Effect of Physically Effective Neutral Detergent Fiber and Moisture Content in Different Fermented Days of Fermented Total Mixed Ration on *In Vitro* Digestibility

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ABSTRACT: The experiment aimed to observe the effectiveness of physically effective neutral detergent fiber (peNDF) and moisture content in different fermented days of fermented total mixed ration (FTMR) on *in vitro* digestibility. The study was designed as 2 × 2 × 4 factorial combinations in a completely randomized design. Total mixed ration (TMR) containing 20 and 25% peNDF with 40 and 60% moisture content was fermented in 0, 7, 14 and 21 days. The result showed that IVDMD was increased approximately 8% on the 20% peNDF with 40% moisture content (70.30%) in 0 days and 6% with 60% moisture content (72.87) in 14 days of fermentation compared to 25% peNDF. Additionally, 40% and 60% moisture in 0 and 14 days were the critical levels that affected to improve the digestion but there were non-significantly affected to FTMR digestibility between 20 and 25% peNDF in all fermented days. Oppositely, it was increased on NDF digestibility when FTMR contained 25% peNDF with 40 and 60% moisture in days 0 and 14; whereas increased or decreased of IVADFD were correlated to peNDF and NDF content in dietary. This result can conclude that 20% peNDF with 60% moisture content with the fermented 14 days is great for *in vitro* dry matter digestibility as the recommended of dairy cattle requirement.
Keywords: fermented total mixed rations, peNDF, moisture, *in vitro* digestibility

Introduction

Fermented total mixed ration (FTMR) is the mixture of concentrate and roughage to reduce feed sorting and to maintain rumen fermentation through the improving of rumen ecology that leads to stimulating microbial activity for digesting more feed. FTMR is made by a fermenting of fresh TMR under anaerobic conditions (e.g. ensilage) in the sealed container or plastic bags for 21 days (Wongnen et al., 2009). In dairy cows, FTMR leads to increase and improve dry matter intake (DMI) and nutrition digestibility including dry matter (DM), organic matter (OM), neutral detergent fiber (NDF) and starch (Yuangklang et al., 2004; Vasupen et al., 2006). Importantly, fermentation total mixed ration is a method to improve and preserve the quality of TMR for long period storage without effect to the growth rate of ruminant (Wongnen et al., 2009).

NRC (2001) recommended that cattle need forage fiber in long particle size of roughage to increase fiber content because it has associated to successfully increase the chewing time and saliva secretion, ruminal pH, acetate or propionate ratio then trends to increase milk fat percentage (Kononoff and Heinrichs, 2003). Physically effective neutral detergent fiber (peNDF) is the proportion of diet that has the

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function to encourage animal in chewing activity and result to maintain proper rumen fermentation. According to Lammers et al. (1996), peNDF can be calculated as a proportion of dry matter retained by the 19-and 8-mm screens of Pann State Particle Separator (PSPS) multiplying by NDF content in the diet ($peNDF_{>8}$). Similarly to Mertens (2000) showed that the physically effective fiber conception is to estimate the fiber content in the diet included roughages' particle length for maintaining rumen ecology. In tropical countries, rice straw is the basal roughage for ruminant and is relatively low in moisture. In fact, moisture had the strongly interaction with the particle size and feed sorting of cattle through the addition of water to dry total mixed ration because it commonly supposed that added water to dry TMR will bind particles together and made it harder for dairy cattle to sort out fine particles. Then the bigger intake of long fibrous particles on the wetter diets likely contributed higher milk fat percentage (Shaver, 2002; Leonardi et al., 2005). For the previous study of dairy cows and heifers has shown that when the moisture content in TMR was increased, it increased dry matter intake (Lahr et al., 1983), less dust (Arzola-Alvarez et al., 2010), reduced sorting (Leonardi and Armentano, 2003) and decreased feed losses due to wind but other researching have reported non-significant effected between high or low moisture content (Fish and DeVries, 2012) but it was able to make animals reduce their feed intake (Felton and DeVries, 2010) and increased feed sorting with the greater dietary moisture (Miller-Chushon and DeVries, 2009). Moreover, Felton and Devries (2010) indicated that wetter diets were prone to spoilage importantly with the higher atmosphere temperature. Variances in consequences of former trails might be related with different kinds of feed ingredients (e.g. F:C ration, roughage sources, and feed particle size), environment impact (temperature and moisture) and water addition in the diet. Therefore the moisture and fiber content used in fermented TMR should consider both its digestive and fermentative characteristics. Additionally, increased moisture content in TMR had the high effect on the starch loss during ensilage (Miyaji et al., 2016). Thus this study was to observe the effectiveness of peNDF and moisture in different fermented days of fermented total mixed ration (FTMR) on *in vitro* digestibility.

Materials and Methods

Treatments and experimental design

The experiment design was 2 × 2 × 4 factorial combinations in a Completely Randomized Design (CRD). The main effects were peNDF (20 and 25%), moisture content (40 and 60%) in TMR formulation and difference fermented in 0, 7, 14 and 21 days.

Preparation of TMR and laboratory analysis

TMR was used rice straw chopped as the peNDF and cassava pulp as the moisture content in different levels following the treatments assigned that were listed in **Table 1**. All treatments including fresh or fermented TMR have used the same ingredients but different the time of fermentation. Additionally, the treatment that contained 25% peNDF with 60% moisture was added the 1.51% oil in feed formulation because the high fiber tended to low of TDN in TMR compared to other treatments (**Table 1**). The fermented TMR amount 5 kg each treatment and each fermented day was fermented in sealed containers with air removing (vacuum pump). Fermented TMR has kept at the normal temperature (30°C ±5) and were opened for each period on days 0, 7, 14 and 21. All TMR samples were taken from the top, center and bottom parts of the sealed container. They were collected in plastic bags then oven dried in a 60 °C for 48 h and was ground to pass through 1 mm screen in Wiley mill in the nutritional laboratory. The rumen fluid was collected from a cow fed basal ration, before morning feeding by stomach tube. The determination of *in vitro* digestibility, F57 filter bags were prepared and were loaded with approximately 0.50-0.55g fermented TMR, heat sealed filter bags were placed into the digestion jars along with prepared buffered rumen fluid and incubated in DAISY^{II} incubator (ANKOM Technology, Fairport, NY) for 48 h at 39°C. Data obtained were analyzed using the general linear model procedure of SAS (1996) according to a completely randomized design. Means were separated by Duncan New's Multiple Range Test at $P < 0.05$ significance level.

Table 1 The calculated rations and chemical composition of experimental diets on *in vitro* study (% DM basis)

Items	Treatments[1]			
	P20M40	P20M60	P25M40	P25M60
Ingredients composition,%				
Soy bean meal	15.59	18.32	16.82	19.39
Cassava pulp	8.50	21.00	8.50	21.00
Rice bran	8.00	6.55	8.00	3.19
Salt	0.50	0.50	0.50	0.50
Dry molasses	6.00	6.00	5.00	5.00
Cassava chip	5.00	11.00	8.39	4.00
Corn meal	25.84	8.00	15.46	7.00
Urea	1.00	1.00	1.00	1.00
Mineral premix	0.40	0.40	0.40	0.40
Rice straw	29.17	27.23	35.93	37.01
Oil	0.00	0.00	0.00	1.51
Total	100.00	100.00	100.00	100.00
Calculated of chemical composition,%				
DM[2]	59.52	39.91	59.60	39.95
Moisture	40.48	60.09	40.40	60.05
TDN	72.11	70.00	70.00	68.85
CP	13.50	13.50	13.50	13.50
ADF	17.62	19.48	20.12	22.58
NDF	31.97	33.74	35.00	38.32
peNDF	20.00	19.62	24.58	25.00
Fat	2.74	1.86	2.39	2.90
Ash	9.14	8.94	10.13	10.03

[1]Treatments: P: peNDF 20 and 25%, respectively; M: Moisture 40 and 60%, respectively.
[2]DM = dry matter, TDN = total digestible nutrients, CP = crude protein, ADF = acid detergent fiber, NDF = neutral detergent fiber, peNDF = physically effective neutral detergent fiber.

Results and Discussion

Dry matter digestion of fermented total mixed ration

The result of *in vitro* dry matter digestibility (IVDMD) was increased approximately 8% on the 20% peNDF with 40% moisture content in 0 days and 6% with 60% moisture content in 14 days of fermentation (**Table 2**). According to **Table 3**, IVDMD highly increased in FTMR in peNDF 20% compared to 25% on the 0 days of TMR (67.22 vs 62.80%, $P < 0.01$). But there was not different between levels of peNDF on IVDMD in 7, 14 and 21 days because during fermented days, starch content was greatly decreased but both grain and NDF were increased during fermentation period and the reducing of starch content in FTMR could be caused by being hydrolyzed and consumed by lactic acid bacteria, yeast and fermented products (Miyaji et al., 2016). Similarly to Nha and Pattarajinda (2017) resulted that TMR that contained 20 and 25% of peNDF were greatly increased on IVDMD (69.11% and 69.00%, $P < 0.001$) but it was decreased IVDMD approximately 10% with 30% peNDF. In the same manner, Yang and Beauchemin (2006) determined that 19% $peNDF_{>1.18}$ of dietary was improved 46.2% NDF digestibility that was maintained a rumen pH of 6.0. Additionally, Tafaj et al. (2005) estimated that to achieve a chewing time of 74 min/kg of DM and to maintain 3.4% milk fat, TMR required containing 28% NDF or 19% peNDF with 60% slowly degradable concentrate. In terms of the peNDF requirement, Mertens (1997) recommended that 19.7% peNDF maintained a milk fat of 3.4%, or 22.3% peNDF kept a stable 6.0 of rumen pH. So the cattle ration should have approximately 22-23% peNDF to assure a chewing time of 150 min/kg for maintaining optimal ruminal fermentation and 3.6% milk fat content. On other hands, moisture content in TMR was also the main factor that affected to digestibility in the rumen and their production. According to **Table 2** and **3**, the critical levels of moisture were 40% and 60% moisture in 0 days (67.19%) and 14 days (69.64%) but in 7 and 21 days were not affected by moisture content. In fact, fermented days of FTMR were the highest interaction with moisture content but 7 days fermentation might be the shorter duration of ensilage time that was non-significantly effected to FTMR quality or nutrient digestibility (Kondo et al., 2016). Moreover, the prolonging of fermented time after 21 to 91 days, the hemicellulose and cellulose losses in silage were small and within the acceptable range of DM losses in low and medium moisture of silage, it was probably due to organic acid hydrolysis during fermentation (McDonald et al., 1991). Miller-cushon and Deveries (2009) were reported that DMI was affected by the dry matter content (57.6%) and wet (49.9%) by DM basis. The cows tended to consume wet diet more compared to the dry diet (60.3 vs 55.1 kg/d) but the more wet diet was greater of water intake when fed the wet diet to the animals. This reason made the cows decreased their DMI when fed the wet diets (32.8 vs 29.6 kg/d). Other trail reported that eating time was higher in dry corn compared high moisture corn diet (Krause et al., 2002). Moreover, Hosseinkhani et al. (2007) concluded that adding the water to TMR diet (32.3% DM) was decreased rumen pH compared to 64.6% DM in TMR (5.93 vs 6.22) however the pH was in the physiological range for a healthy rumen and normal fermentation. According to Felton and Devries (2010) showed that the TMR containing primary haylage and silage as the roughage should contain moisture less than 60% DM because it will be limited the nutrient consumption and will not always discourage cows from sorting. Importantly high moisture TMR is more prone to spoilage during increased environmental temperature in periods (Eastridge, 2006). Thus, there was an interaction increased between peNDF 20% with moisture 40 and 60% content in TMR in 0 and 14 days (70.30 and 72.87%, $P < 0.001$) (**Table 2**). Totally, it could conclude that interaction between 20% peNDF with 40 and 60% moisture containing rice straw as the based in fermented total mixed ration was the ideal levels to improving IVDMD.

NDF digestion of fermented total mixed ration

In vitro neutral detergent fiber digestibility (IVNDFD) was non-significantly difference all between 20 and 25% peNDF in fermented days (**Table 3**) and it was increased on NDF digestibility when TMR contained 25% peNDF with 40 and 60% moisture in 0 and 14 days (**Table 2**). Fermented TMR was related to the roughage with insufficient fermentable substrate or too low a DM content and also the fermentation period. Other researchers determined that fermentation or chemicals composition change of FTMR was non-significantly different after ensilaging for a shorter or the longer storage duration. Similarly, Kondo et al. (2016) reported that NDF content in FTMR did not change between fresh, 30 and 90 days of FTMR (381 vs 380 vs 378g kg^{-1} DM, respectively; $P > 0.05$). Similarly to Nha and Pattarajinda (2007) mentioned that the peNDF levels range in 20 to 25% content in TMR were not difference on NDF digestibility but IVNDFD was highly increased with 30% peNDF (28.2%). Interestingly, peNDF content did not affect to DM and NDF intake significantly (Beauchemin et al., 2003; Oh et al., 2016), but it would be increased the digestibility when peNDF levels increased. The apparent digestibility was significantly higher in treatment that content 30.36% peNDF compared to 29.20% and 27.50% peNDF on DM (66.85 vs 65.56 vs 64.42%, $P < 0.05$) and NDF (44.39 vs 43.79 vs 42.11%, $P < 0.05$) (Oh et al., 2016). According to the recommended of ruminant requirement's range, TRM has to contain peNDF 19-21% (Merten, 1997) and 18.9-21% (Einarson et al., 2004). It was generally agreed by nutritionists that the physical characteristics of the diet and as well as forage particle size, are the essential elements for reducing the risk of sub-acute ruminal acidosis (SARA). For moisture content in TMR was increased by 60% compared to 40% moisture on IVNDFD at 0 days (24.36 vs 25.93%, $P < 0.05$) but 14 days of fermented TMR was highly significant in 40% moisture (22.70%, $P < 0.001$) (Table 2, 3). Nha and Pattarajinda (2017) also determined that increasing moisture 60% in TMR was helpful of increasing of IVNDFD (26.29%, $P < 0.001$). Correspondingly to Felton and Devries (2010) compared 2 levels of moisture included the dry (80.8%) and wet (64.4%), the NDF intake was 6.42 vs 6.15 kg/d for wet and dry ($P = 0.007$). Wet diet tended to be higher in milk fat percentage compared to dry (3.41% vs. 3.31%). In contrast, Miller-cushon and Deveries (2009) resulted that NDF intake was less when cows were on the wet diet (50.1% moisture), however, the consuming of starch concentration was greater in wet diets but cows tended to reduce DMI on the wet diet then it resulted in less NDF and starch intake on diet. Such dropping in nutrient intakes might hypothetically limit milk production. Owens et al. (1998) and DeVries et al. (2008) showed that the imbalance of ration composition in wet ration was able to increase the risk of SARA in ruminant because the ration offered animals the less fiber intake and more starch intake than nutritional estimation. Overall, the interaction between peNDF and moisture content on IVNDFD concluded that 20-25% peNDF with moisture 60% were the critical treatments in 0 and 14 days in fermentation (27.49% and 24.24%). Thus, 20-25% peNDF and 60% moisture content on IVNDFD in the trail has stayed in the range standard recommendation of NRC (NRC. 2001).

ADF digestion of fermented total mixed ration

According to Table 2 and 3 of *in vitro* acid detergent fiber digestibility (IVADFD) showed that 25% peNDF increased approximately 2% compared to 20% peNDF in 0 days (15.8 vs 13.21%) and 14 days (14.30 vs 11.85%) of fermentation but other fermentation days were not affected by peNDF content. Normally, the roughage is lower digestibility and reducing fiber to minimum levels in the diet is often desirable for high performance dairy cows. 25% peNDF in the current research was the optimum level to improve ADF digestibility which was in ranging of Oh et al. (2016) that reported 30.36% pond in TMR increased 1.1% ADF digestibility of fattening heifer compared to 29.20% and 27.50% (36.50 vs 35.70 vs 31.40% DM, respectively) because reducing the peNDF level in TMR was increased the surface area available for microbial attack, thereby accelerating digestion, but fine particles often had a faster passage rate from the rumen such that digestibility was reduced. Yang and Beauchemin (2006) indicated that the increasing of dietary peNDF did not change the nutrient intake including DM, NDF, starch, and ADF but the apparent digestibility were linearly increased when increasing dietary peNDF 11.5% on DM (70.2%), OM (71.5%), NDF (47.2%) and ADF (36.5%). Zebeli et al. (2006) reported that ADF digestibility was linearly increased due to the increasing of peNDF and NDF but ADF was decreased with increasing NFC concentration in the diet. Indeed, increased or decreased IVADFD was related to the level of peNDF and NDF content in ration or digestibility (Nha and Pattarajinda, 2007). As shown in **Table 2 and 3** IVADFD was not influenced by the levels of moisture content in TMR ($P > 0.005$). Thus the low or high peNDF and moisture levels did not interaction effect on IVADFD in all treatments in the trial. In summary, the critical moisture should be less than 65% for TMR with 20 and 25% peNDF because the high-moisture content in TMR does not an active technique to reduced feed sorting but it had harmfully affected to DMI, motivated sorting and also the subsequent in consumption of ration with the different nutrient composition in diets than intended.

The optimum days of fermentation period

Ensiling is a fermentation method of forage preservation with a final pH of 3.8 to 4.2 for optimal quality (Nkosi and Meeske, 2010). According to **Table 4** showed that the fermentation days was affected by levels of peNDF on IVDMD because 20% peNDF increased digestibility on 14 days (69.82%, $P < 0.05$) compared to other days. But when increasing peNDF up to 25%, IVDMD on 0 days was decreased compared to 7, 14 and 21 days (63.09 vs 66.61 vs 66.29 vs 66.40%, $P < 0.01$). Similarly to other researchers that increased the fermentation day, it tended to increased digestibility because fermented rations had greater nutrient digestibility and the fiber fraction could be broken down during the fermentation process leading to improve the microbial digestion in the rumen (Wongnen et al., 2009). In contrast, Shaani et al. (2016) determined that the fiber content in TMR including NDF was increased when TMR ensilage until 60 days compared to fresh TMR (35.5 vs 40.3%, $P = 0.04$) and the IVDMD was increased in fresh TMR (70.5 vs 65.4%, $P < 0.01$). The increasing of IVDMD was probably related to the lower NDF and peNDF content in fresh TMR. There were non-significant different on IVDMD between 40% moisture and ensilage time but it had the highly effected between 60% moisture and ensilage in 0, 7, 14 and 21 days (62.82 vs 67.14 vs 69.64 vs 66.55%, $P < 0.001$). In fact, moisture and ensilage had the interaction because increasing ensilage time 91 days with high moisture up to 70% would result in excessive losses of DM, hemicellulose, cellulose and water-soluble carbohydrates with the higher level of ammonia in silage (Yahaya et al., 2001). The IVNDFD in different fermented days was non-significant on 20% peNDF but it was affected to 25% peNDF and it was highly in 0 days (27.17%, $P < 0.05$). The aspects included in fermentation quality were not only the physiological properties of epiphytic bacteria in the silage (Cai et al., 1999) but the particle size of the silage material as well (Johnson et al. 2003). Similarly to Shaani et al. (2016) revealed that ensilage 60 days of TMR extremely decreased 10% of NDF digestibility (52.8 vs 48.0%, $P < 0.001$) and 8.6% in CP digestion but did not change on hemicellulose (59.8 vs 40.3%, $P = 0.34$) compared to TMR without silage processing. Oppositely, Yuangklang et al. (2004) investigated NDF digestibility was greater with FTMR compared to fresh TMR. The fiber fraction in FTMR was easily digested by microbial bacteria in rumen then led to improving the NDF digestion of ruminant when feeding FTMR. The IVNDFD of 60% moisture was increased on the 0 days of fermentation (25.93%, P < 0.001) compared to other fermented days. In contrast, Cao et al. (2016) indicated that 55% moisture of FTMR increased NDF digestibility compared to fresh TMR (78.5 vs 73.2%, P < 0.05). For IVADFD were non-significant different for all fermented days both peNDF and moisture content (**Table 4**). Wongnen et al. (2009) also concluded that ADF intake (4.68 vs 4.48%, P = 0.69) and digestibility (54.9 vs 54.4%, P = 0.37) was similar between fresh and fermented TMR. Furthermore, Cao et al. (2016) indicated that fresh and fermented TMR with 55% moisture content were not different on ADF composition and digestibility. Additionally, FTMR was noticed as an ideal technique to develop the TMR quality for long-term storage without disadvantageous affecting to animals performance. Overall, it can conclude that 20% peNDF and 60% moisture in 14 days of fermentation were the ideal levels to improve digestibility and also the good method to store and increased the quality of TMR for high performance of ruminant.

Table 2 Effect of FTMR with difference levels of peNDF and moisture content on *in vitro* digestion

Items	Days	Treatments[1]				SEM	P-value		
		P20M40	P20M60	P25M40	P25M60		P	M	F*M*T
IVDMD	0	70.30[a]	64.13[b]	64.08[b]	62.09[b]	0.35	**	**	***
	7	69.33	66.86	65.79	67.43	0.42	NS	NS	NS
	14	66.76[b]	72.87[a]	66.17[bc]	66.40[b]	0.33	NS	***	***
	21	63.83	66.00	65.70	67.10	0.40	NS	NS	NS
IVNDFD	0	21.88[b]	24.37[b]	26.84[a]	27.49[a]	0.35	NS	***	**
	7	21.59	24.38	24.76	22.41	0.48	NS	NS	NS
	14	21.17[bc]	19.34[c]	24.24[a]	22.70[b]	0.31	NS	**	***
	21	24.19	21.70	25.34	21.78	0.55	NS	NS	NS
IVADFD	0	11.77[b]	14.66[a]	14.69[a]	15.67[a]	0.22	*	NS	***
	7	12.52	15.41	14.99	13.74	0.38	NS	NS	NS
	14	11.91[b]	11.80[b]	14.36[a]	14.24[a]	0.25	*	NS	***
	21	13.75	13.35	14.90	13.25	0.47	NS	NS	NS

[1]Treatments: P: peNDF 20 and 25%, respectively; M: Moisture 40 and 60%, respectively.

[a, b, c, d] Means within a row without a common superscript letter differ (P < 0.05).

NS: Non-significant different (P > 0.05). [*]Statistically significant level of 0.05, [**]Statistically significant level of 0.01, and [***]Statistically significant level of 0.001.

Table 3 On *in vitro* digestion of FTMR with difference level of peNDF and moisture content in varies fermented days

Items	Days	peNDF		SEM	Moisture		SEM	P-value	
		20%	25%		40%	60%		P	M
IVDMD	0	67.22^{a}	62.80^{b}	1.15	67.19^{a}	62.82^{b}	1.18	*	***
	7	68.10	66.61	0.85	67.56	67.14	0.90	NS	NS
	14	67.22	63.09	1.07	67.19^{b}	69.64^{a}	1.13	NS	*
	21	64.91	66.40	0.84	64.76	66.55	0.82	NS	NS
IVNDFD	0	23.12	27.17	0.72	24.36^{b}	25.93^{a}	1.08	NS	*
	7	22.99	23.58	1.05	23.17	23.39	1.05	NS	NS
	14	20.26	23.47	0.57	22.70^{a}	21.02^{b}	0.85	NS	***
	21	22.94	23.56	1.39	24.78	21.74	1.21	NS	NS
IVADFD	0	13.21^{b}	15.18^{a}	0.49	13.23	15.17	0.48	*	NS
	7	12.97	14.36	0.73	13.75	14.58	0.71	NS	NS
	14	11.85^{b}	14.30^{a}	0.32	13.13	13.02	0.63	*	NS
	21	13.55	14.08	0.89	14.32	13.30	0.88	NS	NS

[1]Treatments: P: peNDF 20 and 25%, respectively; M: Moisture 40 and 60%, respectively.
[a, b, c, d] Means within a row without a common superscript letter differ ($P < 0.05$).
NS: Non-significant different ($P > 0.05$).
*Statistically significant level of 0.05, **Statistically significant level of 0.01, and ***Statistically significant level of 0.001.

Table 4 Effect of fermentation days on peNDF levels and moisture content in FTMR on *in vitro* digestion

Items	Days	peNDF		Moisture	
		20%	25%	40%	60%
IVDMD	0	67.22^{ab}	63.09^{b}	67.19	62.82^{b}
	7	68.10^{ab}	66.61^{a}	67.56	67.14^{a}
	14	69.82^{a}	66.29^{a}	67.19	69.64^{a}
	21	64.91^{b}	66.40^{a}	64.76	66.55^{a}
SEM		0.51	0.39	0.46	0.47
P-value		*	**	NS	***
IVNDFD	0	23.12	27.17^{a}	24.36	25.93^{a}
	7	22.99	23.58^{b}	23.17	23.39^{ab}
	14	20.26	23.47^{b}	22.70	21.02^{b}
	21	22.94	23.56^{b}	24.78	21.74^{b}
SEM		0.44	0.47	0.51	0.42
P-value		NS	*	NS	***
IVADFD	0	13.21	15.18	13.23	15.17
	7	12.97	14.36	13.75	14.58
	14	11.85	14.30	13.13	13.02
	21	13.55	14.08	14.32	13.30
SEM		0.37	0.37	0.40	0.36
P-value		NS	NS	NS	NS

[1]Treatments: P: peNDF 20 and 25%, respectively; M: Moisture 40 and 60%, respectively.
[a, b, c, d] Means within a column without a common superscript letter differ ($P < 0.05$).
NS: Non-significant different ($P > 0.05$).
*Statistically significant level of 0.05, **Statistically significant level of 0.01, and ***Statistically significant level of 0.001.

Conclusions

Physically characteristics of TMR were the greatest factor for improving proper digestibility and chewing activity as well as for milk's production and their performance. In this study, peNDF and moisture contained in TMR had the strong relationship with IVDMD, IVNDFD and IVADFD. Moreover, the fermenting process in different days was also affected to the increasing or decreasing of digestible nutrient. Thus, it can conclude that 20% peNDF with 60% moisture in 14 days fermentation can be critical for the optimal of dry matter digestibility and neutral detergent fiber. Moreover, the fermented times of TMR are the highest interaction with moisture content because increasing fermented time of high moisture would result in excessive losses of hemicellulose, cellulose, DM and starch during storage.

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