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Research Article

The Effects of Physically Effective Neutral Detergent Fibre Content on Growth Performance and Digestibility in Beef Cattle Fed with Total Mixed Ration

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Abstract

The objective of this study was to investigate the effects of physically effective neutral detergent fibre (peNDF) content on growth performance and digestibility in beef cattle fed with total mix ration (TMR). A total of 54 six-month-old male Holstein beef cattle (averaged weight of 280 kg) were divided into 3 groups each consisting of 18 cattle. Feed ingredients were added to the TMR wagon as follows; wheat straw, alfalfa hay, barley, corn, cotton seed meal, conventional beef feeding, corn silage, beet pulp, molasses and feed additives. TMR was offered daily to animals. The dietary treatments included; a) TMR diet mixed for 7 min (T1); b) TMR diet mixed for 14 min (T2), and c) free choice diet (FCD). The same ingredients feeds of TMR was given to the T1 and T2 groups but in different mixed times. Alfalfa hay and calf grower feed were separately offered animals in FCD. End of first month of trial, the daily feed intake (DFI) and dry matter intake (DMI) were significantly lower in the cattle that received FCD. At the end of the second month, the daily feed intake and dry matter intake were the highest in T1 diets among all the groups. There was no significant effect of different mixing times on n 48-h NDF digestibility (NDFD48) and ADF (ADFD48) digestibility of TMR. The lowest ration cost of 1 kg daily gain was observed for T2 and the daily feed intake cost was lower for FCD group than T1 and T2. It was concluded that mixing time had an effect on dry matter intake (DMI), average daily gain (ADG).

Keywords: *Feedlot cattle, In vitro digestibility, Particle size, peNDF, Total mix ration*

Toplam Karma Yemle Beslenen Besi Sığırlarında Fiziksel Etkin Nötral Deterjan Lifin Büyüme Performansına ve Sindirilebilirlik Üzerine Etkisi

Öz

Bu çalışmada toplam karma rasyon (TMR) ile beslenen besi sığırlarında fiziksel etkin nötral deterjan lifin (peNDF) büyüme performansına ve sindirilebilirlik üzerine etkisi incelenmiştir. Altı aylık yaştaki toplam 54 Holstein erkek besi sığırı (ortalama ağırlıkları 280 kg), her biri 18'er hayvandan oluşan 3 gruba ayrılmıştır. Yem bileşenleri TMR vagonuna; buğday samanı, yonca kuru otu, arpa, mısır, ayçiçeği küspesi, klasik besi yemi, mısır silajı, şeker pancarı posası ve katkı maddeleri sırası ile eklenmiştir. TMR hayvanlara günlük olarak sunulmuştur. Deneme grupları a) 7 dakika karışan TMR (T1); b) 14 dakika karışan TMR ve c) serbest seçenekli yemlemeden (SSY) oluşmuştur. T1 ve T2 grubuna verilen TMR'nin yem bileşenleri aynı iken karışma zamanları farklı olmuştur. Yonca kuru otu ve buzağı büyütme yemi SSY grubuna ayrı olarak sunulmuştur. Denemenin birinci ayı sonunda günlük yem tüketimi (GYT) ve günlük kuru madde tüketimi (GKMT) SSY grubunda önemli derecede düşük bulunmuştur. İkinci ayın sonunda günlük yem tüketimi ve kuru madde tüketimi T1 grubunda en yüksek bulunmuştur. TMR karıştırma süresinin 48 saat NDF (NDFD48) sindirimi ve 48 saat ADF (ADFD48) sindirimi üzerine etkisi olmamıştır. 1 kg canlı ağırlık artışı için rasyon maliyeti en az T2 grubunda saptanmış ve günlük yem tüketim maliyeti FCD grubunda T1 ve T2'ye göre düşük çıkmıştır. Bu çalışmada karıştırma süresinin kuru madde tüketimi, canlı ağırlık artışı üzerine etkisi olduğu sonucuna varılmıştır.

Anahtar sözcükler: *Çiftlik hayvanı, In vitro sindirilebilirlik, Partikül boyutu, peNDF, Toplam karma rasyon*

INTRODUCTION

It is essential to improve management of agriculture and husbandry that will be very economical for the sustainability of the cattle industry. A large number of feeding systems has been used in feedlot management including total mix ration system, pasture system and conventional system [1]. Among these, TMR making is

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prepared to ensure balanced ration and a homogeneous ration for all the feed material [2]. The consistency of TMR can be dependent on many factors such as equipment condition, ingredient-mixing order, nutrient moisture and variability, which plays important roles in the production efficiency [3]. Total mix ration, or complete ration, is an important system for many feedlot performances e.g. daily gain (DG), feed intake and feed conversion ratio (FCR). TMR supplies the correct amount and a blend of balanced nutrients (energy/protein proportional) to cattle in a proper amount time. The advantages of TMR include that it allows cattle to consume the desired proportion of forages, increases feed efficiency, reduces risk of digestive upset and allows accuracy of diet formulation [4,5]. It is critical to point out that auditing of TMR must be controlled. The biggest problems are overfilling wagons, inadequate mixing time and improper loading of fluids while preparing TMR. Inadequate or extra-time mixing influences the feed particle size that stimulates rumination. The greater the amount of saliva they produce, the more their buffering capacity becomes [6]. It is possible to measure feed particle size that uses $peNDF_{\geq 4mm}$ and NDF content value of feeds [7]. $peNDF_{\geq 4mm}$ is the product of NDF concentration to the physical effectiveness factor (pef). Pef varies from 0 to 1. At 0 NDF, there is failure to stimulate chewing, and there is the maximum stimulation when Pef is 1 [8]. The Penn State particle Separator (PSPS) is being used at farms to determine the particle size and total mixed particles [7]. The model of PSPS consists of four screens with circular holes. When a TMR sample is analyzed with PSPS, four groups are formed; feed particle >19 mm (0.75 inch/upper sieve), feed particle >8 to 19 mm (0.31 inch/middle sieve), feed particles 4 mm to 8 mm (0.16 inch/lower sieve) and feed particles <4 mm (bottom pan). Poppi et al.[9] reported that feed particles retained on a 1.18-mm sieve had high resistance to passage from the rumen resulting in increasing chewing and rumination activity. Reduction of particle size increases the release rate from the rumen, and digestibility is reduced [7]. If the consistency of the ruminal mat is better, the passage of feed particles to the omasum is lower [8].

The objective of this study was to evaluate the effects of physically effective neutral detergent fibre content on live weight, dry feed intake, feed conversion ratio, daily gain, NDF and ADF digestibility in beef cattle.

MATERIAL and METHODS

This study was carried out from February to April in 2018 at a private feedlot farm in the province of Afyonkarahisar, located central Anatolia Turkey, 39° north latitude, 31° east longitude.

Experimental Unit

A total of fifty-four Holstein male beef cattle aged 6-7 months and weighed 280 kg were divided into 3 groups of 18 each in a generalized randomized block design based on their live weight. Before placing the male beef cattle to the stall, these animals were weighed on two consecutive days, and then, they were assigned to the groups. Accompanying the vaccination program, the study lasted 60 days, and among these days, the first 7 days constituted the adaptation period. The animals were kept in northside closed feeding pens kept in a shade area to protect them from north-east winds. The dimensions of the pens were 18 x 15 m, with 18 m^2 of concrete in front of the feed bunk. *Ad libitum* fresh water was provided during the experimental trial. The automatic float valve system was cleaned every week. A keystone was used as base that cleaned biweekly with a tractor. Light was provided from 18:00 h to 06:00 h in the pens throughout the study. The total mix ration was prepared as nutritional research council (NRC) requirements [10] by an expert in a horizontal De Laval wagon (12 m^3) with a digital weighing balance. The ration was formulated as monthly due to the variable nutrient requirement of beef cattle based on live body weight. The feed material was added to the TMR wagon as follows; wheat straw, alfalfa hay, barley, corn, cotton seed meal, conventional beef feeding, corn silage, beet pulp, molasses and feed additives. After adding all the ingredients to the TMR wagon, it was mixed for 7 min to prepare the T1 ration and 14 min to prepare the T2 ration. The TMR was offered daily to the animals for feeding. The dietary treatments included; a) TMR diet mixed for 7 min (T1); b) TMR diet mixed for 14 min (T2), and c) free choice diet (FCD). The same TMR ration was given to the T1 and T2 groups but in different times. The FCD group ration consists of alfalfa hay and calf grower feed that were separately offered to the animal. The FCD ration did not mixed in TMR wagon. The animals were fed twice a day in the morning (08:00) and evening (18:00). The feeds were delivered by more than 5-10% to the bunk needed for dry matter intake to ensure an *ad libitum* system. The residual of feeds given the other cows in farm, which was not in the experiment. times. The animals had free access to mineral blocks at all. DFI was measured by weighing feed offered and residue left over with 24 h during the study. FCR was calculated individually as LWG:DMI (kg of live weight gain divided by kg of DMI). The beef cattle were weighed by using a digital weighing machine 2 h before feed delivery at the beginning and every 4th week during the entire experimental period. The average daily gain (ADG) of each cattle was determined by dividing live weight gain by the number of days on feed.

Chemical Analyses and Digestibility

The feed samples were analyzed based on the methodology of the Association of Official Analytical Chemists (AOAC) [11] for DM (method 934.01), ash (method 942.05), ether extract (EE) (method 920.39) and N (method 954.01) contents. NDF and ADF were determined according to the method described by Goering and van Soest [12]. Crude fibre content was determined by the methods of Crampton and Maynard [13]. Non-fibrous carbohydrates (NFC) were calculated by difference NFC= 100 – (%NDF + %CP + %Fat + %Ash) according to the standards of the National Research Council [10]. Forty-eight-h *in vitro* true NDF and ADF digestibility ($NDFD_{48}$ and $ADFD_{48}$) values were determined using a Daisy II Incubator (Ankom Technology, NY, USA) described by Vogel et al.[14]. Approximately 0.5 g of each sample was put into F57 fibre bags (ANKOM Technology, NY, USA) and heat-sealed. The samples were placed into a digestion jar with two buffers and rumen fluid (Buffer A: KH_2PO_4, $MgSO_4$-$7H_2O$, NaCl, $CaCl_2$-$2H_2O$, and Urea; Buffer B: Na_2CO_3 and Na_2S-9H20). Rumen fluid was collected and mixed at Afyon Kocatepe University Animal Research Center from two cannulated nonlactating Brown Swiss that were fed a forage-based diet (60:40 forage:concentrate). After the inclusion of the rumen fluid, all jars were flushed with CO_2 and placed into a preheated incubator (39°C). The incubation process was continued for 48 h with agitation. After the incubation process, the samples were rinsed with cold tap water for about 10 min. Then, the $aNDF_{om}$ and ADF_{om} procedures were performed in a way previously described for Fibretherm FT12 (Gerhardt GmbH&Co. KG, Königswinter, Germany). The digestibility of each sample was then determined via weight differences before and after digestion.

Particle Size Analysis

The particle sizes of TMR were determined by using PSPS. The model of PSPS consisted of four screens with circular holes sized 19 mm (0.75 inch/Upper), 8 mm (0.31 inch/ Middle sieve), 4 mm (0.16 inch/lower sieve) and a bottom pan. Each TMR sample of about 1000 g was placed on the top of the PSPS box. On a flat surface, we shook the PSPS in the north-south direction 5 times, then rotated the box by a one-fourth turn. This series was repeated 8 times, for a total of 40 shakes so that the box was shaken 5 times for each set. The residual of particles in each sieve were weighted on digital scales. The values obtained in each sieve were recorded to calculate the physical effectiveness factor (pef) which was determined by adding particle size retained on the three boxes (19-8-4 mm). The $peNDF_{\geq 4mm}$ content of TMR was calculated by multiplying the neutral detergent fibre (NDF) content of TMR by pef [15]. The proportion of sample DM collected in the ≥4 mm sieve was commonly used as the physical effectiveness factor in the equation [16]. The particle sizes of TMR were determined to repeat 4 replicates per sample and average the results have a representative sample of TMR.

Statistical Analysis

The statistical analyses were carried out with SPSS (Statistical Package for the Social Sciences; Inc., Chicago, IL, USA). All data were subjected to statistical analyses using one-way ANOVA except for digestibility data. The differences among the groups were calculated using Duncan's test [17]. The *in vitro* NDFD and ADFD values of each TMR were evaluated using PROC T-TEST of SAS version 8.3 (SAS Institute Inc., Cary, NC, USA) after log-transforming the digestibility levels. The level of significance was taken as P<0.05 for all data.

RESULTS

The Ingredients and chemical composition of the total mix rations (T1 and T2) are presented in *Table 1*. The effects of $peNDF_{\geq 4mm}$ content on growth performance in male Holstein beef cattle are presented in *Table 2*. At the beginning of the study, the live weights were 218.52, 210.11 and 199.44 kg in T1, T2 and FCD, respectively. The live weights were 260.88, 264.72 and 243 kg in T1, T2 and FCD, respectively at the end of 30th days. At the end of the first month, the daily feed intake and dry matter intake were significantly lower (P<0.05) in the cattle that received FCD (7.57, 6.69 kg/day) than in those that received T1 (10.46, 8.12 kg/day) and T2 (11.03, 8.57 kg/day). Daily weight gain value was found as 1.41, 1.81 and 1.45 kg for the T1, T2 and FCD diets, respectively. The FCR value was 8.12, 8.57 and 6.69 for T1, T2 and FCD, respectively. There were significant differences among the treatments in terms of daily feed intake, dry matter intake and feed conversion ratio (P<0.05). The final live weights were 298.82, 308.61 and 286.11 kg in T1, T2 and FCD, respectively, at the end of the study. At the end of the second month, the daily feed intake and dry matter intake values were the highest in the T1 diets among all the groups *(Fig. 1)*. The mean daily feed intake and dry matter intake were very similar in T1 and T2 (12.78, 9.59; 12.77, 9.58), and these values were lower in the FCD (8.80, 7.77) group. The daily weight gain was the highest in T2 (1.46) followed by FCD (1.44) and T1 (1.26). FCR was lower in the FCD diets in comparison to the other diets.

In the first month *(Table 3)*, the proportion remaining on the upper part (19 mm of sieve size) in T1 was higher than that in T2. The percentage of particles retained on the 19-mm sieve decreased by increasing the mixing time of TMR. Forage particle size reduction resulted in increased DMI

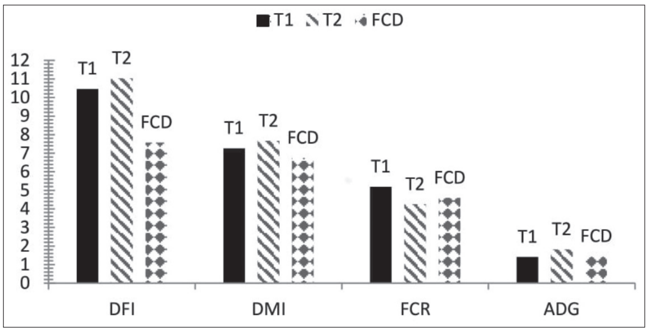

Fig 1. Growth performance in Holstein beef cattle. **DFI:** Daily feed intake; **DMI:** Dry matter intake; **FCR:** Feed conversion ration; **ADG:** Daily gain; **T1:** Total mix ration was mixed 7 min; **T2:** Total mix ration mixed 14 min; **FCD:** The animals was offered feed separetely

***Table 1.** The ingredients and chemical composition of total mix ration*

Diet Formulation/Months	Amount of Supplementation, kg as a Feed Basis	
	First Month	Second Month
Corn silage	0.50	1.00
Wheat straw	0.80	0.84
Alfalfa hay*	1.00	1.25
Barley	0.80	1.20
Corn	0.70	1,00
Sunflower oil	1.50	1.70
Molasses	0.40	0.40
Sugar beet pulp	1.00	1.35
Calf grower feed**	2.50	2.54
Limestone	0.05	0.05
Salt	0.02	0.02
Vitamin- mineral premix[1]	0.01	0.01
Concentrate: forage ratio	64.43;35.56	60.91;39.08
Chemical composition as DM	**First month**	**Second month**
Dry matter	77.70	76.38
Crude protein	18.37	17.17
Ether extract	4.61	4.30
Crude fibre	17.61	17.14
Ash	7.32	6.93
Nötral detergent fibre	32.49	32.04
Acid detergent fibre	20.53	15.26
Hemicellulose	11.96	16.78
[2]Non-fibre carbohydrate	37.21	39.56
[3]Nitrogen free extract (NFE)	52.09	54.56

*[1] Each kilogram of vitamin-mineral mix contains 12.000.000 IU A vit, 20.000 mg E vit, 50.000 mg Mn, 50.000 mg Fe, 50.000 mg Zn, 10.000 mg Cu, 800 mg I, 150 mg Co, 150 mg Se; [2] NFC= 100 – (%NDF + %CP + %EE + %Ash); [3] NFE= 100- (CP+CF+EE+Ash); * Alfalfa hay: DM: 89.92; CP 16.65; EE: 2.35; CF: 21.18; Ash: 9.52; NDF: 41.15; ADF: 29.95; ** Calf grower feed: DM: 90.51; CP 19.21; EE: 3.76; Ash: 5.02; NDF: 18.35; ADF: 9.38*

***Table 2.** Effects of $peNDF_{\geq 4mm}$ content on growth performance in Holstein beef cattle ration*

Groups*	First Month		
	T1[1]	T2[2]	FCD[3]
Initial live weight (kg)	218.52±4.99	210.11±6.30	199.44±3.87
Live weight (kg, 30th day)	260.88±4.46	264.72±4.97	243±4.31
Daily feed intake (kg/d)	10.46±0.64^a	11.03±0.16^a	7.57±0.20^b
Dry matter intake (kg/d)	8.12±0.50^a	8.57±0.12^a	6.69±0.18^b
Feed conversion ratio (DMI/DG) kg/kg	5.80±0.35^a	4.76±0.06^b	4.56±0.12^b
Daily gain (kg)	1.41	1.81	1.45
Groups*	**Second Month**		
	T1	**T2**	**FCD**
Final live weight (kg, 60th day)	298.82±5.10	308.61±4.46	286.11±4.40
Daily feed intake (kg/d)	12.78±0.22^a	12.77±0.64^a	8.80±0.38^b
Dry matter intake (kg/d[4])	9.59±0.17^a	9.58±0.48^a	7.77±0.34^b
Feed conversion ratio (DMI/DG) kg/kg	7.57±0.13^a	6.53±0.33^b	5.55±0.24^c
Daily gain (kg)	1.26	1.46	1.44

[1] T1 = Total mix ration was mixed 7 min; [2] T2 = Total mix ration was mixed 14 min; [3] FCD = The animals was offered feed separately; [4]Total mix ration dry matter is of 77.70 in 1th month; Total mix ration dry matter is of 75.06 in 2th month; Concentrate dry matter is of 87.75; Alfalfa dry matter is 89.95
[a,b,c] Means with different superscript in the same row are different (P<0.05)

(T1: 8.12; T2: 8.57 kg/d). The percentage of the particles retained on the middle part (8 mm of sieve size) was 33.59 in T1, which was higher in comparison to T2 (23.91). The fraction of particles retained in the lower part (4 mm of sieve size) was 47.11and 48.12 in T1 and T2, respectively. The percentage of particles obtained in the bottom sieve decreased in parallel by increasing the mixing time of TMR (T1: 14.66; T2: 14.18). In the second month *(Table 3)*, the proportion remaining on the upper part (19 mm of sieve size) in T1 was higher than that in T2. The percentage of particles retained on the 19-mm sieve decreased by increasing the mixing time of TMR (T1: 13.51; T2: 8.28). Similar DMI values were observed in T1 (9.58 kg/d) and T2 (9.57 kg/d). The percentage of particles retained on the middle part (8 mm of sieve size) was 25.78 in T1, which was quite similar in T1 in comparison to T2 (26.61). The fraction of particles retained in the lower part (4 mm of sieve size) was 41.94 and 34.70 in T1 and T2, respectively, and it was higher than the recommended values [16]. The percentage of particles obtained in the bottom sieve increased in parallel by increasing the mixing time of TMR (T1: 26.93; T2: 27.47).

For all months, there was no significant effect of different mixing times on either *in vitro* 48-h NDF digestibility ($NDFD_{48}$) or ADF ($ADFD_{48}$) digestibility of TMR. However, $NDFD_{48}$ values of the rations mixed for 14 min were numerically higher than those mixed for 7 min for first and second months. $ADFD_{48}$ values of the ration mixed for 7 min were numerically lower than those mixed for 14 min for both months *(Table 4)*.

In this study, daily feed intake cost was found as $ 1.857, 2.120 and 1.847, whereas the ration cost of daily gain was $ 1.528, 1.290 and 1.397 for T1, T2 and FCD, respectively *(Table 5)*.

DISCUSSION

Growth performance and digestibility are essential factors that are related to the physical effectiveness of a

Table 3. Particle size distribution of the total mixed (%)				
Size of Sieve	**Groups**			
	T1		**T2**	
	Proportion Remaining On Each Sieve %	**Compute Cumulative Percentage Undersized[1]**	**Proportion Remaining On Each Sieve %**	**Compute Cumulative Percentage Undersized**
First Month				
19 mm	11.45±0.58	100	7.86±0.51	100.00
8 mm	33.59±1.12	88.55	23.91±2.12	92.14
4 mm	47.11±1.46	54.96	48.42±3.05	68.23
Bottom Pan	14.66±1.00	7.85	14.18±1.79	19.81
[1]$pef_{\geq 4mm}$	0.92		0.80	
NDF (DM %)	32.04		32.04	
[2]$peNDF_{\geq 4mm}$	29.47		25.63	
Second Month				
19 mm	13.51±2.49	100.00	8.28±0.58	100.00
8 mm	25.78±0.82	86.49	26.61±2.02	91.72
4 mm	41.94±2.23	60.71	34.70±1.97	65.11
Bottom Pan	26.93±1.23	18.77	27.47±1.54	30.41
[1]$pef_{\geq 4mm}$	0.82		0.7	
NDF (DM %)	32.49		32.49	
[2]$peNDF_{\geq 4mm}$	26.65		22.74	

[1] Cumulative percentage undersized refers to the proportion of particles smaller than a given size. For example, on average, 95% of feed is smaller than 0.75 inches, 55% of feed is smaller than 0.31 inches and 35% of feed is smaller than 0.16 inches; [1] The pef is calculated as sum of the proportion of particles retained on both 19.0; 8.0-and 4 mm sieves; [2] The $peNDF_{\geq 4mm}$ was calculated multiplying the pef by the NDF content of the TMR

Table 4. The NDF[1] and ADF[2] digestibility of different total mixed rations (%)					
Item		**T1**	**T2**	**SEM**	**P-value**
$NDFD_{48}$ [3]	1st month	42.0240	43.1287	3.0389	0.7500
	2nd month	44.1678	46.7243	3.7482	0.5326
$ADFD_{48}$ [4]	1st month	84.7536	86.3563	2.1267	0.4930
	2nd month	85.0954	86.7157	2.2642	0.5138

[1] Amylase-treated, ash-free aNDFom; [2] Ash-free ADFom; [3] NDF Digestibility (48-h in vitro incubation), % of NDF; [4] ADF Digestibility (48-h in vitro incubation), % of ADF

Table 5. Economic analyses of ration		
Groups	**Daily Feed Intake Cost**	**Ration Cost of 1 kg Daily Gain**
T1	10.92±0.36 TRY/$1.857	8.27±0.42 TRY/$1.528
T2	11.19±0.22 TRY/$2.120	6.98± 0.41 TRY/$1.290
FCD	10.79±0.32 /$1.847	7.56±0.28 TRY/$1.397

Current prices were used in economic analyses. 1 $ is 5.41 TRY (13.11.2018). Price of TMR is first month: 0.961 TRY/kg; Price of TMR is second month: 0.923 TRY/kg; Price of calf rower feed is 1.4 TRY/kg; Price of alfalfa hay is 0.95 TRY/kg

ration or feeding ingredients. Excessive amount of long fibres could limit dry matter intake and digestibility, as a short particle size decreases chewing activity and results in a decline of saliva production and rumen pH [18]. Especially regarding this concern, several studies have been conducted on dairy cows [8,19,20], beef cattle [7,21] and goats [22].

In this study, in the first month, the dry matter intake (DMI) in the FCD group was significantly lower than those in the other groups (P<0.05). The PeNDF value did not affect dry matter intake, but the feed conversion ratio was lower (P<0.05) in T2 in comparison to T1. DMI increased numerically (T1: 8.12; T2: 8.57) with decreasing dietary $peNDF_{\geq 4mm}$ (T1: 29.47; T2: 25.63). The results were consistent with those found by Park et al.[23], who reported that dry matter intake was increased significantly with respect to reduced peNDF$_{\geq 4mm}$. There seems to be a relationship between particle size and DMI. A study by Allen [24] reported that decreased particle size decreases the filling effects of forage and increases the ruminal passage rate. Feed intake may be reduced due to a long particle size that occupies larger volumes per unit of DM weight in the rumen content. In contrast to the other studies [7], DMI was increased linearly by increasing mixing time. According to the results of this study, the daily weight gain of the T2 diets was significantly ($P<0.05$) lower than those in the other groups, which was in accordance with another study by Jang et al.[22] Feed conversion ratio (T1: 5.80; T2: 4.76 kg/kg) was decreased linearly by decreased peNDF$_{\geq 4mm}$ (T1: 29.47; T2: 25.63). The results obtained from this study were in compliance with those found by Oh et al.[7], who reported that feed conversion ratio was decreased by increasing the peNDF$_{\geq 4mm}$ content.

In this study, increased final live weight gain (T1: 298.82; T2: 308.61; FCD: 286.11) resulted in cattle fed T2 having higher daily weight gain than the other groups (T1: 1.26; T2: 1.46; FCD: 1.44).

In the first month, the percentage of particles retained on the 19-mm sieve, 8-mm sieve, 4 mm sieve and bottom pan of the T1 and T2 groups were 11.45, 7.86; 33.59, 23.91; 47.11, 48.42 and 14.66, 14.18; respectively. Kononoff and Heinrichs [15] recommended for high production dairy cows for the particles in the upper sieve to be 2-8%, 30 to 50% in the middle sieve, 10 to 20% on the 4-mm sieve and no more than 30 to 40% in the bottom pan. In this study, the peNDF$_{\geq 4mm}$ values (T1: 29.47; T2: 25.63) and feed conversion ratios (T1: 5.80; T2: 4.76) were decreased linearly by increasing mixing time.

The reduction in the feed conversion ratio might be related to an increase in forage surface area for the microbial attack of the rumen [25], and it causes increased fermentation [26]. Based on the results from a previous study [7], increasing revolution per min (T1: 12.000 rpm; T2: 15.000 rpm) was attributed to reduction in peNDF$_{\geq 4mm}$ value (T1: 21.71; T2: 16.22).

In the second month, the percentages of particles retained on the 19-mm sieve, 8-mm sieve, 4 mm sieve and bottom pan of the T1 and T2 groups were 13.51, 8.28; 25.78, 26.61; 41.94, 34.70 and 26.93, 27.47 respectively. The mixing time (T1: 7 min; T2 14 min) affected the peNDF$_{\geq 4mm}$ value (T1: 26.65; T2: 22.74) and feed conversion ratio (7.57; 6.53). This result was consistent with those found by Oh et al.[7], who reported that the proportion of particles retained on the 19-mm sieve (T1: 14.15; T2: 5.81; T3 1.81) decreased by increasing the mixing time (T1: 3 min; T2: 10 min; T3: 25 min) of TMR. Likewise, feed conversion ratio and feed intake were influenced by peNDF$_{\geq 4mm}$ of TMR along with the NDF contents of forages [8]. Additionally, feeding high NDF in TMR resulted in gut filling effect (bulkiness) in relation to the voluntary intake of the reticulorumen [9,27] with decreasing digestibility [28,29], thereby decreasing feed intake [30,31]. Wang et al.[32], reported that roughage particle size in the diet did not significantly affect the DMI; this could be attributed to a result of the lower roughage percentage (50% DM for forage and silage) in the diet. Possibly, the cattle may prefer to consume longer forage to ensure the sufficient rumen fill or to increase their foraging needs [33].

Although NDF and ADF digestibility values of the diets mixed for 14 min were numerically higher than those mixed for 7 min for both months, the duration of mixing had no significant effect on NDFD$_{48}$ or ADFD$_{48}$. In earlier studies, researchers observed higher rumen passage rates with smaller particle sizes, and they predicted a possible decrease on fibre digestibility in this manner [17,33,34]. However, more recently, Yansari et al.[35] showed that reducing forage particle size had no effect on the digestibility of ADF in mid-lactation dairy cows. This was in agreement with our results. Furthermore, the researchers observed no effect of particle size on the digestibility values of most nutrients such as dry matter, organic matter, non-fibre carbohydrates or crude protein in the same study. On the other hand, Yansari et al.[34] interestingly observed a lower NDF digestibility value for smaller forage particle sizes, contrary to our findings. As it is well-known, increasing DMI is encouraged for the passage rate of digesta in the gastrointestinal tract [25]. In our study, the observed effects of particle sizes on DMI were expected to decrease fibre digestibility. However, contrary to our expectations, no significant effect was observed on fibre digestibility with different particle sizes. Although the 48-h *in vitro* ADF and NDF digestibility model had no kinetic passage rate effect unlike the other kinetic *in sacco* and *in situ* methods, the effects of forage particle size on digestibility might be more relevant for the rate of passage rather than the direct rate of digestibility. This hypothesis might explain the significant effects on DMI and lack of effects by particle size on NDF and ADF digestibility without an outflow rate.

The current prices for diets were used to calculate daily feed intake cost and ration cost. In this study, daily feed intake cost was found as $1.857, 2.120 and 1.847, whereas the ration cost of daily gain was $1.528, 1.290 and 1.397 for T1, T2 and FCD, respectively. The estimated daily feed intake cost was quite similar for the T1 ($1.857) and T2 ($2.120) groups, but the ration cost of 1 kg daily gain in T2 ($1.290) was lower than that in T1 ($1.528) due to high average daily gain (T1: 1.33; T2: 1.63). Small particle sizes had a direct effect on feed intake and daily gain, thereby decreasing ration costs for 1 kg of daily weight gain.

In conclusion, the optimal (standard) value ranges were determined (%) for dairy cattle but not for beef cattle. Statistically significant or insignificant differences were mostly due to individual differences in animals such as age or sex of the animal, physically effective fibre content of the forage and ration ingredient. It is concluded that mixing time is important for dry matter intake, daily gain.

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