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Original Research

Effect of rumen manipulation on the laboratory digestion coefficient of barley straw

Authors:
Shaker A. Hassan and Ibrahim S. Jasim

Institution:
Department of Animal Production, Faculty of Agriculture, University of Baghdad, Iraq.

Corresponding author:
Shaker A. Hassan

ABSTRACT:

This experiment was conducted to study the effect of altering the rumen environment by making combinations of rumen fluids taken from ruminants (sheep and buffalo) to improve the digestion coefficient of dry matter and organic matter for barley straw. The rumen fluid obtained from sheep was mixed with rumen fluid obtained from buffalo and combinations were made in the following percentages: 0:100, 25:75, 50:50, 75:25 and 100:0 (sheep:buffalo) respectively, to estimate their effect (*in vitro*) on digestion coefficient for dry matter (IVDMD) and organic matter (IVOMD) for barley straw. The results showed a significant superiority (P<0.01) at the combination of 25:75 (sheep:buffalo) in laboratory digestion coefficient for dry matter (46.06%) and organic matter (48.56%) for barley straw on the other manipulation ratios 50: 50, 25: 75 and 0:100 (sheep:buffalo) and compared to 100% rumen from sheep (30.43 and 36.45%) respectively. The improvement percentage in IVDMD and IVOMD was 6.37 and 7.05%, respectively. The results also showed a significant decrease (P<0.01) in IVDMD and IVOMD of barley straw at 50:50 proportion (sheep: buffalo) compared to 100% rumen of sheep and 100% rumen of buffalo. The results showed a significant superiority (P<0.01) in IVDMD and IVOMD for barley straw when using 100% rumen fluid obtained from sheep compared to 100% rumen liquid taken from buffalo.

Keywords:

Manipulation, Rumen environment, Combinations, Barley straw.

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INTRODUCTION

Due to the limited areas allocated for the cultivation of feeding materials, which led to a severe shortage in the availability of such feed in many countries, including Iraq and the available areas for grazing is not commensurate with the number of existing animals. Coarse feeds are an important part of ruminant feed, so many researchers have turned their attention to the remains of agricultural crops, industrial residues and available wild plants, therefore many feedstocks were used included corn stalks (Hassan *et al*., 1989), dates residues (Al-Ani *et al*., 1991) and wild reeds (Hassan *et al*., 2010). The main problem with these feedstocks was their lack of energy and protein a well as the high carbohydrate content, however, they are associated with lignin acid. Several methods have been used to improve the nutritional value of these wastes including physical and chemical. Physical processes involving crushing, chipping and feed tablets while chemical processes including the use of sodium hydroxide (NaOH) (Hassan and Tawffek, 2009), calcium hydroxide ($CaOH_2$), ammonium hydroxide (NH_4OH) (Hassan and Hassan, 2006), urea (Hassan *et al*., 2012), biological treatments (Hassan *et al*., 2014) and feed additives.

However, despite the improvement in nutritional value and the increased digestion factor of these feeds, these transactions were accompanied by many health and environmental problems and material costs as well as risks related to some of the above methods. For example, several studies have indicated that the chemical treatment of poor quality coarse feed has resulted in improved nutritional value and increased intake. However, this improvement was associated with an increase in phenolic compounds, a decrease in the number of anaerobic bacteria, an increase in pH inside the animal rumen, in addition chemical treatment leading to increased free lignin. In order to overcome many of the problems related to the use of the above methods, new methods were developed to improve the utilization of these feeds, namely to improve the nature of digestion within the animal's rumen (manipulation of rumen ecology). To improve the rumen environment, it is important to improve digestion efficiency within the rumen, reduce environmental contaminants and thus maintain safe production of the consumer. Therefore, the present study aimed to improve the digestion coefficient of barley straw by improving the fermentation ratios in the rumen liquid through means of direct microbial modification using rumen fluids obtained from different types of ruminants (sheep and buffalo) in addition to study the effect of the improved digestion efficient (*in vitro*) on dry and organic matter of barley straw.

MATERIALS AND METHODS

The experiment was conducted at the Central Laboratory of Graduate Studies at the Department of Animal Husbandry, Faculty of Agriculture, University of Baghdad for the period from 15th of October 2014 to 28 of February 2015. The rumen fluid was used for both sheep and buffalo, obtained from slaughtering house at Al-Shaala, Baghdad. Animals were slaughtered at the age of 2.5 years for sheep and 4.5 years for buffaloes as well, barley straw was obtained from local markets, which were grinded in a laboratory mill with a 1 mm diameter filter. Mixing of rumen fluid for both sheep and buffalo was done as follows:

Chemical analysis

The chemical analysis was carried out at the Central Laboratory for Postgraduate Studies at the De-

Table 1. Ratio of rumen fluid combinations for both sheep and buffalo in the laboratory digestion experiment for barley straw

S. No	Treatments	Barley straw	
		Sheep (%)	Buffalo (%)
1	1	100	0
2	2	75	25
3	3	50	50
4	4	25	75
5	5	0	100

partment of Animal Husbandry at the Faculty of Agriculture, University of Baghdad, for barley straw and reed straw models, which were previously grinded in a laboratory mill with a 1 mm diameter filter and then stored in nylon bags.

Dry matter

The dry material of the reed and barley straw varieties was estimated by taking 1.5-2 g of each sample and placed in a porcelain crucibles after taking the weight of the crucible. It was then placed in an electric oven at 105°C for 24 h, according to AOAC (1984) dry matter ratio according to the following equation:

$$\text{Dry matter ratio (DM\%)} = \frac{\text{Sample weight after drying}}{\text{Sample weight before drying}} \times 100$$

Ash

The percentage of the ash in the cane and barley samples was estimated according to AOAC (1984) by taking 1.5-2 g of the sample in porcelain crucible and then placed in the Furnace at a temperature of 600°C and for three hours after which the crucible was cooled, ash as follows:

$$\text{Ash percentage (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Neutral Detergent Fiber (NDF)

The equivalent fiber extract was estimated in the reeds and barley straw varieties according to Goering and Vansoost (1970) for both reed and barley varieties. The method is summarized as follows:

The solution used in estimating the NDF solution was first prepared from the following materials:

- 61.18 g of EDTA (Ethylene diamine tetra- acetic acid)
- 81.6 g of borax
- 30 g of Sodium Dodecyl sulphate (SDs)
- 10 mL of 2-ethoxyethanol
- 4.56 g of disodium hydrogen phosphate anhydrous

All these substances were dissolved in one liter of water and then used to estimate the NDF solution.

Method of action

- 2 g of feed material was weighed.
- The feed material was taken in a 600 mL flask and add 100 mL of the above solution.
- 2 mL of Decline foam solution was added.
- The beaker was placed in the fiber digestion device and leave boil for an hour.
- The contents of the beaker were emptied into a crucible
- Perforated glass and sample was filtered.
- The crucible was washed with warm water, heated well and then put in furnace at 105°C for 24 h.
- The crucible was weighed after it was cooled.

The percentage of NDF was calculated according to the following equation:

$$\text{NDF (\%)} = \frac{\text{Weight of the sample and crucible after drying} - \text{Weight of the empty crucible}}{\text{Weight of sample}} \times 100$$

Acid Detergent Fiber (ADF)

The acid fiber extract was estimated according to Goering and Van Soest (1970). The method is summarized as follows:

A special solution was prepared to estimate the ADF solution from 20 g of cetyltrimethylammonium bromide (CTAB) and 29 mL of concentrated sulfuric acid (H_2SO_4).

Method of action

- The above materials were dissolved in 1 liter of distilled water.
- 2 g of feed material was weighed.
- The sample was placed in a 600 mL flask and 100 mL of the ADF solution was added.
- The sample was digested in the fiber digestion device for one hour.
- The contents of the beaker were poured into a perforated glass crucible and filtered with an air pump.
- The crucible was washed with hot water and then put in furnace at 105 ° C for 24 h.
- The crucible was weighed after it was cooled.

Table 2. Chemical composition of barley straw based on dry matter (%) (Jasim, 2015)

S. No	Chemical composition	Barley straw
1	Dry Matter (DM %)	96.51
2	Organic Matter (OM %)	87.66
3	Crude Protein (CP %)	2.29
4	Crude Fiber (CF %)	27.47
5	Neutral Detergent Fiber (NDF %)	70.55
6	Acid Detergent Fiber (ADF %)	60.79
7	Acid Detergent Lignin (ADL%)	39.50
8	Total phenolic compounds (mg/100g)	61.42

- The ADF ratio was calculated according to the following equation:

$$\text{ADF}(\%) = \frac{\text{Weight of the sample and crucible after drying} - \text{Weight of the empty crucible}}{\text{Weight of sample}} \times 100$$

Acid Detergent Lignin (ADL)

The extraction solution was a 72% sulfuric acid. The crucible containing the dried sample (which was used in the previous ADF estimation) was placed on a 50 mL Beaker glass container and 72% sulfuric acid was added. Then, the sample was covered and activated using a glass column and left inside the solution. The process of adding acid with stirring continued every 30 minutes for three hours. The glass column was washed with hot boiling water from the sample residue above the crucible and left aside. The crucible was well ventilated and placed in the furnace at 105°C, then cooled in the dryer and weighed for a fourth decimal place (1 mg). The crucible was put in the furnace at 550°C for two hours after that the crucible was cooled and weighed and the lignin extract acid was calculated using the following ADL equation:

$$\text{ADL}(\%) = \frac{\text{Crucible weight after drying} - \text{Crucible weight after burning}}{\text{Weight of sample}} \times 100$$

Determination of raw fiber

The crude fiber estimate is based on the principle that the sample was treated with a diluted acid solution (200 mL H_2O_4 at the concentration of 1.25%) and placed on a thermal source for 30 minutes after the start of acid boiling and then a dilute base solution (200 mL NaOH at 1.25 concentration) was added and placed on a thermal source for 30 minutes after boiling to dissolve all organic matter except raw fiber. The soluble materials are disposed of by filtration, the precipitate was dried, weighed and then burned thereafter the ash was weighed for the purpose of calculating the fiber weight in the sample and depending on the method described in AOAC (1984). Ratio of crude fiber was determined according to the following equation:

$$\text{CF}(\%) = \frac{\text{Crucible weight with precipitate after drying} - \text{Crucible weight with ash after burning}}{\text{Weight of sample}} \times 100$$

Total nitrogen

The total nitrogen of the reed and barley straw was estimated in the protein analysis machine Tecator distilling unit 1002, heating unit 1005 and lab CONCO NO 102565 and based on Kaldal theory (AOAC, 1984). The process of estimating nitrogen, based on the theory of Kaldal, includes three stages:

The digestion phase of the sample with the boiling acid concentration was summarized as follows

0.2 g of sample was weighed and 3 mL of concentrated sulfuric acid was added to the sample and then 1.5 mL of hydrochloric acid was put. Then process of

Table 3. Fermentation variables in rumen fluid for both sheep and buffalo used in the laboratory digestion experiment (Jasim, 2015)

S. No	Rumen fermentation variables	Sheep	Buffalo
1	Ammonia nitrogen NH3-N (mg/100 mL)	2.4	4.5
2	Total volatile fatty acid (TVFA)	4	9.4
3	Total phenolic compounds (mg/100g)	0.162	0.239
4	pH	6. 2	6.8

Table 4. Effect of feed, animal type and percentage of rumen liquid mixture on laboratory digestion coefficient for dry matter (IVDMD %) ± Standard error (Jasim, 2015)

Effective factors	Laboratory digestion coefficient IVDMD (100%)	
Percentages of the rumen fluid combinations	Sheep 100	39.23±1.56[b]
	Buffalo 25+sheep 75	42.48±1.67[a]
	Buffalo 50+sheep 50	36.50±1.49[c]
	Buffalo 75+sheep 25	38.36±1.27[b]
	Buffalo 100	39.40±1.53[b]
Significance level	-	**

** Significant difference at (P<0.01) level; [a, b, c] The averages with different characters for each factor within the column are significantly different at (P<0.01)

gradual titration of the sample was begin and turned to black, until the heat of the concentrated acid was heated for not more than one hour and left for 10-30 minutes then cooled to the room temperature then diluted with distilled water to a size of 50 mL.

The phase of distillation

The release of the nitrogen associated with the sulfuric acid in the previous digestion process in the basal medium and source of heat, in the form of ammonia gas, was summarized as follows:

A 5 mL mixture of boric acid and the previously prepared dye was placed in a volume vial to receive the resulting ammonia by distillation process and the end of the rubber tube connected to the condenser is immersed. Thereafter, 5 mL of the diluted sample was placed in the digestion tube of the Kaldal machine and 5 mL sodium hydroxide was added. It is placed directly in the valves and the steam valve is opened to begin the process of releasing the ammonia gas and receiving it in the boric acid and continuing until at least 30 mL was collected in the volumetric vial and ends the distillation process was then taken for direct correction.

The titration phase is summarized as follows

Is a simple titration of the acid with a base where the resulting vial is labeled after the distillation process is completed directly for correction with hydrochloric acid at a concentration of 0.01-0.5 molar and the volume of the acid consumed from the wafer is measured to neutralize the ammonium ion after reaching to the point of equalization that represented by transferring the color of the solution from bluish green to clear and then to pink color. The percentage of nitrogen in the sample was extracted according to the following equation:

$$N\,(\%) = \frac{(\text{The size of the acid in the sludge} - \text{The size of the acid in the plank}) \times 0.014}{\text{Weight of sample}} \times 100$$

To extract the percentage of crude protein, the following equation was used:

Table 5. Effect of feed, animal type, and percentage of rumen liquid mixture on laboratory digestion coefficient for organic matter (IVOMD %) ± Standard error

Effective factors	Laboratory digestion coefficient IVOMD (100%)	
Percentages of the rumen fluid combinations	Sheep 100	37.32±1.53[b]
	Buffalo 25+sheep 75	40.88±1.60[a]
	Buffalo 50+sheep 50	34.74±1.46[c]
	Buffalo 75+sheep 25	36.40±1.37[b]
	Buffalo 100	37.17±1.59[b]
Significance level	----	**

** Significant difference at level (P<0.01); [a, b, c] The averages with different characters for each factor within the column are significantly different at (P<0.01)

Table 6. Effect of the interaction between feed type and rumen mix ratios on laboratory digestion factor for dry matter (IVDMD %) ± Standard error

Feed	Laboratory digestion coefficient IVDMD (100%)	
Barley straw	Sheep 100	42.14± 0.63[b]
	Buffalo 25+sheep 75	45.60±0.80[a]
	Buffalo 50+sheep 50	39.31±0.43[c]
	Buffalo 75+sheep 25	40.76±0.61[bc]
	Buffalo 100	42.35±0.46[b]
Significance level	----	**

** Significant difference at level (P<0.01). The averages within a single column with different characters differ significantly between them

CP% = N% in the sample × 6.25

Determination of total phenolic compounds

The estimated total phenolic compounds in the reed samples and barley straw as well as samples of rumen liquids by optical method mentioned by Swain and Hillis (1959) on the basis of the amount of tannic acid / 100 g dry material in a straw and reeds, and according to the standard curve of tannic acid, (Figure 1 and 2) and optical absorption measurement of the final solution using the LKB spectrophotometer, Biochrom, Novaspec at a wavelength of 760 nm. Solutions used in the estimation of phenolic compounds were folin-denis reagent and sodium carbonate solution. The estimation method was summarized as follows:

A 600 mL of distilled water was taken, boiled and added to 20 g of the sample in a flask and the nozzle of the beaker was sealed and left for 10 minutes. After it was filtered, the leachate was used. Thereafter, a flask (100 mL) was taken and 75 mL distilled water was added. Then 5 mL of the folin reagent was added and 10 mL of the extraction leachate was added to the sample. After 3 minutes, 10 mL of Na_2CO_3 was added and left for 30 minutes followed by reading in the spectrometer at a wavelength of 760 nm.

Nitrogen ammonia: NH_3-N

The frozen and refined rumen liquid was dissolved to estimate ammonia nitrogen in liquid of rumen samples. Sample of 0.5 mL was put in digestion tubes of Kaldal advice and 0.5 g of magnesium oxide with 10 mL of distilled water was added and placed in a distillation unit of Kaldal device and received ammonia in the receiving solution containing 10 mL of 2% boric acid solution and then the resulting was titrate with diluted hydrochloric acid 0.01 according to AOAC (1984).

Table 7. Effect of overlap between feed type and rumen mix ratios on laboratory digestion coefficient for organic matter (IVOMD %) ± Standard error

Feed	Laboratory digestion coefficient IVOMD (100%)	
Barley straw	Sheep 100	44.10±0.73[b]
	Buffalo 25+sheep 75	47.48±1.05[a]
	Buffalo 50+sheep 50	41.13±0.47[c]
	Buffalo 75+sheep 25	42.43±0.52[bc]
	Buffalo 100	43.99±0.38[b]
Significance level	-	**

** Significant difference at level (P<0.01). The averages within a single column with different characters differ significantly between them

Table 8. Effect of interference between animal type and rumen fluid mixture ratios on digestion coefficient for dry matter (IVDMD %) ± standard error

Animal type	Percentages of the rumen fluid combinations	Laboratory digestion coefficient IVDMD (100%)
Sheep	100	38.61±2.13[ab]
	75+25	42.26±1.94[a]
	50+50	35.66±1.88[ab]
	25+75	36.16±1.95[ab]
Buffalo	100	36.55± 2.37[ab]
Significance level	--	**

** Significant difference at level (P>0.01). The averages within a single column with different characters differ significantly between them

Determination of Total Volatile Fatty Acid concentration (TVFA)

The crushed and filtered rumen liquid was dissolved to estimate the volatile fatty acids in the rumen liquid. A distillation unit was used in the Kaldal device according to the Warner method (1964) by steam distillation. 0.5 mL of the methyl red guide was placed in the receiving flask, then, 1 mL of rumen liquid added to 1 ml of phosphoric acid in 10 mL of distilled water and then steam distillation was done and 50-100 mL of condensate solution was collected in flask. The phenolphthalein detector was added and collected from 50 to 100 mL and then corrected with standard sodium hydroxide 0.01. When the colour changed from red to yellow that indicates the equalization and the amount of the used base for equivalent as well as volatile fatty acids were calculated.

pH

The pH of the rumen was measured using a pH meter digital H1 931400 PW instrument. The measurement was done immediately after the rumen was brought from the massacre. Table 2 and 3 show the chemical composition of barley and yeast fermentation variables for both sheep and buffalo.

Determination of dry matter digestion (IVDMD) and laboratory organic matter (IVOMD)

Method of Tillley and Terry (1963) was used to estimate the in vitro IVDMD and the in vitro IVOMD for all samples after obtaining the rumen fluid for both sheep and buffalo after slaughter directly.

Table 9. Effect of interference between animal type and rumen fluid mixture ratios on laboratory digestion coefficient for organic matter (IVOMD %) ± Standard error

Animal type	Percentages of the rumen fluid combinations	Laboratory digestion coefficient IVOMD (100%)
Sheep	100	40.38±2.30[ab]
	75+25	44.01±2.23[a]
	50+50	37.53±1.84[ab]
	25+75	38.13±1.73[ab]
Buffalo	100	38.41±2.35[ab]
Significance level	--	**

** Significant difference at level (P>0.01). The averages within a single column with different characters differ significantly between them

Table 10. Effect of the interaction between the studied factors (feed type, animal type, percentage of rumen liquid mixture) on laboratory digestion coefficient for dry matter (IVDMD %) ± Standard error

Feed	Animal type	Percentages of the rumen fluid combinations	Laboratory digestion coefficient IVDMD (100%)
Barley straw	Sheep	100	43.30 ± 0.52^{abc}
		75 25 +	46.06 ± 1.31^{a}
		50 50 +	39.63 ± 0.59^{ef}
		25 75 +	40.26 ± 1.08^{def}
	Buffalo	100	41.70 ± 0.65^{cde}
Significance level		--	**

** Significant difference at level (P<0.01). The averages within a single column with different characters differ significantly between them

RESULTS AND DISCUSSION

The main effect of the factors studied in IVDMD and IVOMD

The results in Tables 4 and 5 showed a highly significant effect (P<0.01) for feed type, animal type and percentage of rumen fluid on IVDMD and IVOMD. The quantitative analysis of barley straw was consistent with finding of Hassan and Tawfiq (2009) for non-chemically treated barley straw when mixtures of rumen liquid were used, the results showed that replacing 25% of the rumen fluid obtained from the buffalo instead of the rumen fluid obtained from the sheep resulted in a significant increase (P<0.01) in IVDMD and IVOMD. The substitution of 50% of the rumen fluid taken from the buffalo instead of the rumen fluid obtained from the sheep resulted in a significant decrease (P<0.01) in IVDMD and IVOMD (Tables 4 and 5). When replacement ratio is increased to 75% and 100% of rumen fluid obtained of Buffalo did not differ significantly from rumen fluid taken from sheep (100%).

Effect of the interaction between feed and the ratios of rumen liquid mixtures on the laboratory digestion factor of dry matter and organic matter

Tables 6 and 7 showed the effect of the interaction between feed type and rumen mix in the IVDMD and IVOMD. The results showed a significant increase (P<0.01) in IVDMD and IVOMD of barley straw when the utilized replacement ratio was 25% of the buffalo rumen fluid replaces rumen fluid obtained from sheep. This may be due to the inclusion of buffalo rumen fluid on microorganisms capable of analyzing cellulosic material more than those in sheep or due to a change in the rumen environment (Wanapat *et al*., 2000). The results showed that there was a significant decrease in IVDMD and IVOMD for barley straw when the replacement ratio increased to 50% and 75%, and did not differ sig-

Table 11. Effect of overlap between the studied factors (feed type, animal type, percentage of rumen liquid mixture) on laboratory digestion coefficient for organic matter (IVOMD %) ± Standard error

Feed	Animal type	Percentages of the rumen fluid combinations	Laboratory digestion coefficient IVOMD (100%)
Barley straw	Sheep	100	45.36 ± 0.37^{bc}
		75 25 +	48.56 ± 1.57^{a}
		50 50 +	41.43 ± 0.48^{ef}
		25 75 +	41.79 ± 0.87^{def}
	Buffalo	100	43.50 ± 0.62^{bcde}
Significance level		--	**

** Significant difference at level (P<0.01). The averages within a single column with different characters differ significantly between them

nificantly when the used rumen liquid was 100% of the buffalo compared to 100% of the sheep. The decreased IVDMD and IVOMD in barley straw by increasing the substitution ratio may be due to a large change in the microbial groups, which affected the microbial groups that already found in the rumen fluid of sheep.

Effect of the interaction between the animal type and the ratios of the rumen liquid mixture on the laboratory digestion factor of the dry matter and organic matter

Table 8 and 9 showed the effect of the interaction between the animal type and the percentages of rumen liquid mixture in IVDMD and IVOMD. The results showed no significant difference ($P>0.01$) between the rumen fluid obtained from the sheep (100%) and the rumen fluid of buffalo (100 %) in IVDMD for barley straw. The substitution of 25% of buffalo rumen fluid with rumen fluid obtained from sheep resulted in a significant increase ($P<0.01$) in IVDMD and IVOMD in barley straw.

The effect of the interaction between feed and animal and the ratios of rumen liquid mixtures on the laboratory digestion factor of dry matter and organic matter

Tables 10 and 11 showed the effect of interaction between feed, animal type and rumen mix ratios in IVDMD and IVOMD. The results showed a significant ($P<0.01$) superiority for the substitution ratio 25% for the rumen fluid obtained of buffalo replaces the rumen fluid of sheep in IVDMD and IVOMD on the other replacement ratios (50, 75 and 100%) for buffalo rumen fluid and rumen fluid of sheep (100%) which can be attributed to the difference in rumen pH between sheep and buffalo. Devendra (2007) have pointed that buffaloes were more efficient in many traits, including nitrogen cycling, fiber digestion, and concentration of ammonia nitrogen in the rumen, which is related to the fermentation efficiency and feed intake. Wanapat and Rowlinson (2007) demonstrated that increased level of the ammonia in the rumen to 17.6 mg / 100 mL resulted in an increase in feed intake. Additionally, they noted that ammonia nitrogen in the rumen was the main source of nitrogen for microbial protein synthesis. Ammonia nitrogen in rumen fluid obtained from buffalo was more concentrated than rumen fluid obtained of sheep as well as total volatile fatty acid concentration Table 3.

CONCLUSION

The development of the rumen environment through the creation of combinations of sheep and buffalo liquid 75: 25 (sheep: buffalo) resulted in improved laboratory digestibility of dry matter and organic matter of barley straw, while 50: 50 (sheep: buffalo) reduced laboratory digestion of dry matter and organic matter for barley straw.

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