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Use of total mixed ration citrus pulp silage as a new feeding strategy for growing lambs

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Abstract

This study was conducted to evaluate the effects of using total mixed ration silage based on citrus pulp on feed intake, growth performance, serum metabolites and ruminal fermentation of growing female lambs. For this purpose, sixty-eight female Zel lambs (5-6- months) with an initial body weight of 20.65 ± 3.71 kg were randomly divided into two equal groups (n = 34). Two isonitrogenous and isoenergetics diets were formulated based on corn silage (TMRC) and citrus pulp silage (TMRPS), respectively. To prepare TMRPS, first the citrus pulp and wheat straw were poured into A feed mixer, after which the concentrate was added. After thoroughly homogenising all components, the resulting mixture was ensiled into a permanent horizontal silo (a concrete bunker) and was covered with plastic shortly after the silo was filled. Each diet was randomly assigned to a group of lambs. Feed was offered two times a day (at 08:00 and 16:00 h) *ad libitum*. Daily dry matter intake (DMI) averaged 1067 g for the TMRC and 924 g for the TMRPS group. No significant differences were observed in the Kleiber ratio (6.9 *versus* 6.6) and average daily gain (ADG; 86 g *versus* 91 g) between the TMRC and TMRPS group. There was no difference in serum glucose and total protein concentration between treatments, but serum concentrations of urea-N and cholesterol in lambs fed the TMRC diet (80.5 and 47.1 mg dl^{-1}, respectively) were higher than in lambs fed the TMRPS diet (65.5 and 22.8 mg dl^{-1}, respectively). Ammonia-N content of the rumen fluid was reduced by changing the diet from TMRC to TMRPS. It can be concluded that TMRPS can be used as a low-cost diet for lambs without negative impact on the growth performance.

Keywords: citrus pulp, growth performance, rumen fermentation, sheep

1 Introduction

The agro-industrial by-products have been increased in the north of Iran (Fazaeli *et al.*, 2020), which resulted in some problems for industry owners and, more broadly, for the human environment. However, most of these agro-industrial by-products can be converted into an acceptable feed for livestock with only little processing (Chaudhry & Naseer, 2006). This reduces the potential risks to the environment and, due to the low initial price, lowers the feed costs thus enhancing the efficiency in farm animal feeding. Citrus pulp is a by-product of juice production and can be fed to dairy sheep, without negative impact on their production performance (Fegeros *et al.*, 1995).

Annually, more than three million tons of citrus fruits are produced in Iran, of which about 59 % are harvested in Mazandaran province (Teimouri Chamebon *et al.*, 2017). About 100,000 tons thereof are channelled into processing industries whose main products are fruit juice and concentrate. More than half of this volume leaves the production line as waste or pulp, of which about 60-65 % is skin, 30-35 % is pulp, and up to 10 % is the grain (Teimouri Chamebon *et al.*, 2017). According to Arbabi *et al.* (2008), this by-product can be used in ruminant diets. Yet, seasonal availability, low dry matter percentage (15-20 %), rapid decay, loss of soluble nutrients with the affluent, as well as transport and environmental problems limit the possibility of using the fresh matter for feeding ruminants (Kordi *et al.*, 2014). A solution to this problem is storage of citrus pulp in the form of silage (Chaudhry & Naseer, 2006), which works well under the climatic conditions in the North of Iran. While the moisture content recommended for ensiling of forage and food materials is 65-70 %, fresh citrus pulp contains 85-88 %

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of moisture. Thus, high-quality silage is only obtained if material with a high absorption capacity, like for example wheat straw, are added to the citrus pulp (Migwi *et al.*, 2001).

Another option is preparing a total mixed ration silage (TMRS), which can reduce feed costs in livestock rearing units (Kondo *et al.*, 2016). TMRS is based on silage and concentrate additives, whose ingredients are formulated according to the animals' nutrient requirements (Hasanah *et al.*, 2017). In cases where the moisture content of the base feed (such as citrus pulp) exceeds the threshold required for ensiling, moisture absorbers such as cereal straw are used (Migwi *et al.*, 2001). The primary studies in this field were conducted in the United States in the 1960s, while in the last decade scientists in Italy, Japan, South Korea, China, Vietnam, Thailand, Indonesia, South Africa, Argentina, and Brazil have addressed TMRS as well (Bueno *et al.*, 2020). Today, TMRS production is common for the storage and optimal use of high-moisture by-products as ruminant feed in Japan (Wang & Nishino, 2013).

Water-soluble carbohydrates are the main substrates for microbial growth during silage fermentation. As a result of microbial activity organic acids, mainly lactic acid, accumulate and lead to a reduction in pH (Bueno *et al.*, 2020). Miyaji *et al.* (2017) reported that the digestibility of starch in a mixed ration increased as a result of ensiling, which likely improves energy availability and production performance.

There is concern about the breakdown of protein in silages from food industry by-products, as well as forages and other substrates. However, although protein breakdown and conversion to non-protein nitrogen also occurs in TMRS, this process is of limited extent since TMRS has usually a high dry matter concentration of 40-60 % (Bueno *et al.*, 2020).

Feeding TMRS saves labour for feed preparation and delivery (Bueno *et al.*, 2020), confers uniformity of the total mixed ration (Rao *et al.*, 2014), increases the palatability of non-palatable components in the prepared silage (in response to fermentation and its effect on reducing unpleasant odor and taste) and enhances the aerobic stability of the prepared silage at the time of opening and during daily feeding (Wang *et al.*, 2012). The aerobic stability of the prepared silage is even observed under high environmental temperatures such as in summer (Nishino *et al.*, 2004). Also, the possibility of preparing individually wrapped (plastified) TMRS bales and providing these to farmers is another advantage of this technology (Nishino *et al.*, 2003; Weinberg *et al.*, 2011).

In Iran, the National Animal Sciences Research Institute has recently started to investigate TMRS production. The present study aimed to prepare a TMRS based on the citrus pulp for feeding lambs because of its cheap and high availability in the north of Iran.

2 Materials and methods

The study started in the winter of 2019 at the Gavdasht National Research Station located in Babol city, Mazandaran Province, and ended in the summer of 2020. Babol is located at an altitude of 54 m above sea level (latitude: 36°23′ N, longitude: 52°53′ E). The average annual temperature and rainfall in this area are 18.5 °C and 652 mm.

2.1 Experimental diets

The citrus pulp was purchased from the Neshtarood Juice Factory. Two experimental rations were prepared (Table 1).

Table 1: *Ingredients and chemical composition of the experimental diets (DM basis).*

	Experimental diets (%)			
	TMRC[1]		*TMRPS*[2]	
	as-fed	*DM*	*as-fed*	*DM*
Ingredient				
Corn silage	68.45	35.00	0.00	0.00
Fresh citrus pulp	0.00	0.00	78.88	36.64
Wheat straw	10.31	21.00	9.19	27.00
Wheat bran	5.84	12.00	5.10	15.00
Sugar beet pulp	2.83	5.79	2.00	5.75
Corn grain	5.93	12.00	2.47	7.25
Barley grain	6.00	12.00	1.40	4.10
Urea (46 % N)	0.68	1.50	0.50	1.60
Diammonium sulfate	0.00	0.00	0.15	0.49
Dicalcium phosphate	0.22	0.48	0.00	0.00
Vitamin & mineral mix[3]	0.10	0.23	0.10	0.23
Chemical composition ($g\,kg^{-1}$)				
Crude protein	-	126.40	-	127.20
Calcium	-	5.90	-	7.40
Phosphorus	-	2.80	-	2.50
Metabol. energy ($MJ\,kg^{-1}$)[†]	-	10.00	-	9.70

[1] TMRC: Total mixed ration maize silage. [2]TMRPS: Total mixed ration citrus pulp silage. [†]Metabol. energy: Metabolizable energy
[3]Supplies per kg of diet: 99.2 mg Mn, 50 mg Fe, 84.7 mg Zn, 10 mg Cu, 1 mg I, 0.2 mg Se, 9000 IU vitamin A, 2000 IU vitamin D, and 18 IU vitamin E.

A total mixed ration based on citrus pulp (TMRPS) and a total mixed ration based on corn silage (TMRC). Both rations were formulated and balanced regarding the nutrient requirements of Zel female lambs (NRC, 2007) as well as the nutrient composition tables of livestock feed in Iran (Gholami *et al.*, 2018). To prepare TMRPS, citrus pulp and wheat straw were poured into the feed mixer, and then the concentrate was added. After thoroughly homogenising all components, the resulting mixture was ensiled into a permanent horizontal silo (a concrete bunker with about

20 t capacity of fresh crop) and was covered with plastic shortly after the silo was filled for 60 days (Daniele *et al.*, 2013). The compaction density of silage was approximately 105 kg DM m^{-3}. In order to prepare TMRC ration, corn silage was mixed with other diet components every day and provided to animals.

2.2 Laboratory analysis

The chemical composition of fresh citrus pulp (15.00 % DM, 6.90 % crude protein, 22.00 % NDF, 1.40 % Ca, 0.10 % P, and 10.73 MJ ME kg^{-1}, all DM basis) and TMRPS was measured 0, 30, and 60 days after ensiling. Following AOAC (2002), the dry matter content of the samples was determined using an oven (DM, method 930.15), and ash through incineration in a muffle furnace (method 924.05). Nitrogen (N) was measured using the Kjeldahl procedure (Kjeltec-UDK, Gerhardt, Germany). The concentration of N was multiplied by 6.25 to calculate the crude protein (CP) concentration (method 984.13). To determine the concentration of macro-minerals, a digestive extract was prepared using perchloric acid 72 % (1 ml) + nitric acid 65 % (2 ml; AOAC, 2002). The phosphorus content was measured using a spectrophotometer (CEcill CE 3041, Biorad, USA) and the calcium content was measured via flame atomic absorption method (GBC 932 PLUS, Leipzig, Germany). The aNDF content was measured using heat-resistant α-amylase without sodium sulfite (Van Soest *et al.*, 1991).

In order to measure pH, 50 g TMRPS was put into an electric mixer; 100 ml of distilled water was added and mixed for 10 min. Afterwards, the mixture was passed through a filter and extracted. The obtained extract was used to measure pH (CG 804- pH meter, SANA, Germany), and ammonia-N concentration was measured using phenol-hypochlorite (Broderick and Kang, 1980). To determine the digestibility of diets, Tilly and Terry's (1963) method was used and the results were used to estimate the metabolizable energy content of the diets (Table 2; AFRC, 1993).

2.3 Animal management

Sixty-eight, 5 to 6 months old female Zel lambs (20.65 ± 3.71 kg initial BW) were used in this study. Once weighed, the lambs were randomly divided into two groups (n = 34), and each group was housed in a closed building (10 m × 7 m). The lambs were randomly assigned to two dietary treatments, namely TMRC and TMRPS. A period of 10 days was allowed for the lambs to adapt to the diets. Then, the lambs were weighed again with this weight recorded as the starting weight of the experiment. Next, the feed of each group of lambs was offered to the lambs twice (08:00 and 16:00 h) a day for 120 days. The lambs were provided *ad libitum* access to feed and water during the trial. The amounts of feeds offered and orts (removed at 07:00 next day) were weighed daily. Individual feed intake was recorded through subtracting the weight of the daily offered feed from refused quantities for all animal group and then dividing it by 34. All lambs were weighed individually at the end of each month after 16 h of feed deprivation. Average daily gain (ADG) of individual lambs was calculated based on the sum of average daily gains of every month record divided by 4 (4 = times of BW recording). The Kleiber ratio was calculated as the ratio of ADG to metabolic body weight ($BW^{0.75}$) at the end of the trial (Talebi, 2012).

2.4 Ruminal fermentation parameters

Four mature, ruminally fistulated Chall rams were used to measure ruminal fluid parameters including pH and ammonia-N concentration. The animals were housed in individual metabolic crates (0.6 m × 1.3 m) during 21 days (14 days of adaptability and 7 days for sample collection). The rams were cared for according the guidelines of the Iranian Council of Animal Care (1995). Rumen samples were collected from each ram before the morning feeding and three hours after, through the rumen fistula. The rumen content was filtered immediately after sampling using fustian cloth. In order to determine the concentration of ammonia-N, 5 ml ruminal fluid and 5 ml 0.2 N hydrochloric acid were

Table 2: *Digestibility of total mixed ration citrus pulp silage during storage (DM basis).*

	Storage period		
Chemical composition (%)	*0 days*	*30 days*	*60 days*
Digestible dry matter	69.55 ± 0.45	75.25 ± 1.06	71.13 ± 1.59
Digestible organic matter	70.55 ± 0.35	76.20 ± 0.19	71.25 ± 1.90
Digestible organic matter (in dry matter)	64.90 ± 0.40	70.84 ± 0.18	66.30 ± 1.77
Metabolizable energy (MJ kg^{-1})*	10.90 ± 0.65	11.90 ± 0.30	11.30 ± 0.03

*ME (MJ/ kg DM) = 0.168 DOMD (digestible organic matter in dry matter; AFRC, 1993).

Table 3: *Chemical composition and fermentation characteristics of total mixed ration citrus pulp silage during storage (DM basis).*

	Storage period				*p-value*	
Chemical composition (%)	*0 days*	*30 days*	*60 days*	*SEM*	*Linear*	*Quadratic*
Dry matter	27.93	28.05	28.15	0.069	0.259	0.967
Organic matter	92.88	93.05	93.03	0.039	0.099	0.221
Crude protein	12.53	12.51	12.37	0.090	0.539	0.776
NDF	38.13	37.62	37.78	0.108	0.173	0.139
Fermentation characteristics						
NH_3-N (%)	8.66	8.72	8.77	0.075	0.628	0.986
pH	4.60	3.84	3.81	0.049	0.001	0.029

mixed in 15- ml Falcon tubes and centrifuged (Sigma -2-16-P-Germany) at 4000 × g for 20 min. The supernatant was transferred into 1.5- ml plastic microtubes to determine ammonia-N, using a phenol-hypochlorite assay according to Broderick and Kang (1980).

2.5 Blood metabolites

Blood samples of the lambs were taken on day 120 to determine the concentration of serum metabolites. For this purpose, five lambs were randomly selected from each treatment and 10 ml blood were collected from the jugular vein (Venogect tubes, Fartest., Iran). The samples were kept in a refrigerator at 4 °C for 24 h and then centrifuged (8311024, Parsia., Iran) at 3000 × g for 20 min. The supernatant was transferred into 1.5- ml plastic microtubes to determine serum metabolites. Serum samples were kept in the freezer at -20 °C until further analysis. The serum concentrations of total protein, glucose, urea- N, and cholesterol were measured using an autoanalyzer system with com-mercial kits (Far assamed, Co., Iran) according to Kerscher and Ziegn Born (2001).

2.6 Statistical analysis

The statistical model for performance data analysis is:

$Y_{ij} = \mu + T_i + \beta_i$ (BW (+ e_{ij}

Where, Y_{ij}= value of each observation in treatment *i*

μ= Mean of the studied traits

T_i= effect of treatment i (ration)

$\beta 1$= linear function coefficient of the studied trait from the weight of the beginning of the test

BW = Test start weight

e_{ij}= effect of test error (residual error).

The statistical model for serum metabolites and rumen parameters data analysis is:

$Y_{ij} = \mu + T_i + e_{ij}$

Where, Y_{ij}= value of each observation in treatment i

μ= Mean of the studied traits

T_i= effect of treatment i (ration)

e_{ij}= effect of test error (residual error).

Analysis of variance was performed using SAS version 9.1 statistical software (SAS, 2002). Data on performance (body weight change and kleiber ratio), serum metabolites, and rumen parameters were analysed as a completely randomized design. The polynomial contrast statement of the SAS program was used to acquire linear and quadratic (non-linear) time effects on TMRPS quality (Table 3). The treatment mean values were compared by LSD method and GLM procedure. Since the lambs were group-fed, analysis of variance of intake data was not possible. Differences were considered significant at $p < 0.05$.

3 Results

The changes in the chemical composition and fermentation properties of TMRPS over time are reported in Table 3. None of the changes in the concentration of dry matter, organic matter, crude protein, and NDF during storage of the ensiled material were significant ($p > 0.05$).

Table 4: *Effects of TMRC and TMRPS diet on feed intake and growth of lambs (n = 34 per diet).*

	Experimental diets			
Variable	*TMRC**	*TMRPS†*	*SEM*	*p-value*
Dry matter intake (kg DM/animal*day)	1067	924	-	-
ME intake (MJ/animal*day)	10.70	8.96	-	-
Initial body weight (kg)	19.7	21.6	0.68	0.150
Final body weight (kg)	32.0	32.5	0.74	0.460
Average daily gain (g/day)	86	91	6.2	0.460
Kleiber ratio‡	6.86	6.62	0.225	0.613

*TMRC: Total mixed ration maize silage.
†TMRPS: Total mixed ration citrus pulp silage.
‡Kleiberratio: The Kleiber ratio was calculated as the ratio of ADG to metabolic body weight ($BW^{0.75}$) at the end of the trial.

Due to the group-feeding of lambs in the treatments, it was not possible to statistically compare their dry matter intake (DMI). However, the mean DMI of TMRC-fed lambs was numerically higher than that of TMRPS-fed lambs (Table 4).

Table 5: *Effects of TMRC and TMRPS diets on rumen fermentation parameters (n = 4).*

*Variable**	*Experimental diets* *TMRC†*	*TMRPS‡*	*SEM*	*p-value*
Rumen fluid pH				
0 hour	7.00	7.20	0.05	0.60
3 hour	6.90	6.70	0.19	0.36
NH_3-N (mg dl^{-1})				
0 hour	11.20^a	5.00^b	1.79	0.03
3 hour	21.30^a	16.50^b	2.52	0.01

*Rams were sampled immediately before and 3 h after the morning feeding.
†TMRC: Total mixed ration maize silage.
‡TMRPS: Total mixed ration citrus pulp silage.

The changes in pH and ammonia-N of ruminal fluid of fistulated rams fed the TMRC and TMRPS before morning feeding and three hours after feeding are outlined in Table 5. Ruminal ammonia-N concentration at both measurement times was significantly lower when rams were fed the TMRPS diet than when fed the TMRC diet ($p < 0.05$).

Table 6: *Effects of TMRC and TMRPS diets on serum metabolites of lambs (n = 5 per diet).*

Variable	*Experimental diets* *TMRC†*	*TMRPS‡*	*SEM*	*p-value*
Glucose (mg dl^{-1})	68.7	66.0	5.70	0.74
Total protein (g dl^{-1})	6.65	6.77	0.19	0.66
Cholesterol (mg dl^{-1})	80.5^a	65.5^b	50.90	0.04
Urea-N (mg dl^{-1})	47.1^a	22.8^b	1.84	0.01

†TMRC: Total mixed ration maize silage.
‡TMRPS: Total mixed ration citrus pulp silage.

No significant difference was observed for serum glucose and total protein concentration between lambs fed the TMRC and the TMRPS diet (Table 6). The blood cholesterol and urea-N levels of lambs fed the TMRC diet were significantly higher than those of the lambs fed the TMRPS diet ($p < 0.05$).

4 Discussion

In line with our findings, Miyaji *et al.* (2017) reported a non-significant reduction of the crude protein content of two types of TMRS based on corn and rice after 7, 14, 30, 90, and 210 days of ensiling. Our results also concurred with those of Wang *et al.* (2012) who reported that the crude protein content in a total mixed ration silage based on distillers grains and corn straw did not change during the test. Although breakdown of protein and conversion to non-protein nitrogen also occurs in TMRS, the extent of these processes is limited by the usually high dry matter concentration (40-60 %) of the silages (Bueno *et al.*, 2020). The rate of breakdown of protein varies depending on the type of ensiled forage and feed, concentration of fermentable carbohydrates and dry matter, carbohydrate fermentation rate, pH reduction intensity, and ambient temperature. The optimal pH for the activity of protein-decomposing enzymes is within 5.5-6 (Muck, 1990); thus, the low pH in our TMRPS may explain the stable crude protein content over time.

There are limited and varied reports on changes in NDF content after ensiling, ranging from breakdown to stable concentrations. In a TMRS containing 50 % of dry matter which was ensiled for 140 days, the NDF concentration decreased from 38 % to 34 %, while no change was observed when dry matter concentration of the TMRS was 64 % (Weinberg, 2011). Similarly, Kondo *et al.* (2016) reported that NDF concentrations in TMRS did not change during 30 days or 90 days of ensiling. Again, NDF hydrolysis and variations in the fiber content during ensiling are mainly dependent on TMR components, moisture, and time of ensiling (Bueno *et al.*, 2020). Ammonia-N content of TMRPS did not change during ensiling, while its pH declined linearly. In the early days of ensiling, pH dropped from 4.6 to 3.85 and then remained almost constant, similar to the results reported by Wang *et al.* (2012). The reduction in pH in the early days of silage preparation and its relatively constant values thereafter can be ascribed to the fermentation of starch and consequent increase in lactic acid concentration (Wang *et al.*, 2012).

Examining the effects of TMRS feeding in Holstein dairy cows, Miyaji & Nonaka (2018) reported that DMI of TMRS-fed cows compared to that of cows fed with a normal total mixed ration (TMR) but was higher by trend. In some studies (Fazaeli *et al.*, 2020), it has been reported that feeding livestock with TMRS instead of fresh TMR could promote the digestibility of DMI, organic matter, and NDF. Thus because of increasing digestibility of the diet due to ensiling, can reduce retention in the rumen and thus increases DMI (Miyaji & Nonaka, 2018).

No statistically significant difference was observed between TMRC and TMRPS in the ADG of the lamb's groups. While no comparable study with TMRPS is available for lambs, our results are consistent with the findings of other studies investigating the effects of citrus pulp silage on daily weight gain of fattening Zel lambs (Teimouri Chamebon *et al*., 2017; Fayz *et al*., 2016). Abedeini *et al*. (2012) reported that the complete replacement of barley grains with dried citrus pulp in the diet of fattening lambs reduced their ADG, but this was not confirmed by the present results. The very similar nutritional quality of the TMRPS and TMRC diets may be one of the reasons for the lack of a significant difference in lambs' ADG as well as in the Kleiber ratio.

In general, the pH of the ruminal fluid of the fistulated rams in the present study was within the desired physiological range (Dehority, 2003). Before feeding, the pH content of the ruminal fluid in rams fed TMRPS was numerically higher than that of rams fed TMRC; after feeding, the rumina pH of animals receiving TMRPS was numerically lower than that of animals fed the TMRC diet. However, no statistically significant difference was observed between the treatments. Ruminal production of ammonia is due to the activity of proteolytic bacteria and ammonia-producing bacteria (Chen & Russell, 1989); starch-rich diets foster these bacteria and thus elevate the concentration of rumen ammonia-N (Beizai *et al*., 2013). The higher ruminal ammonia-N concentration in animals fed the TMRC diet might be attributed to a high starch content of the ensiled maize cobs. In addition, a reduction in ruminal ammonia-N concentration of lambs fed the TMRPS diet may be due to the fact that an increase in the available energy in the rumen has caused more bacteria to use ammonia, which in turn reduces ammonia-N concentration (Novozamsky *et al*., 1974).

The serum glucose concentration is affected by the concentration of propionic acid in the blood, as propionate is a precursor to the synthesis of blood glucose in ruminants (Busquet *et al*., 2006). A normal blood glucose level of 50-80 mg dl^{-1} has been reported for lambs (Kaneko, 1989), as also confirmed by the results of the present study. Bayat Koohsar *et al*. (2010) reported that feeding different levels of dried citrus pulp to dairy cows had no significant effect on the serum cholesterol concentration; in the present study, on average lambs fed TMRPS ingested less DM than lambs fed the TMRC diet; thus the lowered blood cholesterol concentration might be related to lower DMI.

The urea-N concentration in the blood of lambs fed the TMRPS diet was lower compared to lambs fed the TMRC diet. This might have been due to a higher breakdown of carbohydrates in the rumen, allowing ruminal bacteria to use rumen degradable protein more efficiently (Beizaei *et al*., 2014). Blood urea-N is a key indicator for an animal's protein supply status and its ruminal function (Hess *et al*., 2000); its high concentration usually reflects inefficient utilisation of dietary nitrogen (Nousiainen *et al*., 2004).

5 Conclusions

Although the DMI of lambs fed TMRC was higher compared to lambs fed TMRPS, the ADG was not affected by the diet. Feeding TMRPS to lambs had no impact on rumen pH but it reduced the concentration of ammonia-N in rumen fluid and urea-N in the serum. Thus, it can be concluded that TMRPS can be used as a low-cost diet for lambs without adverse effects on the growth performance.

Conflict of interest

The authors declare that there is no conflict of interest.

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