein, Germany). Feed samples and rumen contents were separated into fractions using six sieves ranging from 9.5 to 0.15 mm, while four sieves ranging from 3.35 to 0.15 mm were used for intestinal contents and feces. Comparing the results of feed and feces sieving revealed that the influence of diet particle size on particle distribution in the rumen or post-ruminal contents was minimal. This suggests that larger particles are chewed more efficiently (33).

Kononoff et al. (2003) conducted a feeding experiment as well and analyzed the effects of different particle sizes of alfalfa haylage. The particle size distribution of the feed was determined using the PSPS, while digestive contents and fecal samples were analyzed using the Analysette 3 PRO Vibratory Sieve Shaker (Fritsch, Oberstein, Germany). The authors adapted the procedure from Beauchemin et al. (1997), utilizing six sieves ranging from 13.2 to 0.15 mm (55) to analyze individual fractions. The smaller particle sizes of the feed and rumen content were not reflected in the feces: the proportion of material retained on the 1.18 mm sieve remained consistent.

Maulfair et al. (2011) particularly assessed the impact of varying particle lengths in the feed on the particle size distributions in manure. Cows were fed four rations, differing in the length of dry grass hay. The feed was analyzed using the ASABE separator, while rumen and fecal

samples were examined using a vibratory sieve shaker (AS 200 Digit Analytical Sieve Shaker, Retsch GmbH, Haan, Germany) containing six sieves similarly to Yang et al. (2001). In addition to the retained material, they also evaluated the soluble fraction, i.e. the portion that passes through the sieve with the smallest pore size. The calculations using solely retained particles also indicated that fecal particle size does not change depending on the particle size of the diet. However, when soluble particles were included, a significant contrast was found: As the particle size in the TMR increased, the particle size in the feces decreased (50). It is possible that the soluble DM in feces increases due to greater chewing activity with larger particles in the ration, leading to more saliva secretion and, consequently, more liquid containing small particles leaving the rumen (56).

Khorrami et al. (2022) examined the effects of different starch levels in feed on ruminal pH, fecal pH, and fecal particle size distribution, and whether these factors could be used to detect subacute ruminal acidosis (SARA). While TMR was evaluated using the PSPS, the feces were analyzed using both laboratory sieving and an on-farm method. However, the aim was not to validate the on-farm method, but rather to compare sample preparation and to determine the ideal time after feeding for measurements. In the laboratory, the samples were wet sieved using an automatic sieve shaker (AS 200 Digit Analytical Sieve Shaker, Retsch GmbH, Haan, Germany) containing five sieves with pore sizes ranging from 4.0 to 0.15 mm. The on-farm method was conducted using three of these sieves (ranging from 2.0 to 0.5 mm). The material was manually sprayed with water, and the particles were separated without shaking the sieves. The particle size distributions from on-farm sieving (using fresh samples) and the laboratory method (using frozen, thawed and subsequently dried samples) showed some differences, but both methods demonstrated similar trends in how particle size distribution changed with diet and time post-feeding (39).

Custom sieves specifically developed for on-farm analysis are also employed. Kljak et al. (2019) utilized the Nasco Digestion Analyzer (NDA; Nasco, Fort Atkinson, WI, USA) to evaluate the effects of diet manipulations and to assess different sampling times post-feeding. The device consists of three screens with pore sizes of 4.76 mm, 3.17 mm, and 1.59 mm, and requires larger amounts of manure (200 g compared to 15-30 g for several laboratory methods). The wet sieving was followed by drying of the retained material and determination of DM and neutral detergent fiber (NDF). These evaluations revealed that the percentage of

retained DM depends on forage quality and fiber content, but not on the particle size of the feed (57).
On-farm sieving is also conducted using tools not specifically designed for this purpose. The use of a kitchen strainer with a 1.66 mm mesh size is described as an easily executable method for examining manure (58).

3.3.2. Dry sieving

In dry sieving, samples are typically washed in nylon or polyester bags in washing machines, then freeze-dried, and subsequently sieved using a vibratory sieve shaker (59–63). Hence, this procedure falls under the category of laboratory methods.
Kornfelt et al. (2013) evaluated the effects of harvest time of silage on particle size distribution in rumen contents and feces using dry sieving and image analysis. After fecal samples of 30 g were washed and freeze-dried, the particles were separated into seven fractions using a vertical sieve shaker (AS 200 Digit Analytical Sieve Shaker, Retsch GmbH, Haan, Germany). The retained material was then scanned with an HP Scanjet 8300 Scanner (Alleroed, Denmark) to accurately determine particle length (PL) and particle width (PW). The size determination of these techniques differ: sieving allows for the calculation of arithmetic and geometric mean particle sizes, while image analysis enables precise measurement of both particle length and width. However, the particle size values obtained by sieving were consistent with the results from image analysis, and the combination of both methods allowed for a more accurate determination of particle size and shape (59).

Whether wet and dry sieving methods can be directly compared is unclear, as dry sieving separates fecal particles by width, while wet sieving sorts them by length (64).

3.3.3. Comparison between laboratory and on-farm methods

In the scientific literature, to my knowledge, there is only one study that compares wet sieving under laboratory conditions with on-farm sieving to validate the latter. Both sieving systems for the evaluation of TMR and for fecal assessment are compared.
The on-farm methods for manure analysis include a monolayer screen with a 1.18 mm mesh size, as well as the Cargill Digestion Analyzer (MN, USA), which consists of three sieves with descending pore sizes (4.76 mm, 3.17 mm and 1.58 mm). Under laboratory conditions, the

manure was wet-sieved through a stack of six screens (4.75 mm, 2.36 mm, 1.18 mm, 600 µm, 300 µm and 150 µm) using a vibratory sieve shaker (Octagon 200, Endecotts Limited, Lombard Road, London, SW193TZ, England).

The DM retained differed significantly among the used methods: laboratory sieving (15.2%), monolayer sieve (26.5%), and multilayer sieve (21.4%). The laboratory method consistently retained less DM compared to the other methods.

Both, the monolayer and multilayer sieves overestimated the percentage of fecal particles larger than 1.18 mm compared to the laboratory method. The fraction retained by the 1.18 mm screen was inversely proportional to the number of screens used. Fewer screens (monolayer sieve) retained more particles, while more screens (laboratory method) retained fewer particles. This is likely due to the stratification of particles and the formation of a fiber mat when fewer screens are used.

The laboratory method showed a narrower range of retained fractions (12.7% to 18.2%) compared to the monolayer sieve (17.8% to 33.8%) and multilayer sieve (18.9% to 29.6%). The results indicated that the accuracy of the measurements decreased with fewer screens, suggesting that field methods struggle to effectively separate particles of different sizes (44).

However, there are no comparative data available where laboratory and on-farm procedures match in terms of the number of sieves and pore sizes. In our experiment, we aimed to align the laboratory and on-farm methods as closely as possible, specifically by using three sieves with the same pore size for both.

Furthermore, the application of on-farm fecal sieving devices in calves was examined. Theoretically, it is viable to monitor calf feeding with minimal time and cost through on-farm manure analysis. Given that previous trials have mainly been conducted with adult cows or heifers (57), this study aims to evaluate whether this approach is applicable to calves and whether it produces comparable results to the laboratory method.

The objective of this study is to validate an on-farm fecal particle size separator. The laboratory method serves as the standard against which the results of the on-farm device are compared. Additionally, differences in the agreement between the two methods are assessed across different age groups (calves and adult cows).

4. Materials and methods

4.1. Study design and animals

This study was conducted at the teaching and research farm “Kremesberg” (VetFarm), and at the Center for Animal Nutrition and Welfare at the Department for Farm Animals and Food System Science, of the University of Veterinary Medicine, Vienna. Fecal samples were collected within a larger study on heat stress in calves and the use of two different concentrates for calf rearing. The animal study protocol was approved by the Ethics and Animal Welfare Committee of the University of Veterinary Medicine, Vienna, in accordance with the University’s guidelines for good scientific practice and authorized by the Austrian Federal Ministry of Education, Science, and Research (ref. BMBWF 2023-0.300.442, 28 April 2023) in accordance with current legislation.

The newborn calves were separated from their dams. Within one hour after birth calves were bottle fed four L of their mother’s colostrum. In order to enhance the immunity transfer, calves received additional two L twelve hours later. On the first day, calves were housed in individual calf boxes (120 x 60 x 50 cm) containing fresh bedding material to minimize the risk of infection. The following three days calves were sheltered in calf igloos with ad libitum access to milk, water and hay. From then on, calves were group-housed in two pens, each with 8-15 animals. In the beginning, calves received milk replacer ad libitum through an automatic calf feeder. The amount of milk replacer (Sanolac Startino, Sano, 50% skimmed milk powder content) was gradually reduced until weaning. During this period, calves had ad libitum access to hay and water.

The approximately 80 cows kept at VetFarm were housed in a free-stall barn with individual cubicles (2.6 × 1.25 m) and straw bedding. The examined animals were provided with a feeding ration consisting of grass silage, corn silage, grass hay and concentrates (Kuhkorn Kompakt 19, Garant-Tiernahrung Ges.m.b.H., Pöchlarn, Austria). Since the animals were in different stages of lactation, they received varying amounts of concentrate feed. In addition, they had ad libitum access to mineral blocks and clean, fresh water.

4.2. Fecal sample collection and analysis

As part of daily vital parameter checks, fecal samples were taken from randomly selected calves during rectal temperature measurements. A total of 20 samples of approximately 200 g each was collected from 7 calves aged between 20 and 125 days. At the same time,

20 samples of 200 g fecal material from 8 randomly selected adult dairy cows were collected. However, these were not induced but spontaneously voided fecal samples. It is important to note, that no feces were gathered from the ground to avoid distorting the particle size with bedding material. Sampling was not conducted according to a specific schedule, and samples from both the calves and the cows were collected at varying times of the day. The samples were either assessed immediately after collection or frozen in disposable palpation gloves at -20 °C for later analysis.

4.2.1. On-farm analysis

The fecal material was processed using a fecal particle size separator (Digestion check, Sweep Agro, 4861 DJ Chaam, The Netherlands). The three sieves had mesh sizes of 5 mm, 3 mm and 1 mm.

At the beginning, the empty sieves were wetted, weighed five times, and the average was calculated. The frozen samples were thawed and thoroughly mixed. Then, approximately 150 g from each sample were weighed in measuring cups and soaked in one L of warm water for about 15 minutes or until no clumps were visible in the feces. Based on a study by Khorrami et al. (2022) (39) and the results from previous test runs, the amount of 150 g was determined to be suitable for the device. Simultaneously, the remaining 50 g were placed in small plastic bags, labeled, and frozen at -20 °C for later laboratory analysis. Once the fecal sample was dissolved in water, it was stirred with a spatula and poured into the separator (Figure 1). The three screens were arranged in descending order of mesh size. The sample was then evenly rinsed over a drain gate from a distance of approximately 15 cm with a consistent stream of water, ensuring that the entire material was covered (Figure 2). This process was continued until clear water emerged from the top screen and no small particles were visible. Then, the 5 mm sieve was removed and the same procedure was conducted with the 3 mm and 1 mm sieves. Subsequently, the sieves containing the retained particles were weighed individually and the results were logged.

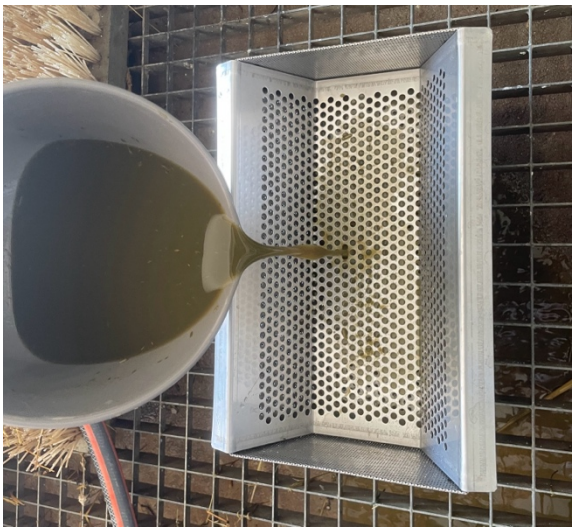

Figure 1 Transfer of the dissolved sample into the fecal particle size separator.

Figure 2 Rinsing the sample with a steady stream of water.

4.2.2. Laboratory analysis

The comparison method was conducted in the laboratory at the Campus of the Vetmeduni using an automatic sieve shaker (AS 200 Digit Analytical Sieve Shaker, Retsch GmbH, Haan, Germany, 200 mm of diameter).

Similar to the on-farm procedure, the three sieves with 5 mm, 3.15 mm and 1 mm mesh size were wetted and weighed individually five times. The average was used for later calculations. Of the previously frozen fecal samples of 50 g, 15 g (39) of each sample were weighed and

soaked in one L of warm water for ten minutes. The dissolved feces were then transferred into the upper sieve (5 mm). The measuring cup previously containing the sample was rinsed and cleaned with the spatula to ensure that no particle residues remained. Each sample was then shaken for three minutes at an amplitude of 50% = 1.5 mm under a constant and consistent water flow (Figure 3). The duration of three minutes was established through preliminary tests, during which no small particles were observed in the top sieve after this period, and the water running through the 1 mm sieve was clear. In the first attempt, the individual sieves containing the retained particles were weighed immediately after the three minutes. However, the values exceeded the original 15 g used. A possible explanation is that the particles retained water, causing the weight to increase. Therefore, the samples were analyzed again. This time, after the initial three minutes of shaking with the settings mentioned above and water flow, an additional three minutes of shaking without water flow followed before weighing.
After, all measurements both from the on-farm and the laboratory method were transferred to an Excel spreadsheet for further calculations.

Figure 3 Analysis of the fecal samples using an automatic sieve shaker.

4.3. Statistical analysis

The statistical analysis was conducted using IBM SPSS Statistics (International Business Machines Corporation, Statistical Package for the Social Sciences, Version 29.0.2.0., NY, USA). A comparison of methods was performed between cow and calf data, as well as separate comparisons using only calf data and only cow data. The data were assessed for

normality using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Subsequently, either a paired t-test or a Wilcoxon signed-rank test was applied. Correlations were calculated using either Pearson's r or Spearman's rho. To illustrate the agreement between the two methods, Bland-Altman plots were generated. Additionally, linear regression analyses were conducted.

5. Results

One dataset from the group of adult cows was excluded, resulting in a sample size of n = 39.

5.1. Calves and cows

Table 1 Descriptive statistics of combined data

	N	Range	Minimum	Maximum	Median	Mean	Std. Deviation
Retention on-farm (%)	39	65.4	14.0	79.4	58.1	57.76	14.62
Retention lab (%)	39	78.6	16.2	94.8	47.0	48.35	19.83

The descriptive statistics for the data from cows and calves are summarized in Table 1. For the on-farm method, the range of retained material was between 14.0% and 79.4%. The mean was 57.76%. For the lab, a range between 16.2% and 94.8% was observed. The mean was 48.35%. The distributions of this dataset, as well as those of the calves and cows separately, are shown in Figures 4-6.

The Kolmogorov-Smirnov test indicated a significant deviation from normality for the on-farm data. In contrast, lab data did not show a significant deviation from normality.

Since the on-farm data were not normally distributed, a Wilcoxon Signed Ranks Test was performed to compare the two methods. The results indicated that the lab showed significantly lower results compared with the on-farm test. Out of the 39 cases, 26 showed negative ranks (retained material in % in the lab < retained material in % on-farm), while 13 showed positive ranks.

Bland-Altman plots were generated to highlight the differences and similarities between the two methods (Figure 7). The graphic shows a mean difference of 9.41% and a range from -26.38% to 45.21%.

The correlation between on-farm and lab was assessed using Spearman's rho. A significant positive correlation was found, indicating a moderate relationship between the two methods.

The regression model showed a correlation coefficient R of 0.472, indicating a moderate relationship between both procedures. The R Square value was 0.222, suggesting that approximately 22.2% of the variance in on-farm results is explained by laboratory outcomes.

5.2. Calves separately

Table 2 Descriptive statistics of calf data

	N	Range	Minimum	Maximum	Median	Mean	Std. Deviation
Retention on-farm (%)	20	61.3	14.0	75.3	54.1	54.06	18.78
Retention lab (%)	20	78.2	16.6	94.8	49.9	49.58	22.26

The descriptive statistics for the calf data are summarized in Table 2. For the on-farm, the mean retained material was 54.06%, with a range between 14.0% and 75.3%. The lab results showed a mean of 49.58%, with a range between 16.6% and 94.8%.

The Kolmogorov-Smirnov and Shapiro-Wilk tests indicated that both on-farm and lab did not significantly deviate from normality.

Therefore, the paired samples t-test was applied. It showed a mean difference of 4.49% suggesting that, on average, the on-farm values were slightly higher than those from the lab method. However, the standard deviation indicates considerable variation around this mean difference. The results were not statistically significant, indicating that the differences may also be due to random chance.

The Bland-Altman plot shows a mean difference of 4.49% and a range from -34.77% to 43.74% (Figure 8).

The correlation between on-farm and lab was significant, with a Pearson correlation coefficient of $r = 0.535$ ($p = 0.015$), indicating a moderate positive relationship.

The regression analysis indicated a moderate correlation ($R = 0.535$) and an R Square value of 0.286, suggesting that approximately 28.6% of the variance in on-farm can be explained by lab.

5.3. Cows separately

Table 3 Descriptive statistics of cow data

	N	Range	Minimum	Maximum	Median	Mean	Std. Deviation
Retention on-farm (%)	19	27.0	52.4	79.4	60.2	61.65	6.93
Retention lab (%)	19	70.2	16.2	86.4	46.7	47.05	17.42

The descriptive statistics for the cow data are presented in Table 3. The on-farm had a mean of 61.65%, with a range between 52.4% and 79.4%. For the lab, the mean was 47.05%, with a range between 16.2% and 86.4%.

The Kolmogorov-Smirnov and Shapiro-Wilk tests indicated that both the lab and on-farm data did not significantly deviate from normality.

The paired samples t-test revealed a mean difference of 14.6%, suggesting that the retention rate using the on-farm method was, on average, significantly higher than that of the lab method. The standard deviation indicates a moderate level of variability in the differences observed among the samples.

The Bland-Altman plot shows a mean difference of 14.6% and a range from -14.82% to 44.02% (Figure 9).

The correlation between the approaches was significant, with a Pearson correlation coefficient of $r = 0.522$ ($p = 0.022$), indicating a moderate positive relationship.

The regression analysis indicated a moderate correlation ($R = 0.522$) and an R Square value of 0.273, suggesting that approximately 27.3% of the variance in on-farm can be explained by lab.

5.4. Comparative figures

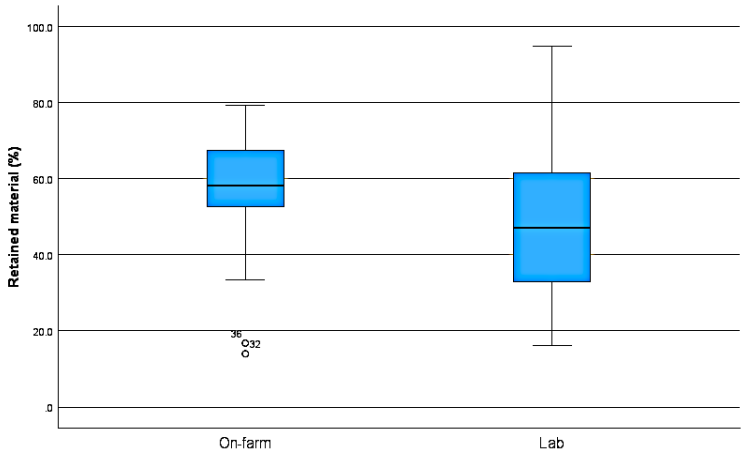

Figure 4 Cows and calves

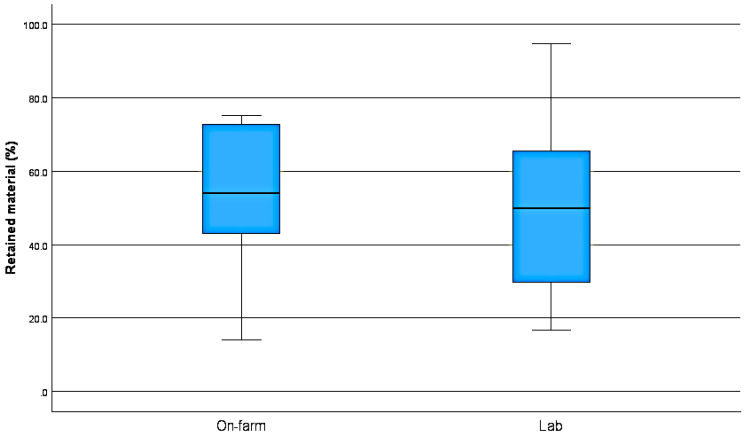

Figure 5 Calves

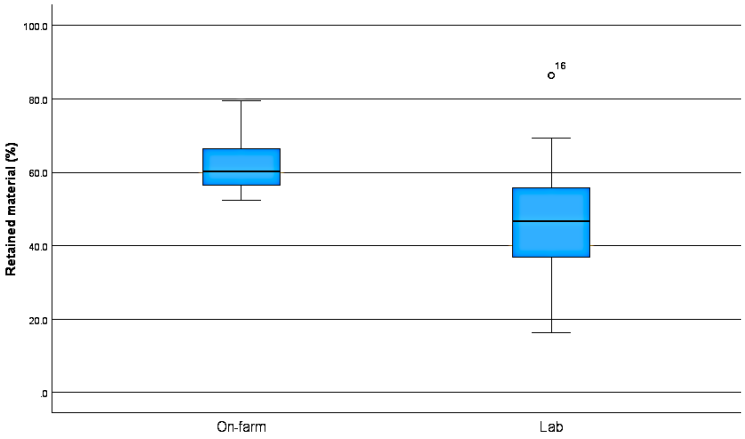

Figure 6 Cows

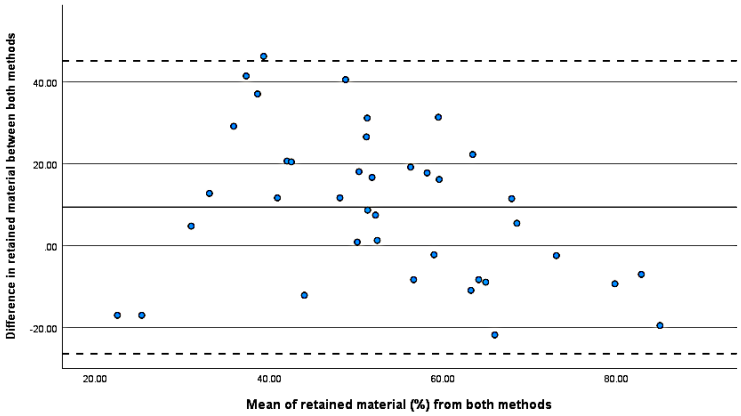

Figure 7 Cows and calves

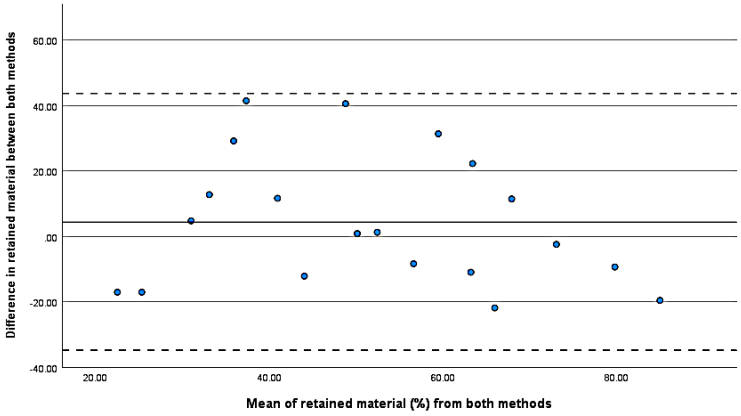

Figure 8 Calves

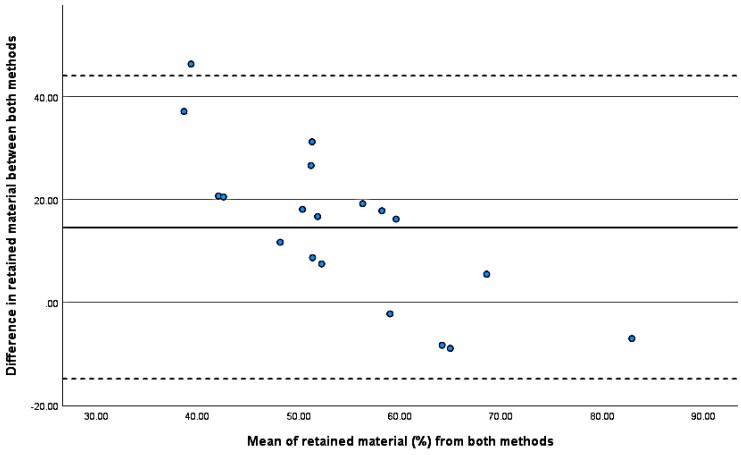

Figure 9 Cows

6. Discussion

6.1. Procedural differences between laboratory and on-farm sieving

Consistent with Carta (2010), the mean amount of retained material in the laboratory was lower than on-farm. These differences are significant, both in the combined data of cows and calves and in the data of cows. Carta (2010) attributed the significant differences to the varying number and pore sizes of the sieves used. While six sieves with pore sizes ranging from 4.75 mm to 150 µm were utilized in the laboratory, the on-farm method employed three sieves (ranging from 4.76 mm to 1.58 mm) or a single sieve (1.18 mm). Due to the reduced number of screens, the separation of particles by size on-farm was only partially effective. Larger particles formed a fiber mat due to the water (64), resulting in fewer particles being able to pass through the smallest pore size (44). In our experiment, however, both the number and pore sizes were consistent between the laboratory and on-farm conditions yet the results varied. For cows, the on-farm mean was significantly higher at 61.65%, while the laboratory mean was 47.05%. Similarly, for calves, the on-farm mean of 54.06% exceeded the laboratory mean of 49.58%, but this difference was not statistically significant. These findings indicate that alternative factors may account for the observed differences.

A significant source of error in on-farm sieving methods arises from the lack of mechanical shaking, which is consistently applied in controlled environments. In laboratory settings, sieves are shaken uniformly and continuously, promoting a more efficient separation of particle sizes. This is reflected in the wider range of retained percentages observed in the laboratory method compared to the on-farm sieving, particularly when considering the cow data separately (Table 1-2). The differences suggest that clustering or adhering of particles can be prevented in the laboratory through consistent agitation. In contrast, on-farm sieving relies on subjective assessments, where the material on the top sieve is visually evaluated, and the clarity of the outflowing water is judged to estimate particle separation. This manual and subjective evaluation increases the risk of human error. In comparative studies on feed sieving, this factor has been highlighted as a potential source of variability when employing manual sieving techniques (41). Consequently, initial efforts were dedicated to standardizing the operation of the PSPS by providing detailed guidelines for the shaking procedure (42,45). There is a lack of comparable studies regarding the optimal implementation of on-farm fecal sieving devices.

The median, which is considered a more robust measure than the mean, further indicates consistently higher retention percentage with the on-farm tool compared to the laboratory method (Table 1-3). In the on-farm method, 150 g of sample was mixed with one L of water, whereas only 15 g was used in the laboratory for the same volume. This discrepancy may have resulted in incomplete dissolution of particles in the on-farm samples and the lack of agitation during sieving likely further hindered efficient particle separation. Furthermore, in the laboratory, the samples were first shaken for three minutes with water, followed by an additional three minutes without water to ensure accurate measurements, as initial tests showed that without this step, the weighed material exceeded the original sample weight. On-farm, however, the excess water was not removed, which likely contributed to the higher retention percentage observed in the on-farm results.

6.2. Variability in sample composition

In addition to human influences and potential errors in the execution, the sample composition appeared to impact the results. Interestingly, there are differences in the agreement between both methods when comparing calves (Figure 8) and cows (Figure 9). The Bland-Altman plot is a graphical method used to assess the agreement between two quantitative measurement techniques. It plots the differences between the two methods against their mean, allowing for visual identification of systematic bias and the extent of variability between the measurements. The central horizontal line represents the mean difference (bias), while the upper and lower limits of agreement (set at ±1.96 standard deviations of the differences) indicate the range within which most differences between the two methods are expected to fall. The differences were calculated as on-farm minus laboratory values. Positive differences in the plot indicate that more material was retained on-farm than in the lab, negative differences indicate the opposite. A small bias and narrow limits of agreement would suggest good agreement between the methods, while a significant bias or wide limits would indicate poor agreement.

For the calves, the differences between the laboratory and on-farm sieving methods ranged from +43% to -34%, with a mean difference of +4%. Out of the 20 samples, 11 showed positive differences, indicating higher retention on-farm compared to the laboratory, while 9 exhibited the opposite trend. The distribution of points showed no clear pattern suggesting that the variability is consistent across the range of measurements.

This lack of a distinct trend may be related to the developmental stage of the calf rumen and the composition of their feces. The near-zero mean difference suggests that, on average, there is no strong bias toward either method, but the wide range of differences indicates that individual measurements can vary considerably, potentially due to inconsistencies in fecal particle size and composition in calves. The age ranging from 20 to 125 days resulted in variations in feeding practices, with some of the calves already weaned. Consequently, rumen development and gastrointestinal tract conditions varied, potentially leading to differences in fecal composition.

For the cows, the differences between the laboratory and on-farm sieving methods ranged from +44% to -14%, with a mean difference of +14%. The distribution of data points suggests a pattern: At lower mean retained percentages (up to 62%), the differences are predominantly positive, with the exception of one measurement. Above 62%, with one exception, only negative differences are observed. Furthermore, among the samples from adult cows, there are only four cases where retention in the laboratory exceeded that on-farm. However, in both groups, the results did not consistently cluster near the bias line, which would have indicated systematic, and thus predictable, differences between the methods.

The generally higher retention on-farm (in 15 out of 19 cases) may be explained by the presence of excess water and associated microbial aggregates and small or soluble particles, artificially increasing the retained percentage on-farm. Maulfair et al. (2011) investigated the soluble fraction to assess the impact of varying particle lengths in the feed on particle size distributions in manure. While calculations involving solely retained particles indicated that fecal particle size remains unchanged regardless of the particle size in the diet, the analysis including soluble particles revealed differences. An increase in particle size in the TMR resulted in a decrease in fecal particle size. The authors attributed this phenomenon to an increase in soluble DM in feces, which occurs due to enhanced chewing activity with larger particles in the diet, leading to increased saliva secretion and, consequently, a greater quantity of liquid containing smaller particles exiting the rumen (56). The fully developed rumination behavior and the exclusive provision of solid feed in adult cows may account for the differences in results observed between young animals and cows (Figure 5, Figure 6). In contrast, the lab method, which involved shaking the sample dry for three minutes, likely removed this excess water and the soluble particles and microorganisms it contains, leading to lower retained percentages.

Similar to the calf group, the feeding regimen for the cows also varied. The animals were in different stages of lactation and received varying amounts of concentrate feed, which may

have influenced the fecal particle size distribution. It has been demonstrated that a high starch content reduces the proportion of small fecal particles, while increasing the proportion of larger and soluble particles (39). Additionally, the timing of sample collection in relation to feeding is a significant factor and should always remain consistent. Khorrami et al. (2022) observed differences in the particle size distribution between 8 hours post-feeding and immediately before feeding time (39).

6.3. Applicability and improvement approaches for practical use

Despite the observed discrepancies between the on-farm device and the laboratory method, the sieves can still be considered a practical and cost-effective tool for visual assessment of feces. Their ease of use makes them accessible for routine evaluations in the field.
Khorrami et al. (2022) examined the impact of varying dietary starch levels and demonstrated the relationship between the proportion of small particles in feces and the critical rumen pH threshold used in SARA diagnostics. The manual on-farm sieving was performed using three screens with pore sizes of 2 mm, 1.18 mm, and 0.5 mm, and both ruminal and fecal pH were measured. The authors observed that as the rumen pH value approached the critical threshold of 5.8, the proportion of smaller particles (0.5 to 1.18 mm) decreased. Furthermore, a higher proportion of large particles was observed with increased concentrate levels, possibly due to a decreased rumen pH and reduced fiber degradation. The measurement of particle distribution in feces offers a non-invasive, on-farm method to approximate fermentation conditions in the rumen (39).
This is also applicable to calves: feeding concentrate increases the concentration of VFAs, primarily propionate and butyrate, which stimulate papilla growth (29). At the same time, the rumen pH decreases, increasing the potential for rumen acidosis (31). An appropriate on-farm tool and established threshold values for the retained particles would enable the farmer to immediately detect changes and intervene.

Introducing standardized procedures, similar to those used for feed sieving, would be beneficial to promote consistent use of fecal sieving tools on farms. The standard for determining particle size in chopped forages is the ASABE particle separator. This standardized method reduces human error and eliminates the need for sample pre-treatment. However, its substantial weight and size limit its application to laboratory environments (41).

Hence, the PSPS was developed based on the ASABE separator as a portable and convenient method for forage evaluation. The construction of the device, as well as the sample volume and sieving procedure, are modeled after the reference laboratory method. The precise guidelines for operation are designed to minimize human error. Despite aligning the procedure with the laboratory method and providing specific operational guidelines, variations in shaking frequency among users resulted in differing particle distributions. Following studies on shaking frequencies and moisture content, the instructions were further refined to improve consistency and accuracy (42).

In contrast, there are no standardized procedural guidelines for conducting fecal sieving, neither for the laboratory method nor for the on-farm approach. For our hypothesis, we assumed that the laboratory method, as we conducted it, serves as the standard against which we compare the laboratory device. However, the literature presents various examples of laboratory methods with differing numbers of sieves, pore sizes, sample volumes, sieve durations, and shaking frequencies (33,38,39,50).

In our experiment, there was a considerable variation in sample volume between laboratory (15 g) and on-farm (150 g). While the sample volume for feed sieving using the ASABE separator or the PSPS is in relation to one another, the use of 15 g of manure in the laboratory method is based on specifications found in the literature. A sample size of 15 g may not be representative, potentially leading to random variations in composition that significantly influence the retained percentage and particle size distribution.

For on-farm methods, the absence of a shaking component seems to be a significant factor contributing to the observed differences. In such an environment, feces must be wet-sieved, as drying would undermine the objective of an easily implementable method. However, guidelines for the removal of excess water could potentially lead to more uniform results. Similar to feed sieving, detailed protocols for manual shaking could effectively remove the water while minimizing errors between different users.

The sieving duration was subjectively determined for each trial and not uniform. However, the validity for employing a standardized sieving duration without visual inspection seems questionable in this context, as Kljak et al. (2019) observed that prolonged washing led to the passage of large particles.

Additionally, it may be necessary to utilize different pore sizes for various research objectives or age groups. To facilitate the detection of SARA through fecal particle distribution, it was necessary to assess smaller particles than those measurable with the on-farm device

Digestion check (Sweep Agro, 4861 DJ Chaam, The Netherlands). Sieves with pore sizes of 2 mm, 1.18 mm, and 0.5 mm were utilized to quantify the proportion of smaller particles that correlated with the critical rumen pH threshold. (39).

A suitable on-farm tool, accompanied by precise procedural instructions and guidelines for interpreting the results (e.g. established threshold values), represents a cost-effective, on-site resource that enables the farmer to directly assess feeding practices and intervene when necessary. However, further studies are needed to define such standards. Moreover, consistent recommendations regarding the materials and methods under laboratory conditions could enhance the comparability of results across different studies.

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