

## The effect of physically effective fibers on the digestibility of total mixed rations using different indicators

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### ABSTRACT

Digestibility rate is an essential parameter of total mixed ratio (TMR) quality because forage NDF varies widely in its degradability in the rumen, and digestibility influences animal daily intake and productive performance. In this sense, the physical effectiveness of the meal largely determines the dynamics of decomposition, the kinetics (passage), and ultimately, the rate of utilization of the ration. The main goal of the research is to evaluate the effect of reducing the particle size of TMR on the total digestibility rate of cows in early lactation according to the Penn State Particle Separator (PSPS) measuring method. The diet is based on an ad libitum diet of complete mixed meals (TMR) based on corn silage. During the adaptation period, cows were given three different meals ( $T_0$ ,  $T_1$ , and  $T_2$ ), which had similar chemical composition but differed in their content of physically effective fibers (peNDF), distribution, and particle size ( $6.9 \pm 2.4$ ,  $5.5 \pm 2.3$ , and  $5.4 \pm 2.3$  mm, respectively). The trial was conducted at Dairy Farm "Spreča," where the adaptation period lasted 21 days. After this, feces collection was observed. The content of physically effective fibers through meals was 23.5%, 21.8%, and 20.0% at the 4 mm level and 17.5%, 14.0%, and 12.4% at the 8 mm sieve level. The natural contented indicators lignin (ADL) and acid-insoluble ash (AIA) methods were used to test digestibility. The results indicated that physical effectiveness positively affects nutrient digestibility, except for structural carbohydrates (NDF fiber fraction), which showed a negative correlation.

**Keywords:** dairy cows, total mixed ration, fiber effectiveness, *in vitro* digestibility

### INTRODUCTION

The aim of using Total Mixed Ration (TMR) is to ensure a steady flow of nutrients in the rumen. The balance between energy and protein in TMR enhances feed utilization (Zebeli et al., 2012; Schingoethe, 2017). Assessing physically effective fiber (peNDF) in diets is a relatively new concept that complements the traditional measurement of crude fiber. Extended homogenization and grinding of total mixed rations diets have been shown to reduce the average particle length and, consequently, the level of physically effective fiber. It emerged from the need to ensure adequate amounts of coarse fiber, which

is crucial in creating optimal fermentation conditions in the rumen. This concept is essential for the nutrition of high-yielding cows, especially during early lactation, when the ration typically contains a high proportion of easily digestible concentrates. While general recommendations exist, the criteria for applying this concept are not uniform, given the significant variability in nutritional conditions across different farms. Digestibility refers to how effectively the animal uses the ration, comparing the consumed amount to the amount excreted in feces. It is influenced by the kinetics of food passage through

the digestive tract, known as retention time. The feed's chemical composition, physical structure, the interaction between feeds, and processing methods are the key digestibility factors. A well-formulated ration should consider chemical composition and physical parameters, as these impact the efficiency of fiber digestion in the rumen. The concept of physically effective fiber (peNDF) was created to assess chewing activity and saliva production by analyzing bulky feed particle size and distribution. Recent research shows mixed results regarding the impact of particle size on dry matter digestibility (DMD). Some studies (Kononoff and Heinrichs, 2003; Beauchemin and Yang, 2005) suggest that larger particle sizes in corn silage can enhance DMD, while others (Maulfair et al., 2011) report a decline in digestibility, with values dropping significantly. Additionally, the size of physically effective neutral detergent fiber (peNDF) appears to have a neutral effect on DMD (Plaizier et al., 2008; Krause et al., 2002). Variability in DMD can also be attributed to factors like the ratio of forage to concentrated feed (F:C), the digestibility of starch sources (barley vs. corn), and the non-fiber carbohydrates (NFC) to neutral detergent fiber (NDF) ratio. Kononoff and Heinrichs (2003) note that grinding feed can improve organic matter digestibility (OMD), starch, and NDF (NDFD) by increasing the feed's surface area and enhancing microbial activity in the rumen. Zebeli et al. (2012) indicate that moderately reducing feed particle length can benefit fiber digestibility and ration uniformity, helping to prevent selective feeding. When evaluating the fiber supply in ruminant diets, the physical effectiveness of neutral detergent fiber (peNDF) is crucial. This concept integrates chemical properties (like neutral detergent fiber) and structural characteristics (such as particle size) of fiber, both of which stabilize rumen fermentation and acid-base balance (Mertens, 1997; Tafaj et al., 2007). The interactions of peNDF are complex, affecting feeding behavior, rumen contents, rumination, and fermentation characteristics, which complicates the evaluation of peNDF as a standard for assessing fiber supply (Zebeli et al., 2012). Despite this, using peNDF in diet formulation enhances the assessment of fiber needs in dairy cows. Concentrate feeds have fiber properties

different from roughage, resulting in smaller particle sizes and higher specific gravity. This leads to shorter rumen retention and lower digestibility (Allen and Grant, 2000). Thus, the research aims to investigate how manipulating the physically effective fibers and nutritive modifications in the total mixed rations influences the digestibility of dry matter and associated nutrients.

## MATERIALS AND METHODS

The study was conducted on "Spreča" farm doo, which focuses on a modern, free-range method of breeding and keeping animals. Located at (44°26'29"N; 18°45'02"E) in the Kalesija municipality of Bosnia and Herzegovina, the farm's cow selection was based on breed, age, stage of lactation, body weight, and production characteristics. Thirty multiparous high-producing early lactation (31.0 ± 7.4 days in milk) Holstein-Friesian (HF) cows were selected and divided into three groups (ten cows each) with an average body weight (550 ± 15 kg) and milk production (30.0 ± 6.0 kg/day). They were housed in semi-open barns with ad libitum access to total mixed rations (TMR) and water, with the rations formulated according to NRC (2001) recommendations. Cows were fed three times daily, with corn silage as the primary component, supplemented with meadow hay, grass hay, and concentrate mixtures. Tables 1 and 2 present the detailed structure and composition of the rations. Successive sampling occurred after a 21-day adaptation period. The experiment evaluated three mechanically controlled ration treatments. Each group of cows received a specific ration (TMR). The first group received the control ration (T<sub>c</sub>), which involved 5 minutes of preparation (in a mixer trailer). The preparation time for the other two rations (T<sub>1</sub> and T<sub>2</sub>) was 10 minutes each. The aim was to reduce the levels of effective fibers in the rations. In the third meal (T<sub>2</sub>), the composition changed by removing the hay and compensating with additional corn silage to further lower its effectiveness, which did not significantly affect the energy content of the rations. An increase in crude protein content in the T<sub>2</sub> ration aimed to compensate for the lower effectiveness in enhancing digestibility. Using a reduction technique, 3 kg TMR samples were collected

daily for five consecutive days after serving. The samples were collected in well-compressed PVC bags and stored at -25 °C until analysis (ISO 6497:2002). The Animal Feed and Feeding Laboratory (Faculty of Agriculture and Food Science, University of Sarajevo) conducted complete preparation and physical and chemical analysis. Prior to laboratory analysis, samples were pre-dried (Memmert UE 500) at 55 °C for 24 hours and ground (Foss Tecator Cyclotec™ 1093) to a particle size of 1.00 mm. Samples were continuously collected from each cow (for five days for TMR and three days for feces, rectally), ground, and stored according to laboratory procedures. In the rations and feces, dry matter content was determined according to ISO 6496:1999 (Memmert UE 500); crude ash content (Ash) was measured according to ISO 5984:2002 (Nabertherm N 7/H); organic matter content (OM) was calculated as the difference between DM and Ash:  $OM = DM - ASH$ ; crude fiber content (CF) was measured according to ISO 6865:2000 (ANKOM A200 fiber analyzer); ether extract (EE) content was determined according to ISO 6492:1999 (Soxhlet unit); crude protein content (CP) was analyzed according to ISO 5983-1:2005 and 5983-2:2005 (Foss Tekator™ digester and Kjeltect™ 2200 Auto distillation unit); neutral detergent fiber (NDF) was measured according to (ISO 16472:2006); acid detergent fiber (ADF) and lignin (ADL) were measured according to ISO 13906:2008 (ANKOM A200 fiber analyzer); starch content (ST) was measured according to ISO 6493:2000 (KRÜSS Polarimeter P1000); and non-structural carbohydrate (NSC) was calculated using the model ( $NFC\% = 100 - (NDF\% + CP + EE\% + ASH\%)$ ). Net energy for lactation (NEL) was calculated using a mathematical model according to Weiss (1998) and Jayanegara et al. (2019). The apparent digestibility of TMR dry matter and nutrients was analyzed using the indicator method, which involved measuring the content of acid detergent lignin (ADL) and acid-insoluble ash (AIA) following ISO standards 13906:2008 and 5985:2002. The Penn State Particle Separator (PSPS, ver. 2013) was used to analyze the size and distribution of particle fractions in

total mixed rations (on an as-fed basis). The PSPS setup includes four compartments containing sieves of 19 mm, 8 mm, and 4 mm mesh sizes, along with a flat bottom. Using sieving techniques (Lemmers et al. 1996) and formula calculations (Heinrichs, 2013), the physical effectiveness factor (pef) and the physically effective fiber (peNDF) were determined based on the material retained on the 8 mm and 4 mm sieves ( $peNDF_{8mm} = pef_{8mm} \times NDF\%$ ;  $peNDF_{4mm} = pef_{4mm} \times NDF\%$ ). The *in vitro* dry matter digestibility (IVDMD) method simulates rumen conditions (Goering and Van Soest, 1970). Approximately 0.5 g of pre-dried samples ground to 1 mm were placed in F57 bags and incubated at  $39.5 \pm 0.5$  °C in rumen fluid (pH = 6.80) for 48 hours using the Ankom® Daisy II Incubator under anaerobic conditions. The liquid content of the rumen was collected using an esophageal probe from cows fed TMR rations. IBM® SPSS Statistics software (Version 20.0) was used for the statistical analysis. Descriptive statistics reported mean values  $\pm$  standard deviations for TMR and fecal samples across five and three replicates. An analysis of variance (ANOVA) was conducted to evaluate differences between treatment means, followed by Tukey's LSD test for multiple comparisons ( $P < 0.05$ ).

## RESULTS AND DISCUSSION

The structure and chemical composition of the ingredients of TMR treatments are detailed in Tables 1 and 2. The dry matter content in diets  $T_c$  (47.1%) and  $T_1$  (46.8%) was not significantly different, while diet  $T_2$  had a noticeably lower dry matter content of 43.1% ( $P < 0.05$ ). Nutrient concentrations in diets  $T_c$  and  $T_1$  were similar, indicating that extended grinding time had little effect on nutritional parameters. Significant differences were observed in diet  $T_2$ , which showed variations in parameters such as ash content (8.19%), organic matter (91.81%), and protein (18.08%). Starch content was consistent across all diets ( $P = 0.299$ ), providing a reliable energy source ( $P = 0.182$ ), while crude fat content was around 4% ( $P = 0.134$ ) and ADF was approximately 19% ( $P = 0.212$ ), meeting the NRC (2001) requirements.

**Table 1.** The chemical composition of complete mixed ration ingredients (dry matter basis)

Parameters, %	Meadow hay	Corn silage	Grass haylage	Conc. mix. *	Conc. mix. **	Conc. mix. ***
Dry matter	94.51	31.49	22.82	87.00	87.60	89.10
Organic matter	93.48	92.22	88.59	91.59	91.34	82.92
Crude ash	6.52	7.78	11.41	8.41	8.66	17.08
Crude protein	5.36	7.18	6.61	22.57	25.50	41.77
Ether extract	2.80	3.83	3.66	7.72	6.22	3.42
NDF	58.05	41.19	68.43	12.79	14.29	14.66
ADF	38.02	25.98	42.83	5.72	7.23	7.49
Hemicellulose	20.03	15.21	25.60	7.05	7.07	7.17
Cellulose	33.21	23.24	39.41	4.43	5.27	6.30
Lignine	4.80	2.75	3.42	1.30	1.95	1.19
Starch	-	28.27	-	35.17	34.54	8.01
NFC	27.27	40.03	9.89	48.49	45.32	23.06
NEL (MJ/kg)	4.75	6.75	4.08	8.29	8.15	8.10
pH	-	3.80	3.91	-	-	-
Lactic acid	-	6.90	8.16	-	-	-
Acetate acid	-	2.44	2.32	-	-	-
Butyric acid	-	0.09	0.62	-	-	-
Ammonia	-	0.12	0.40	-	-	-

NDF – neutral detergent fibers; ADF – acidic detergent fibers; NFC – non-fiber carbohydrates; TDN – total digestible matter; NEL – net energy for lactation; MJ – megajoule; kg – kilogram.

\* Concentrate mixture ingredients: cereals and their by-products, oil by-products, monocalcium phosphate, iodized salt, limestone, premix (minimum Ca 0.9-1.1%; P 0.6-0.8%; Na 0.2-0.3%; Vit. A 20000 IU; K 3.0 mg; B<sub>1</sub> 2.0 mg; B<sub>12</sub> 5.0 mg; choline 0.1 mg; Fe 100 mg; Zn 120 mg; I 2.0 mg; Se 0.3 mg; BHT (BHA) 100 mg, yeast cells (*Saccharomyces cerevisiae*, strain Sc47) 10<sup>10</sup>/kg; propylene glycol.

\*\* Concentrate mixture ingredients (pelleted): corn 32.0%; wheat 5.0%; soybean meal 25.0%; full-fat soy (toasted) 5.0%; rapeseed meal 10.0%; sugar beet molasses 2.0%; rumen-protected methionine hydroxy analogue 0.2%; fat (bypass) 2.5%; protein (bypass) 5.0%; salt 1.2%; mono propylene glycol 8.0%; CLA (conjugated linoleic acid) 1.4%; limestone 1.6%; mono-calcium phosphate 0.3%; magnesium oxide 0.3%; premix 0.5% (vit. A 15750 IU; D3 4200 IU; E 91 mg; copper sulfate pentahydrate 42 mg; zinc oxide 126 mg; BHT, EQ 126 mg).

\*\*\* Concentrate mixture ingredients: wheat 2.0%; sunflower meal 30.0%; soybean meal 36.0%; rapeseed meal 15.0%; yeast 5.0%; salt 2.0%; limestone 5.5%; phosphonal forte (monoammonium phosphate 69%, bentonite 31%); 2.4%; slow-release urea 1.2%; magnesium oxide 0.25%; premix 0.75% (vit. A 33750 IU; D3 9000 IU; E 195 mg; copper sulfate pentahydrate 90 mg; BHT, EQ 300 mg).

In this study, the nutrient content of the first two rations did not significantly differ, while the third ration showed marked differences due to changes in feed proportions (Table 2). The dry matter content was 47.0% for T<sub>c</sub> and 46.7% for T<sub>1</sub>, while the T<sub>2</sub> ration had a lower value of 43.1% due to the exclusive use of corn silage. The rations generally met the NRC (2001) standards,

although there were slight deficits in protein content in the first two meals. The first two meals had similar NDF contents of 32.16% and 33.99%, while the third meal had a lower NDF level of 30.69%. The percentage of NDF from forages in all rations exceeded the minimum requirement of 75%. The starch and crude fat contents remained consistent across all meals.

**Table 2.** Ingredients structure, and chemical composition of total mixed rations for dairy cows during early lactation (DM basis)

Item	TMR treatments			P*	SEM**
	T <sub>c</sub>	T <sub>1</sub>	T <sub>2</sub>		
Ingredients, %					
Grass hay	14.4	14.4	-		
Corn silage	39.9	39.9	52.8		
Grass haylage	3.5	3.5	3.5		
Conc. mixture1	17.7	17.7	16.0		
Conc. mixture2	17.8	17.8	18.4		
Conc. mixture3	6.8	6.8	9.3		
Forage ratio	57.8	57.8	56.4		
Concentrate ratio	42.2	42.2	43.6		
Chemical composition, % (mean±SD)					
Dry matter	47.10 <sup>a</sup> ± 0.44	46.71 <sup>a</sup> ± 0.53	43.09 <sup>b</sup> ± 0.42	0.000	0.343
Crude Ash	7.73 <sup>a</sup> ± 0.09	7.71 <sup>a</sup> ± 0.26	8.19 <sup>b</sup> ± 0.04	0.010	0.131
Acis-insoluble ash	1.14 <sup>a</sup> ± 0.02	1.14 <sup>a</sup> ± 0.03	0.91 <sup>b</sup> ± 0.05	0.000	0.029
Organic matter	92.37 <sup>a</sup> ± 0.09	92.43 <sup>a</sup> ± 0.27	91.81 <sup>b</sup> ± 0.04	0.007	0.134
Crude protein	15.33 <sup>a</sup> ± 0.13	15.44 <sup>a</sup> ± 0.05	18.08 <sup>b</sup> ± 0.09	0.000	0.077
Ether extract	4.24 ± 0.04	4.05 ± 0.18	3.92 ± 0.23	0.134	0.136
NDF	32.17 <sup>ab</sup> ± 2.20	33.99 <sup>a</sup> ± 0.63	30.69 <sup>b</sup> ± 1.52	0.044	1.298
ADF	19.29 ± 0.42	18.61 ± 0.45	19.13 ± 0.44	0.212	0.358
ADL	2.62 <sup>a</sup> ± 0.08	2.49 <sup>a</sup> ± 0.07	2.81 <sup>b</sup> ± 0.06	0.019	0.152
NFC	38.43 ± 2.22	38.50 ± 0.56	39.69 ± 1.29	0.258	1.239
Starch	28.79 ± 2.92	26.43 ± 0.34	27.22 ± 0.33	0.299	1.395
NEL, MJ/kg	6.74 ± 0.03	6.78 ± 0.03	6.75 ± 0.02	0.182	0.022

DM – dry matter; NDF – neutral detergent fiber; ADF – acid detergent fiber; NFC – non-fiber carbohydrates; NEL – net energy of lactation; \* level of significance; \*\* standard mean error

However, the energy values of the meals were slightly lower than the NRC recommendations for early lactation, which is 6.8 MJ/kg DM, due to the high proportion of silage (both corn and grass) included in the rations. Furthermore, the levels of pH (3.8), NH<sub>3</sub> (1.7% of SP), and lactic acid (6.9% of DM) in corn silage fell within the recommended ranges.

Rations T<sub>c</sub> and T<sub>1</sub> have identical structures and chemical compositions, with the only difference being the particle size and distribution due to their preparation times, which are 5 minutes for ration T<sub>c</sub> and 10 minutes for ration T<sub>1</sub>. Ration T<sub>2</sub> has a similar particle size distribution to T<sub>1</sub>, but its structure has been slightly modified to enhance digestibility. Unlike the other rations, T<sub>2</sub> does

not contain hay; this was compensated for by increasing the corn silage content. Adjustments were made to boost the protein content in the concentrate portion while slightly reducing the energy level. As a result, the ratio of forage to concentrate changed from 58:42% in rations  $T_c$  and  $T_1$  to 56:44% in ration  $T_2$ . Despite these adjustments, all rations maintained their voluminous character. The longer preparation times for rations  $T_1$  and  $T_2$  led to slightly lower effectiveness values (pef) and less physically effective fibers (peNDF), attributable to the lower NDF content.

Overall, the modifications to improve digestibility also resulted in lower values for effective fibers. Sieving particles for TMR size and distribution analysis is a modern technique that enhances ration design. Although it has not been implemented in our production practices, it is a highly desirable addition to chemical analysis for improving forage nutritive assessment. The particle size distribution remaining on the sieves and the average particle size for the tested forages and TMRs are presented in Table 3. The percentages of corn silage particles retained on the sieves are as follows: 1.8%, 57.8%, 38.0%, and 2.4%. With an average particle size of 8.94 mm, it is classified as finely ground forage (6-10 mm). Compared to the PSPS recommendations for corn silage, the proportion of particles larger than 19 mm was lower at 1.8%, while the proportion of particles larger than 4 mm was higher, averaging 38%. For reference, Kmicikewycz and Heinrichs (2015) reported a slightly higher proportion of finer particle fractions in their research. The proportions of the grass haylage fractions were 41.1%, 47.4%, 10.8%, and 0.7%. The average particle length measured 16.10 mm, which exceeds the upper limit for moderately chopped coarse forages, set at 10-15 mm. As preparation time for the rations increased, the proportions of particle fractions for the 19.0 mm, 8.0 mm, and 4.0 mm sieves and the bottom sieve decreased. The upper 19.0 mm sieve exhibited particle fractions of 7.1%, 2.0%, and 2.2% for the rations, respectively, aligning with the recommended 2-8% range. For the 8.0 mm sieve, the percentages were 47.37%, 39.13%, and 37.90%, fitting within the 30-50% range. The lower 4.0 mm sieve averaged 18.8%, 23.1%, and 25.0%, slightly above the recommended 10-20%

range. At the bottom sieve (< 4.0 mm), proportions were 28.8%, 35.9%, and 34.8%, consistent with  $T_1$  and  $T_2$  recommendations (30% to 40%). In summary, there were notable variations in TMR particle sizes between ration  $T_c$  and the other two rations. Stojanović et al. (2009) found a positive correlation between forage particle size and distribution and their contribution to ration structure, affecting the overall ration values. Table 3 shows that extended grinding time significantly increased finer particles in the  $T_1$  ration compared to the control  $T_c$  ration, resulting in a lower effectiveness factor (pef). This also led to reduced physically effective fiber and a lower proportion of peNDF relative to total NDF on the 4 mm and 8 mm sieves. The geometric mean particle size reduced from 6.9 mm (control) to 5.5 mm ( $T_1$ ) and 5.4 ( $T_2$ ), indicating extended mechanical preparation.

In contrast, the changes in the third meal ( $T_2$ ) did not significantly affect particle size distribution or effectiveness factor compared to  $T_1$ . These parameters had no significant differences, as the meals were prepared in the same 10-minute intervals. In the third meal, the structural change reduced NDF content, directly impacting the amount of physically effective fibers compared to the  $T_c$  and  $T_1$ . Assessment of the particle size and distribution of ration feeds can vary due to differing farm production conditions that impact feeding performance, including total chewing time, salivation, buffering, feed passage, consumption, and digestibility (Stojanović et al., 2011). Slightly higher values were observed on the 4 mm sieve for meals  $T_1$  and  $T_2$ , while lower values were noted at the bottom of the sieve for ration  $T_c$ . According to the PSPS recommendations (Heinrichs and Kononoff, 2002), all the obtained values fall within acceptable ranges for optimal TMR particle distribution. Higher values were found on the 4 mm sieve for meals  $T_1$  and  $T_2$ , while lower values were noted for meal  $T_c$ . Considering all observations, the ratios can fall within acceptable ranges based on PSPS recommendations (Heinrichs and Kononoff, 2002) for optimal TMR particle distribution. The average particle lengths for all three rations were 6.91 mm, 5.48 mm, and 5.49 mm, respectively. Therefore, the first ration can be classified as fine, while the other two are categorized as

too finely. Finely ground rations may negatively impact digestive health and production performance. In a study published by Heering et al. (2023), increasing the mixing time of TMR resulted in a decrease in the geometric mean particle size as well as a decrease in the effective fiber (peNDF), which is confirmed by this study. Zebeli et al. (2010) found that the proportion of  $\text{peNDF}_{8\text{mm}}$  in the total mixed ration is crucial for maintaining regular rumen activity, particularly fiber digestion, with recommended values ranging from 10.9% to 18.8%. The optimal recommended  $\text{peNDF}_{8\text{mm}}$  content in the diet should be between 13.7% and 17.0% to 18.5% to maintain an ideal pH of 6.2. Higher levels may reduce dry matter intake (DMI) and limit feed consumption. Controversies surrounding the relationship between peNDF, rumen pH, and dry DMI reveal the challenges in determining the optimal peNDF levels for high-producing lactating cows at an 8 mm particle size sieve. Zebeli et al. (2010) indicate that the proper content of  $\text{peNDF}_{8\text{mm}}$  in the diet indicates overall chewing activity and physical rumen filling. The optimal range for peNDF at this size is 14.9 - 18.0%. Among the tested diets, only the  $T_c$  ration meets this criterion

(17.52%). In contrast, the  $T_1$  and  $T_2$  have lower  $\text{peNDF}_{8\text{mm}}$  values (13.97% and 12.41%), respectively. For the peNDF content at a 4 mm particle size, the NDF content in the tested rations is 73.21%, 64.17%, and 65.17%. All of these fall within the defined range for this parameter (60-70%). Mertens (1997) reported that to maintain a 3.4% milk fat concentration, the peNDF at 4 mm should be at least 20.0%. For optimal rumen pH ( $\text{pH} \geq 6$ ), peNDF must also be a minimum of 20%, particularly in concentrated diets (50-60%). Mertens (1997b) recommends lactating cow diets contain 22.3% peNDF at 4 mm to ensure adequate chewing activity and rumen function. Zebeli et al. (2006a) suggest the ideal peNDF value is around 20%. The diets analyzed showed peNDF values of 23.55%, 21.81%, and 20.00%, while peNDF at 8 mm ranged from 17.52% in the first diet to 12.41% in the third. Tables 4 and 5 present feces' chemical composition and nutrients' apparent digestibility throughout the digestive tract. Generally, feces from experimental treatments  $T_1$  and  $T_2$  exhibit slightly lower DM values. Additionally, a slight increase in ash, ADF, AIA, ADL, and crude protein values was observed across the treatments.

**Table 3.** Particle size (mm) and distribution (%) of forage and total mixed rations ( $T_c$ ,  $T_1$ , and  $T_2$ ) fed to early lactation cows (as-fed basis)

Screen	Corn silage	Grass haylage	Treatments*		
			$T_c$	$T_1$	$T_2$
Upper sieve (19 mm)	1.8 ± 0.7	41.1 ± 0.8	7.1 <sup>a</sup> ± 1.3	2.0 <sup>b</sup> ± 0.9	2.2 <sup>b</sup> ± 0.6
Middle sieve (8 mm)	57.8 ± 4.0	47.4 ± 0.6	47.4 <sup>a</sup> ± 1.0	39.1 <sup>b</sup> ± 1.7	37.9 <sup>b</sup> ± 2.4
Lower sieve (4 mm)	38.0 ± 4.2	10.8 ± 0.5	18.8 <sup>a</sup> ± 0.7	23.1 <sup>b</sup> ± 0.8	25.0 <sup>b</sup> ± 2.1
Bottom pan	2.4 ± 0.5	0.7 ± 0.2	26.8 <sup>a</sup> ± 0.9	35.9 <sup>b</sup> ± 1.4	34.8 <sup>b</sup> ± 1.9
Particle size, mm**	8.1 ± 1.9	16.1 ± 1.8	6.9 <sup>a</sup> ± 2.4	5.5 <sup>b</sup> ± 2.3	5.4 <sup>b</sup> ± 2.2
$\text{pef}_{4\text{mm}}$	0.98 ± 0.1	0.99 ± 0.1	0.73 <sup>a</sup> ± 0.1	0.64 <sup>b</sup> ± 0.1	0.65 <sup>b</sup> ± 0.1
$\text{pef}_{8\text{mm}}$	0.60 ± 0.1	0.88 ± 0.1	0.54 <sup>a</sup> ± 0.1	0.41 <sup>b</sup> ± 0.1	0.40 <sup>b</sup> ± 0.1
$\text{peNDF}_{4\text{mm}}$	40.2 ± 0.2	67.9 ± 0.2	23.5 <sup>a</sup> ± 0.3	21.8 <sup>b</sup> ± 0.5	20.0 <sup>c</sup> ± 0.6
$\text{peNDF}_{8\text{mm}}$	24.6 ± 1.9	60.5 ± 0.2	17.5 <sup>a</sup> ± 0.2	14.0 <sup>b</sup> ± 0.3	12.4 <sup>b</sup> ± 0.7
$\text{peNDF}_{4\text{mm}}$ %NDF	97.7 ± 0.5	99.3 ± 0.2	73.2 <sup>a</sup> ± 0.9	64.2 <sup>b</sup> ± 1.4	65.2 <sup>b</sup> ± 1.9
$\text{peNDF}_{8\text{mm}}$ %NDF	59.7 ± 4.7	88.4 ± 0.3	54.4 <sup>a</sup> ± 0.7	41.1 <sup>b</sup> ± 0.8	40.4 <sup>b</sup> ± 2.2

$T_c$ ,  $T_1$  and  $T_2$  – control, first and second treatment; pef – physical effectiveness factor; NDF – neutral detergent fiber; peNDF – physically effective NDF; \* level of significance at 0.05; \*\* geometric mean.

**Table 4.** Chemical composition of experimental cow's feces

Parameter, %	Treatment			P	SEM
	T <sub>c</sub>	T <sub>1</sub>	T <sub>2</sub>		
Dry matter	14.40 <sup>a</sup> ± 0.18	13.80 <sup>ab</sup> ± 0.57	13.55 <sup>b</sup> ± 0.86	0.029	0.350
Crude ash	11.94 ± 0.52	12.63 ± 1.40	13.11 ± 1.29	0.233	0.659
AIA	2.69 <sup>a</sup> ± 0.18	3.11 <sup>b</sup> ± 0.33	3.13 <sup>b</sup> ± 0.36	0.040	0.173
Organic matter	88.06 <sup>a</sup> ± 0.52	87.36 <sup>a</sup> ± 1.40	86.89 <sup>a</sup> ± 1.29	0.233	0.659
Crude protein	13.92 <sup>ab</sup> ± 1.17	13.12 <sup>a</sup> ± 0.55	14.19 <sup>b</sup> ± 1.25	0.030	0.599
NDF	59.51 ± 2.31	58.59 ± 1.60	57.32 ± 2.64	0.261	1.285
ADF	29.37 <sup>a</sup> ± 2.47	33.54 <sup>b</sup> ± 0.94	32.14 <sup>b</sup> ± 2.11	0.007	1.130
ADL	6.91 <sup>a</sup> ± 0.78	7.55 <sup>ac</sup> ± 0.35	8.39 <sup>b</sup> ± 0.69	0.004	0.365
NFC	19.17 <sup>a</sup> ± 4.52	15.90 <sup>ab</sup> ± 0.51	15.03 <sup>b</sup> ± 0.87	0.041	1.544

AIA – acid insoluble ash; NDF – neutral detergent fiber; ADF – acid detergent fiber; ADL – acid detergent lignin; NFC – non-fiber carbohydrates; P – level of significance; SEM – standard mean error

The assessment of nutrient digestibility revealed an increase in digestibility for all essential parameters included in the study (Table 5). Similar findings on apparent digestibility using the indicator method were reported by Stojanović et al. (2014) and Yang and Beauchemin (2005), although they contradict some other studies (Fernandez et al., 2004). Similar research and results on the chemical composition of feces, but on fattening cattle, were published by Cunha et al. (2021). This study also confirms that prolonged mixing of TMR diets numerically and statistically negatively affects the digestibility of NDF and ADF while positively affecting the digestibility of CP and NFC. The conflicting results regarding the impact of peNDF on digestibility can be attributed to various nutritional factors. Finely ground feed particles, often used in complete mixed rations, increase the active surface area for microorganisms, thus enhancing nutrient digestibility. However, fine particles generally lead to a shorter retention time of digesta in the reticulorumen, which may reduce digestibility in the rumen. The fermentability of concentrate feeds may also contribute to this increase in digestibility. Previous studies suggest that barley-based concentrates can improve the overall digestibility of finely ground diets. In this study,

the higher digestibility observed in diet T<sub>2</sub>, despite its low peNDF content, resulted from increased corn silage and concentrate feed. Yang and Beauchemin (2006) noted that bacterial nitrogen (N) flow through the small intestine increases quadratically with rising peNDF levels in the diet. This finding underscores that concentrate fermentability is a key nutritional factor. At the same time, the particle size of the diet, which determines the DMI, indirectly affects microbial protein (MP) biosynthesis. Additionally, the reduced microbial protein synthesis linked to lower peNDF content in the diet is supported by the slightly lower crude protein digestibility coefficient observed in ration T<sub>2</sub>. The influence of peNDF on total digestibility is much more pronounced in fiber digestion than in starch digestion. In cases of starch, low rumen degradation can be compensated for by high intestinal digestion (Beauchemin et al., 2001). Conversely, the amount of fiber that can be digested in the small intestine is significantly limited. The apparent digestibility results demonstrate that the digestibility of dry matter and related nutrients is higher for diets consisting of smaller particles with lower peNDF content, based on both indicators (Table 5). Similar findings regarding apparent digestibility were reported by Haselman et al. (2019).

**Table 5.** The impact of reducing peNDF on digestibility in early lactation rations

Parameter, %	Treatment			P	SEM
	T <sub>c</sub>	T <sub>1</sub>	T <sub>2</sub>		
IVDMD <sub>48h</sub>	63.45 <sup>a</sup> ± 0.82	63.91 <sup>ab</sup> ± 1.56	65.09 <sup>b</sup> ± 1.17	0.071	0.704
Ash-insoluble in acid as a marker					
Dry matter	56.07 <sup>a</sup> ± 1.45	56.45 <sup>a</sup> ± 1.27	58.62 <sup>b</sup> ± 1.28	0.010	0.772
Organic matter	65.94 <sup>a</sup> ± 0.67	66.37 <sup>a</sup> ± 0.62	68.39 <sup>b</sup> ± 0.68	0.000	0.381
Crude protein	67.19 <sup>a</sup> ± 1.21	67.88 <sup>a</sup> ± 1.28	69.76 <sup>b</sup> ± 2.34	0.045	0.959
NDF	40.56 ± 5.05	41.41 ± 2.77	39.98 ± 3.09	0.683	2.179
ADF	36.38 ± 1.94	36.25 ± 1.38	35.93 ± 1.46	0.884	0.931
NFC	85.45 <sup>ab</sup> ± 1.96	85.29 <sup>a</sup> ± 0.86	86.89 <sup>b</sup> ± 0.26	0.081	0.776
Acid detergent lignin as a marker					
Dry matter	55.02 <sup>a</sup> ± 0.54	55.05 <sup>a</sup> ± 1.24	56.31 <sup>b</sup> ± 0.79	0.040	0.521
Organic matter	64.66 <sup>a</sup> ± 0.81	65.89 <sup>b</sup> ± 0.28	67.05 <sup>c</sup> ± 0.79	0.000	0.390
Crude protein	66.46 <sup>a</sup> ± 0.79	67.30 <sup>a</sup> ± 0.81	69.03 <sup>b</sup> ± 1.42	0.002	0.606
NDF	39.04 ± 4.66	39.88 ± 2.48	37.25 ± 1.87	0.341	1.770
ADF	34.26 ± 1.52	34.00 ± 2.36	34.14 ± 1.76	0.973	1.104
NFC	84.81 ± 2.03	85.25 ± 0.65	86.29 ± 0.54	0.151	0.734

IVDMD – *In vitro* dry matter digestibility at 48 hours; NDF – neutral detergent fiber; ADF – acid detergent fiber; NFC – nonfiber carbohydrates; P – significant level at 0.05; SEM – standard mean error.

However, the digestibility results for the third ration vary depending on the indicator applied. Specifically, when the AIA indicator is utilized, the digestibility values continue to rise; however, a contrasting trend is observed with the ADL indicator, which yields slightly lower values on average based on the nutrient tested. Previous experience in measuring digestibility through internal indicators (such as AIA, ADL, Cr<sub>2</sub>O<sub>3</sub>, etc.) shows that the AIA method generally provides more reliable measurement results, as it is more resistant to digestive agents.

In contrast, ADL tends to yield relatively lower digestibility values due to the partial degradation of lignin observed in the gastrointestinal tract (Thonney et al., 1979; Taniguchi et al., 1985). Therefore, the preference for AIA as a digestibility indicator is justifiable. The digestibility results align with the concentrations of ADL

and AIA found in feces, which increase across meals (6.91%; 7.55%; 8.39% and 2.69%; 3.11%; 3.13%). The presence of silages as the only forage feed in the third ration influenced the increase in starch concentration while decreasing the concentrations of NDF and ADF, indicating that the increased protein content can explain the improved ration digestibility. It is also suggested that finely chopped rations may lead to a higher passage rate.

This research focuses on fiber residence time in the rumen, as its degree of digestion is relatively limited after leaving the reticulo-rumen. In contrast, protein and starch continue to degrade with the action of intestinal enzymes. Measuring the digestibility of nutrients at the rumen level would yield more reliable and credible results than measurements taken across the entire digestive tract, which is outside the scope of this study. Heering

et al (2020) determined the pronounced effects of a slight increase in the concentration of peNDF in the diet, among other things, on the digestibility of nutrients in cows fed diets with a high content of peNDF. Also, the results of research by Li et al. (2020) support this research, where they confirm the positive impact of lower fiber effectiveness on better total tract digestibility. The increase in the digestibility of DM and OM in both markers, but also in IVDMD, resulted in increased digestibility of SP, NFC (mostly starch), but also due to a slight change due to an increase in the C:F ratio. Li et al. (2020) confirmed similar results in their research. Opposite results on the digestibility of NDF and ADF in TMR diets with prolonged mixing (7 vs. 14 min) were published by Gümüş and Bayram (2019). In conclusion, it can be stated that reducing particle size positively affects nutrient utilization in TMR rations  $T_1$  and  $T_2$ . In conclusion, this study confirms that prolonged mixing of TMR rations numerically and statistically negatively affects the digestibility of NDF and ADF while positively affecting the digestibility of CP and NFC, i.e., overall DM, both in marker and *in vitro* analyses. It is also assumed that a significant portion of nutrients is digested outside the rumen. Chesson and Forsberg (1997) note that grinding feeds increases the active surface area for microbial action, thereby enhancing digestibility. This is supported by the finding that, on average, about 60% of NDF is present in feces from all three meals, with no significant statistical differences. In a similar experimental setup, Kononoff and Heinrichs (2003) reported a lower average of 45.6% NDF. According to the model proposed by Zebeli et al. (2010), optimal digestibility of ADF is achieved by feeding diets with a minimum of 13.7% physically effective NDF with a particle size of 8 mm, the lower limit for lactating cows. An increase in NDF and ADF digestibility with a decrease in particle size, particularly with a higher concentrate level in the diet, is typical for lactating cows during early lactation (Stojanović et al., 2013).

## CONCLUSION

Based on particle size and peNDF content, these rations have been categorized as either fine or overly finely ground. Previous literature suggests that the negative effects of low fiber effectiveness can be mitigated by including a significant proportion of roughage in the ration (greater than 50%). Minor changes to the ration's structure did not significantly impact the average particle distribution. Reducing fiber effectiveness in the  $T_1$  ration did not affect overall digestibility, as evaluated using different markers. Using silage as the sole source of roughage in ration  $T_2$  reduced the peNDF content below recommended levels. It also positively affected the digestibility of dry matter (DM), organic matter (OM), and crude protein (CP). These findings highlight the importance of preserving forages such as hay, silage, and haylage in an appropriate physical form, following PSPS recommendations. This is particularly crucial since the physical form of these feeds significantly influences the formulation of Total Mixed Ration (TMR) diets. The effectiveness (fineness) of the diet should be assessed alongside the chemical characteristics of fiber (NDF), but it must also be considered within a broader context.

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