Dry Matter and Crude Protein Degradability of Some Common Feeds and Total Mixed Ration in F1 Boran- Fresian Bulls Using in situ Methods

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Abstract
The study was conducted at Holeta agricultural research center with objectives to evaluate dry matter and crude protein degradability and degradability parameters of some common feeds (natural pasture hay, wheat bran, cotton seed cake and noug seed cake) and total mixed ration (TMR contained natural pasture hay (50%), wheat bran (14%), noug (Guizotia abyssinica) seed cake (14%), cotton seed cake (8%), molasses (11%), urea (1%) and salt (2%) about 3 g of DM equivalent of samples were weighed in sealed nylon bags (10 x 20cm size and a pore size of 53 µm) that were incubated in the rumen of three ruminally fistulated F1- Boran-Fresian steers (body weight=510±57 kg and age= 13±3 months) that fed natural pasture hay ad libitum and supplemented with 2 kg concentrate mixture contained with 50% wheat bran, 40% noug seed cake, 9 % cotton seed cake and 1% salt on dry matter basis, once per day in the morning. Three ruminally cannulated were housed in 1.5 x 2.0 m pen. The sieve size of the sample was 2mm. The bags with 3 g sample were placed into the ventral rumen after morning feeding in quadruplicate at each time point in each steer for 6, 12, 24, 48, 72, and 96 h. Washing loss was determined by washing duplicate feed samples in the water thank for 30 minutes. Wheat bran, noug seed cake and concentrate mixture had the highest DM and CP degradability ($P<0.05$) but, natural pasture hay had the lowest DM and CP degradability ($P>0.05$). The lag time of DM and CP were lowest and highest in natural pasture hay and wheat bran, respectively ($P<0.05$). Wheat bran and concentrate mixture had higher degradable fraction (a, b and a + b), rate and extent of degradation and effective degradability at ($P<0.01$). In contrast, natural pasture had lower degradability parameters than others feeds ($P>0.05$). In addition, wheat bran had the highest effective degradability ($P<0.05$), but hay had the lowest one. Generally, DM and CP degradability and degradability parameters of TMR were ranged at the degradability values of natural pasture hay and concentrate mixture.
Keywords: Dry matter, Crude protein, In situ degradability, Steers.

1. INTRODUCTION
Natural pasture is the primary feed resources in the mixed crop-livestock production areas (Driba *et al*., 2014) while its nutritional quality is poor in terms of crude protein content and higher fibrous (Seyoum *et al.,* 2007).

Total mixed ration (TMR) or complete ration is used with complete feed, total blended ration. It is a quantitative mixture of all dietary ingredients, blended thoroughly to prevent separation and sorting, and formulated to specific nutrient content (Seung-Uk *et al.,* 2017).

Feeding dietary components as TMR is a frequently used feeding strategy among high-yielding dairy cow herds worldwide. The variations with respect to DM and CP degradability kinetics of test feeds among published information may be due to several factors such as agronomic practices, processing technologies and nature of feeds, breed, species, age and sex of animals under study (Lalatendu *et al*., 2015). Availability of nutrient in ruminants depends on nutrient digestion dynamic in rumen-reticulum. Digestion of nutrients is function of ruminal degradation and passage rate. Using *in situ* and *in vitro* method ruminal degradation of nutrients is measured (Ørskov, 1994). Information in ruminal degradation and passage rate in the TMR feed is important for used to balance of ruminant ration; however, application of this data to formulation of rations is unusual in Ethiopia, because of a little available information about ruminal availability of TMR feed in dairy nutrition .Therefore, this study was carried out in order to determine ruminal degradation, passage rate, ruminal and total mean retention time of some current feeds and total mixed rations in F1 Boran –Frisian bulls.

2. Methodology
2.1. Description of the Study Area
The study was conducted at Adea Berga dairy farm research, which is the sub-center of Holeta Agricultural Research Center .The sub center is located in the central highlands of Ethiopia at $9^0$16'N latitude and $38^0$23'E longitude, 70km west of Addis Ababa and 35km North west of Holeta on the main road to Muger. It lies at an altitude of 2500 meters above sea levels. It is characterized by cool sub-tropical climate with the mean annual

temperature and rainfall of 18^0Cand 1225mm, respectively.

2.2. Animal and Diets

Three Boran –Fresian F1 steers, with permanent rumen fistula, were maintained in individual pens (2*1.5m) managed under Holeta agricultural research barn.

The steers had an average age of 13±3 months and 510±57 kg weight. The steers were fed natural pasture hay *ad libitum* and supplemented with 2 kg concentrate mixture (50% wheat bran, 40% noug (*Guizotia abyssinica*) seed cake, 9 % cotton seed cake and 1% salt) once per day in the morning. The daily dry matter intake was about 10 kg and the diet refusals were collected and weighed daily, and amount of diet offered was adjusted to a minimum of a 20% refusal rate. The animals were fed concentrate mixture twice daily (6:00 AM and 6: PM) in equal parts in 12-hour intervals to maintain a relatively stable rumen environment and animals had free access to fresh water.

2.3. Feeds and Total Mixed Ration Preparation

Natural pasture hay was harvested at 50% blooming stage which is also the usual agronomic practice at the farm. *Andropogon Virginicus and Pennisetum Setacium grasses* and *Trifoilum Hybridum* legume were the dominant species of the natural pasture hay. After drying, the natural pasture hay was stored in ventilated house. Wheat bran, Cotton seed cake, Noug seed cake (*Guizotia abyssinia*), urea, molasses and salt were purchased from food processing industries in Addis Ababa city and Mojo town. Total mixed ration (TMR) was prepared by using the following procedures; -

Step 1: Sun dried natural pasture was chopped (5cm) and weighed.

Step 2: Wheat bran, Cottonseed cake, Noug seed cake, salt and urea was weighed and mixed.

Step 3: Molasses and water (three fold of molasses amount) was weighed and thoroughly mixed.

Step 4: After weighing and mixing concentrate mixture with chopped natural pasture hay, the mixture of molasses and water was sprayed and uniformly mixed. For the preparations of 100 kg of TMR 50:50 hay and concentrate mixture including urea and salt were used (Table 1).

Table 1: Proportion of total mixed ration (DM basis)

Feed	Proportion
Natural Pasture Hay	50
Wheat Bran	14
Cotton Seed Cake	8
Noug Seed Cake	14
Molasses	11
Urea	1
Salt	2

2.4. In Sacco Degradability

In-situ DM and CP degradability (%) of TMR and individual ingredient (hay, wheat bran, noug seed cake, and cotton seed cake and concentrate mixture) was evaluated by using the nylon bag technique. All feed samples were sun dried and ground to pass through 2mm sieve seize. The nylon bag used had 10 x 20 cm size and a pore size of 53 µm. Three grams of feed samples were incubated for 6, 12, 24, 48, 72 and 96 hr to determine the DM and CP degradability and degradability parameters. TMR contained 1.5g chopped natural pasture hay , 0.3g wheat bran, 0.39 g NSC, 0.24g CSC, 0.48g molasses, 0.03 g urea and 0.06 g salt. Concentrate mixture also contained 0.3g wheat bran, 0.39 g NSC, 0.24g CSC, 0.48g molasses, 0.03 g urea and 0.06 g salt. The samples were incubated in the rumen of three fistulated F1 Boran- Frisian steers in duplicates just before the morning meal. After each incubation period, the bags were removed from the rumen of the animals and immediately put in ice water to stop microbial activity and then after hand washed under a running tap water until the water becomes clear. Afterwards the bags with residues were oven dried at 65°C for 72 hrs and again weighed to determine the dry matter content of residues. Washing loss ("0-hour" degradability of soluble nutrients) was determined by washing duplicate feed samples in the water thank for 30 minutes. The duplicate bags were dried in the same way to determine DM contents of the feed samples.

DM degradability at a time t = $\frac{\text{DM in the feed} - \text{DM in the reresidue x 100}}{\text{DM in the feed}}$

The degradability constants were determined using the exponential equation;

P = a + b $(1-e^{-ct})$ as described by Ørskov and McDonald, (1979) using the Neway Excel-program (Chen, 1995).

Where;

P = DM degradability at a time t. a= highly soluble and readily degradable fraction;

b= representing insoluble but fermentable fraction; c= the rate of degradation of B;

t = time relative to incubation (h); e = 2.7182 (Natural logarithm base).

Potential degradation (PD) of DM was estimated as (a+ b), While ED=Effective degradability was calculated according to Dhanoa, (1988) using the formula ED = a + [bc/(c + k)] at rumen outflow rates (k) of 0.02, 0.05 and $0.08h^{-1}$.

2.5. Chemical Analysis

For chemical composition analysis, frozen total mixed ration with average 40% moisture content was thawed and dried in a forced-air oven at 70 ºC for 48 h. Offered feed and refusal were also collected and dried in a forced air oven at in similar procedure. Feeds were ground to pass through 1 mm sieve size. DM content was determined by oven drying the samples at 105 ºC for 24 hours. Ash content was determined by combusting sample at 550 ºC in the furnaces. N content was determined using Kjeldahl method according to AOAC (2005) and CP was calculated as Nx6.25. ADF and ADL were determined by the methods of Van Soest and Robertson (1985). NDF was determined by using the procedure of Van Soest *et al*. (1991). INVOMD of feeds was determined by two stage procedures of Tilley and Terry (1963) at Holeta animal nutrition research laboratory. The metabolizable energy contents of the feeds were estimated from *in vitro* organic matter digestibility as described by McDonald *et al*. (2002). ME (MJ/kg) = 0.16 IVOMD, Where: IVOMD= in vitro organic matter digestibility.

2.6. Statistical Analysis

Statistical analyses were carried out using SAS (2003). For all statistical analyses, significance was declared at *P*< 5%. Tukey mean separation test was used for multiple feed comparisons. The model used was: Yijk=μ+Fi+Mj+ +eij.

Where, Yijk is the dependent variable, μ is the population mean for the variable, Fi is the fixed effect of feed sources (i= 5; natural pasture hay, wheat bran , NSC, CSC andTMR) and Mj is the fixed effect of rumen incubation period.

3. Results

3.1. Chemical Composition of Feeds

The chemical composition of the ingredients is shown in Table 2. Molasses and hay had lower CP content. Noug seed cake had high CP content. The NDF and ADF contents of hay were high as compared to other feeds but it had the lowest IVOMD and ME. However, higher IVOMD and ME was observed in wheat bran compared to other feeds. The CP, IVOMD and ME contents of TMR was higher than hay.

Table 2: Chemical composition of the ingredients and total mixed ration

Items	Feeds								
	Hay	WB	CSC	NSC	M	Urea	Salt	TMR	
								Offer	Refusal
Proportion (%)	50	14	8	14	11	1	2	100	100
DM	93.5	92.1	94.2	93.2	74.5	-	-	92.1	89.9
Ash	8.2	6.8	5.6	11.5	5.1	-	-	9.2	10
CP	5.2	17	24	36.8	5	247	0	15.8	14.9
NDF	65.6	49.6	42.7	37.8	-	-	-	46.6	63.2
ADF	45.6	26.7	24.9	27.7	-	-	-	32.8	37.8
ADL	9.4	3.9	6.3	4.4	-	-	-	9.4	12.5
IVOMD	47.5	78.3	62.9	70.2	-	-		54.3	51.2
ME	7.6	12.5	10.1	11.2	-	-	-	8.7	8.2

Except dry matter (DM), all values are represented on DM basis, CP=Crud protein; NDF= Nutrient detergent fibre; ADF=Acid detergent fibre; ADL=Acid detergent fibre; IVOMD= In vitro organic matter digestibility; ME=Metabolizable energy; WB=Wheat bran; CSC=Cotton seed cake; NSC=Noug seed cake; M=molasses and TMR=total mixed ration.

3.2. Dry Matter, Crude Protein Degradability and Degradability Parameters

3.2. 1. Dry matter and crude protein degradability

The DM and CP degradability are shown in Table 3. WB and NSC had higher (P<0.05) DM degradability at 96 hr incubation period as compared with other feed ingredients and TMR, while hay had lower DM degradability at 96 hr incubation period. WB, NSC and CM had higher (P<0.05) CP degradability at 96 hr incubation period compared with other feeds after 96 hr incubation period.

Table 3: In situ dry matter and crude protein disappearance (%) of TMR and ingredients

Parameter	Ingredients	Incubation times(hr)						
		0	6	12	24	48	72	96
DM	Hay	10.4	23.7	27.6	38.7	49.0	58.2	64.8[e]
	NSC	23.5	40.4	52.0	65.8	77.2	84.2	87.57[a]
	CSC	17.0	24.8	42.9	44.1	61.4	72.1	74.9[c]
	WB	26.2	53.4	69.2	76.2	79.9	85.2	89.50[a]
	CM	20.2	46.7	55.3	69.7	79.2	82.9	84.43[b]
	TMR	18.2	32.8	38.0	46.3	56.0	65.5	70.15[d]
	SE							0.71
CP	Hay	6.3	22.9	31.3	37.3	52.1	58.3	65.56[d]
	NSC	10.6	31.2	43.4	51.8	76.8	81.4	90.16[a]
	CSC	9.3	7.9	27.6	29.8	52.0	59.6	81.00[b]
	WB	12.1	41.5	58.3	70.6	76.8	82.5	91.67[a]
	CM	11.9	21.4	50.8	58.4	69.7	78.6	93.1[a]
	TMR	8.9	22.4	29.3	41.6	51.0	62.4	72.67[c]
	SE							1.3

All values are represented on DM basis. DM= Dry matter; CP=Crud protein; WB=Wheat bran; CSC=Cotton seed cake; CM= Concentrate mixture; NSC= Noug seed cake; M=molasses; TMR=total mixed ration and Hr=Hour and SE=standard error. [a,b,c,d, e]. Means bearing different superscripts in the same columns are significantly different (P<0.05).

3.2.2. Dry matter and crude protein degradability characteristics

The dry matter and crude protein degradability characteristics are given in Table 4. Wheat bran had the highest (P<0.05) washing loss (a) while the highest (P<0.05) insoluble but degradable fraction (b) was for concentrate mixture. Cotton seed cake, wheat bran and concentrate mixture had the highest (P<0.05) degradation rate (c). The highest (P<0.05) DM and CP potential degradability (a+b) was found concentrate mixture. ED value of CP and DM for concentrate mixture was also higher (P<0.05) than the other ingredients and TMR. The lowest washing loss and the longer lag time of DM and CP in hay were recorded.

Table 4: In situ degradability parameters of dry matter and crude protein (DM basis)

Items	Feed	Parameters (%)							
		a	b	c	a+b	L	ED at Kp=		
							0.02	0.05	0.08
DM	Hay	13.1[d]	50.9[bc]	0.02[c]	63.9[d]	3.2	45.6[d]	40.1[d]	38.9[d]
	NSC	26.2[b]	52.2[b]	0.04[b]	78.2[b]	1.5	62.9[b]	51.5[b]	45.6[b]
	CSC	20[c]	48.4[c]	0.05[a]	68.4[c]	1.6	56.4[bc]	46.6[c]	41.1[c]
	WB	29.3[a]	49.3[bc]	0.06[a]	78.6[b]	0.00	73.4[a]	63.3[a]	57.5[a]
	CM	20.4[c]	61.5[a]	0.06[a]	81.8[a]	0.00	54.9[c]	48.5[c]	37.7[d]
	TMR	21[c]	50.3[bc]	0.01[d]	71.3[bc]	0.00	52.1[cd]	41.36[d]	39.4[d]
	SE	0.85	2.1	0.001	3.1	-	0.67	0.58	0.43
CP	Hay	8.3[c]	53.2[d]	0.03[c]	61.5[d]	1.6	43.7[d]	31.8[cd]	26[cd]
	NSC	9.8[b]	74.5[a]	0.03[c]	84.3[ab]	0.94	52.6[c]	36.3[c]	29.1[c]
	CSC	9.3[b]	60.5[c]	0.02[d]	69.5[c]	0.6	40.7[d]	26.8[d]	21[d]
	WB	12.2[a]	71.3[b]	0.07[a]	81.5[b]	0.89	65.9[a]	52.5[a]	44[a]
	CM	10.1[b]	75.4[a]	0.03[c]	87.8[a]	0.23	59.2[b]	41.4[b]	33.6[b]
	TMR	8.4[c]	58.9[cd]	0.05[b]	67.3[cd]	0.36	45.3[cd]	28.4[d]	22.1[d]
	SE	0.39	1.23	0.002	2.1	-	0.43	0.32	0.21

DM= Dry matter; CP=Crud protein; WB=Wheat bran; CSC=Cotton seed cake ; CM= Concentrate mixture ;NSC= Noug seed cake; M=molasses; TMR=Total mixed ration ;SE= Standard error; ED = effective degradability; Kp= represents the rumen out flow rate of particle ;L=lag time(%/h) a=washing loss (%); b= degradability of water insoluble(%); a+b=potential degradability (%) and c is rate constant (fraction /hour).Means in each column with different letters have a significance difference (P<0.05).

4. DISCUSSION

4.1. Chemical Composition of feeds

CP content of natural pasture hay in the present study is comparable with other reports (Dawit *et al.,* 2013 and Dereje *et al.* 2017). The ADF, ADL, IVOMD and Metabolizable energy contents of the natural pasture hay in the present study is consistent with the report of Seyoum *et al.* (2007) and Getu et *al.*(2012) while the CP content in the current study is lower than result of Tesfaye (2017).

CP content of molasses in the present study is comparable with other reports (Dawit *et al.*, 2013), but higher than the report of Sani *et al.* (2016).

In WB the values of CP, NDF, ADF, IVOMD and ME in the present study is comparable with the results of Fekede *et al.* (2015) , Ajebu *et al.(* 2016) and Dereje *et al.*(2017) while DM, ash and ADL contents were higher than with the report of Getu *et al.* (2012) and Dawit *et al.* (2013).

The DM, ash, CP, NDF, ADF and lignin contents of CSC in the current study is similar with earlier reports of Zewdie *et al.* (2011) and Dereje *et al.* (2017). The IVOMD and ME content of CSC higher than the result of Zewdie *et al.* (2011). The IVOMD and ME of CSC in the current study were comparable with the value reported by Getu *et al.* (2012) and Dawit *et al.* (2013) but lower than the result of other researcher (Zewdie *et al.*, 2010, Dereje *et al.*, 2017).

The DM, ash and CP contents of NSC in this study is higher than other reports (Kasahun *et al.,* 2012) and Ajebu *et al.* (2016), while the fiber fractions (NDF, ADF and lignin) were lower than with the report of Getu et *al.* (2012). The IVOMD and ME contents of NSC in the current study were comparable with the value reported by Getu *et al.* (2012) and Dawit *et al.* (2013) but lower than the result reported by Dereje *et al.* (2017).

The DM, ash and CP contents of TMR in this study is consistent with other finding (Shiriyan *et al*., 2011; Muhammad *et al.,* 2012 and Raja *et al.*, 2013) while the fiber fractions (NDF, ADF and lignin) were higher than the values reported by researchers (Lailer *et al.,* 2010 ; Pachauri *et al,*.2010 and Arto *et al* ., 2014). The possible reason for this variation could be related with due to difference in chemical composition and proportion of the ingredients.

4.2. Dry Matter and Crude Protein Degradability and Degradability Characteristics

4.2.1. Dry matter and Crude Protein degradability

The result of DM and CP degradability of all feeds in the present study is in agreement with other researchers (Mc Donald *et al*., 2002; Lalatendu *et al*., 2015 and Seung-Uk *et al.,* 2017) who indicated that as incubation time increases DM and CP degradability of the feed also increases through long microbial and enzymatic activity of the feed in the rumen.

Dry matter disappearance of native pasture hay at zero hour degradability (a) (11.4 %) was higher than the value of 2% which was reported by Tesfaye, (2017). According to Getu *et al.* (2012), maximum DM disappearance of native pasture hay (58.1%) was observed after 96 hour incubation which is comparable with the current report (57.8 %). Lower CP degradability of natural pasture hay in this study was observed as compared with the report of Martineau *et al*. (2016).

In this finding DM and CP disappearance of WB at zero hour were lower than the report of Lalatendu *et al*. (2015). According to Lalatendu *et al*. (2015), maximum DM disappearance of WB (85.6%) was observed at 48 hour incubation while in the current study the maximum DM degradability (89.5 %) appeared at 96 hr incubation.

In contrast to the present study Lalatendu *et al*. (2015) reported higher DM and CP disappearance of CSC at zero hour (32.4%), 24 hour (73.14 %), and 48hour (89.74 %). Maximum DM disappearance of CSC (74.9%) was observed at 96 hour but maximum value (89.74%) was reported at 48 hour by Lalatendu *et al*. (2015). Getu *et al.* (2012) reported lower values (11.8%) of zero hour degradability and 56% of degradation after 24 hour incubation for NSC as compared with the results of the present study.

In this study DM disappearance of concentrate mixture at zero hour (20.2%) was comparable with the value of 23.1% reported by Hamidi *et al*. (2010), while the current value was higher than (2 %) the report of Tesfaye (2017). In the finding of Hamidi *et al*. (2010) the higher DM disappearance of TMR feed at zero hour (32.9 %) was observed as compared as the current value (18.2%) but CP degradability in the current finding is comparable with the report of Hamidi *et al*. (2010).

4.2.2. Dry matter and crude protein degradability characteristics

In native pasture hay rapidly soluble nutrients (a=13.1%), degradable fraction (b=50.9%) and degradation rate(c=2%) in the current study is comparable with the report of Tesfaye (2017) who found the value of rapidly soluble nutrients (a=13 %), degradable fraction (b=47%) and degradation rate(c=2%) but the values of lag time (L) and effective degradability is not comparable. The longer lag time in hay is a reflection of its higher fiber content than the other feeds (Tolera and Sundstøl, 2001). Long lag time in the degradation of fibrous feeds is caused by the time taken for adherence of cellulolytic organisms to the substrate (Ørskov, 1994).

In natural pasture hay CP degradability parameters of "a" and b in the present study is higher than the result of other researcher (Yao-Ming Wu and Jian-Xin Liu, 2016; Tesfaye, 2017) while "c", and ED were comparable with the finding of Seyoum *et al*. (1995) and Tesfaye, (2017) but, lag time is lower than in the report of Yao-Ming Wu and Jian-Xin Liu, (2016) and Tesfaye, (2017) this variation related with stage of harvesting, harvesting place and fiber content of the hay.

The values of "a", "b" and "ED" in DM degradability of concentrate mixture of the current study is comparable with the result of Tesfaye (2017) who found 21%, 68.28% and 52% ,respectively, but lag time was not comparable. Hamidi *et al*. (2010) reported comparable lag time of concentrate mixture with current study.

CP degradation kinetics of concentrate mixture in this study is also not comparable with the report of Lalatendu *et al*. (2015).

In NSC the value of "a" for DM degradability in the present study is higher than the report of Getu *et al.* (2012) who found the value of 16.5% with higher values of b, c and ED, 67, 5 and 83.55 %, respectively. Additionally, in NSC "a", "b", and "ED" characteristics of CP in the present study is comparable with the result of Seyoum *et al*.(1995) but lower than as compared with the report of Getu *et al*. (2012).

"ED" and "a" of DM characteristics in WB reported in the current study is comparable with the report of Mondal *et al.* (2008) who found 75% and 28.99% respectively, but the value of" b" is not comparable. In contrast to the current finding, Lalatendu *et al*. (2015) reported WB had higher values of 'a' (40.66%) and lower value of b (32%) and ED (56%) of DM disappearances. The CP degradation kinetics of WB as reported by Lalatendu *et al*. (2015) and Getu *et al*. (2012) was also different from our observations.

In this finding, the values of DM degradation kinetics of "a" and "ED" of CSC were lower than as the value of 32.7% and 64% reported by Lalatendu *et al*. (2015), respectively, but the values of 'b' was quite comparable with the findings of Getu *et al.* (2012) and Mondal *et al.* (2008). Our observations were also dissimilar in terms of DM and CP disappearance kinetics of CSC with the report of Getu *et al.* (2012).

In this study, the lower values of lag time and "a" of DM and CP degradability in TMR was observed as compared with the report of Hamidi *et al*. (2010) but, the values of b and ED is slightly higher than as compared with the earlier of Hamidi *et al*. (2010) and Seung-Uk *et al.* (2017). Generally, the variations with respect to DM and CP degradability kinetics of test feeds among available information may be due to several factors such as agronomic practices, processing technologies and nature of feeds, naylon bag, diet, season, breed, species, age and sex of animals under study (Lalatendu *et al*., 2015) and (Mc Donald *et al*. 2002).

5. Conclusion

It can be concluded that WB and NSC had higher DM degradability by ruminal microbes. Feeds such as CSC and TMR could be used as source of by-pass protein in dairy rations due to their least CP degradability. Feed in the form of TMR had higher DM and CP degradability than natural pasture hay.

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