



EFFECT OF DIFFERENT SOURCES OF PECTIN-RICH BY-PRODUCTS SUBSTITUTED FOR BARLEY GRAIN ON THE PERFORMANCE OF HOLSTEIN DAIRY COWS

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ABSTRACT

This study investigated the effect of feeding pectin-rich by-products, as a replacement for barley grains, on the performance of Holstein dairy cows. Eight cows were assigned to four dietary treatments in a 4 × 4 Latin square design and fed for 21 days. The experimental diets consisted of 370 g.kg⁻¹ roughages and 630 g.kg⁻¹ concentrates. The diet contained 100 g.kg DM⁻¹ barley grains (BG, control diet), sugar beet pulp (SBP, treatment 2), wheat bran (WB, treatments 3) or dried citrus pulp (DCP, treatment 4). There were no significant differences ($P > 0.05$) among the treatments for dry matter intake (DMI) (kg.d⁻¹), milk production, milk composition and blood metabolites. Eating behaviour was not affected by the treatment ($P > 0.05$). However, the differences among the treatments for eating behaviour as min.kg⁻¹ of neutral detergent fiber intake (NDFI) were significant ($P < 0.05$). These data suggest that the substitution of these by-products instead of barley grains for up to 10 % of in diet DM for dairy cows did not have any adverse effects on animal performance.

Key words: barley grain; dried citrus pulp; pectin; starch; sugar beet pulp

INTRODUCTION

The grain is often substituted for forage in high producing dairy cow diets to increase intake and milk yield. Reducing dietary NDF concentration usually increases DMI, probably by lowering the filling effect of the diet (Allen, 1997). However, increasing dietary starch can also negatively affect feed intake and milk production. Feeding less forage NDF reduces chewing time, and fiber digestibility is reduced when dietary starch concentration is increased (Grant and Mertens, 1992). Also, the grains are costly, and their inclusion into the animal diet further increases animal production cost. In the past, efforts have been made to minimize the use of grains in the diet

of animals by substituting various agricultural by-products. There are several alternative strategies for increasing dietary energy content with a lower risk of its adverse side effects. One approach is to utilize carbohydrate sources in ruminant's diets to ferment faster and more extensive than forage NDF, and that mimic some of its beneficial effects, but do not have the same adverse impact as starch fermentation (Afghahi and Esteghamat, 2015).

Adding non-forage NDF to low-forage diets might reduce the adverse effects of increased starch fermentation without increasing the filling effect of the diet to the same extent as forage NDF (Allen, 2000). Beet pulp, dry citrus pulp and wheat bran are by-product feeds that contain a high concentration

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of neutral detergent soluble fiber (NDSF), especially pectin substances. Dry beet pulp contains 250 g of pectin/kg DM (Thibault *et al.*, 1991). In citrus pulp, pectin comprises approximately 450 g.kg⁻¹ of cell wall component (Sunvold *et al.*, 1995), and the content of total pectin in wheat bran is about 0.35 % on DM basis (Bailoni *et al.*, 2003). Pectin, a valuable constituent of ruminant's feed, is degraded rapidly and extensively in the rumen but, unlike starch, yields little lactate, causing less of a decline in rumen pH (Barrios-Urdaneta *et al.*, 2003). Some of the results in related reports about replacing SBP for grain in the total mixed ration (TMR) are variable. Dann *et al.* (2014), partially replacing corn grains with SBP or wheat middlings, did not find any significant effects on ruminal fermentation, chewing behaviour, milk production and composition. In a recent study, Ertl *et al.* (2016) evaluated the impact of a complete substitution of common cereal grains and pulses with a mixture of WB and SBP in a high forage diet on dairy cow performance. They showed that dietary treatment did not affect milk production, milk composition, feed intake, or total chewing activity.

Bayat Kohsar *et al.* (2010) showed that replacing barley grains with DCP at 5, 10 and 15 % level (DM basis) in the diet of lactating dairy cows not affected the milk yield, milk composition, feed intake, and glucose and urea nitrogen concentration in blood plasma. However, other studies showed that 10–20 % DCP in the diet decrease the feed intake in dairy cows (Miron *et al.*, 2002; Bayat Kohsar *et al.*, 2010).

During the processing of wheat, about 20 % of the DM results in the by-products such as wheat bran, which indicates their high availability (Ertl *et al.*, 2016). The use of wheat bran in livestock diets is recommended due to higher energy content than forages. In comparison with forages, wheat bran NDF is fermentable, and more than 62 % of rumen microorganisms are accessible. Therefore, wheat bran can effectively be included into the diets of dairy and fattening sheep and cattle as a part of forage or grains (Afghahi and Esteghamat, 2015; Oba and Allen, 1999).

The objective of this study was to evaluate the effects of different sources of pectin-rich by-products on performance, plasma metabolites and chewing activity in early lactation Holstein dairy cows.

MATERIAL AND METHODS

Eight primiparous Holstein dairy cows (60 ± 18 days post-partum, weighing 521 ± 48 kg) were assigned to four groups of two cows each, according to calving date, lactation number, and daily milk yield and assigned randomly to one of four diets in a 4 × 4 Latin square design. The measured parameters were: feed intake, milk production and composition, blood metabolites and chewing behaviour during the duration of four 3-week periods. Each experimental period lasted for 21 days, including a 14-day adaptation and seven days for sample collection.

Each cow was assigned to one of four diets with 370 g of forage/kg DM (barley silage and alfalfa hay), and 630 g of concentrate/kg DM, comprised of: 1) 100 g of barley grain (BG)/kg DM (control diet), 2) 100 g of sugar beet pulp (SBP)/kg DM, 3) 100 g of wheat bran (WB)/kg DM and 4) 100 g of dried citrus pulp/kg DM (DCP) and diets formulated by NRC (2001) software. The feed ingredients and chemical composition of diets are shown in Table 1.

The diets were fed individually, in tie stalls, as TMR, in two equal parts at 08:00 and 16:00 h in amounts to ensure 10 % refusals. Feed refusals were removed when the cows left the tie stalls for intake estimation. Cows were milked daily at 05:00, 13:00 and 21:00 h.

Sample collection and analysis

Animals were weighed at the beginning and the end of each measuring period. Feeds and orts were sampled daily during the collection period and were composited by period. The mixed samples of TMR and feed refusal were dried in an oven (60 °C, 48 h), then ground to pass through a 1-mm screen and stored for later analysis. The dry matter content of samples was determined by drying in an oven at 100 °C to a constant weight (AOAC 2005, method 934.01). Ash (method 942.05) and CP (Kjeldahl N × 6.25) were determined by the block digestion method using a copper catalyst and steam distillation into boric acid (method 2001.11) on 2100 Kjeltac distillation unit as described in Association of Official Analytical Chemists (AOAC) (2005). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by Van Soest *et al.* (1991). Milk yield was recorded daily. Milk samples were taken from each milking time during the last three days of

each period and analyzed for protein, fat, lactose, solids-not-fat and total solids with a Milko-Scan 605 analyzer (Foss Electric, Hillerød, Denmark). Feed intakes and feed refusals were collected before the morning feeding and weighed daily during the measurement period. Dry matter intake was calculated by the difference between the total amount of DM offered and refused. Blood samples were drawn from the jugular vein into evacuated tubes on the last day of each experimental period at

about 3–4 h post-feeding. The collected serum was frozen at -20 °C after centrifuging, later analyzed for glucose, urea N, cholesterol, triglyceride, alkaline phosphate and albumin using an auto-analyzer (Biosystems A-15; 08030 Barcelona, Spain).

Ruminal fluid samples were taken using a vacuum pump 4 hours after the morning feeding to determine pH and NH₃-N concentrations. Approximately 100 ml of ruminal content were strained through four layers of cheesecloth, and pH

Table 1. Ingredient and chemical composition of the experimental diets

Item	Treatments ¹			
	BG	SBP	WB	DCP
Ingredients, g.kg DM ⁻¹				
Alfalfa	200	200	200	200
Barley silage	170	170	170	170
Soybean meal	100	105	95	115
Cottonseed	70	70	70	70
Corn grain	210	210	210	210
Cottonseed meal	110	105	115	95
Barley grain	100	0	0	0
Sugar beet pulp	0	100	0	0
Wheat bran	0	0	100	0
Dried citrus pulp	0	0	0	100
Fat	20	20	20	20
Limestone	7	7	7	7
Vitamin-Mineral mix ²	10	10	10	10
Salt	3	3	3	3
Chemical composition, (% DM)				
CP	18.00	17.09	18.01	17.80
NDF	30.43	32.61	32.71	30.38
Forage NDF	17.95	17.95	17.95	17.95
Soluble NDF	9.00	10.50	8.75	11.30
ADF	20.13	21.97	20.91	20.91
NFC ³	37.60	35.40	34.50	37.50
Starch	22.30	17.50	19.20	17.60
Sugar	5.30	5.90	5.50	7.70
NDSF ⁴	10.00	12.03	9.50	12.30
Ether extract	6.10	6.90	6.30	6.10
Ash	7.90	8.30	8.30	8.40
Ca	0.80	0.90	1.00	1.01
P	0.50	0.50	0.50	0.50
NEI ⁵ , MJ.kg ⁻¹	6.73	6.69	6.73	6.71

¹BG: barley grain, SBP: sugar beet pulp, WB: wheat bran, DCP: dried citrus pulp; ²Contained (/kg of premix; DM basis): 330.000 IU of vitamin A, 60.000 IU of vitamin D, 1.000 IU of vitamin E, 16 % Ca, 8.5 % P, 6.3 % Na, 4.5 % Mg, 2.100 mg Zn, 1.500 mg Mn, 535 mg Cu, 12 mg Se, 45 mg I; ³NFC: non-fiber carbohydrates; ⁴NDSF: Neutral detergent soluble fiber; ⁵NEI was calculated based on NRC (2001).

was measured immediately with a portable digital pH meter (Metrohm 691). For determination of $\text{NH}_3\text{-N}$, 10 ml of filtered rumen fluid was added to 10 ml of 0.2 N HCl (vol/vol) and was frozen immediately at $-20\text{ }^\circ\text{C}$ and then analyzed by the procedure of Weatherburn (1967).

Eating and ruminating behaviour observations were recorded visually every 5 min for 24 h. The time spent on each animal observation was not more than 5 sec. Eating, ruminating and total chewing times were determined and expressed in minutes per day. Time (shown in minutes) expended in each activity was calculated by the number of observations recorded, multiplied by 5. Total chewing time was

considered as the sum of eating and rumination times (Krause *et al.* 2002). Eating, ruminating and total chewing times were also expressed as minutes per kilogram of DM and NDF intakes. Animals were weighed on day 21 before feeding, and then the diet given to each cow was changed.

Statistical analysis

Data were analyzed using the GLM procedure of SAS (2003). Data about DMI, milk production, milk composition, blood metabolites and eating behaviour were analyzed as a Latin square design. The model included treatment effects, period effect, random effects of cows and experimental

Table 2. Dry matter intake, milk production, and composition and feed efficiency for dairy cows

Item	Treatments ¹				SEM	P-value
	BG	SBP	WB	DCP		
DMI, kg.d ⁻¹	19.34	19.63	19.74	18.69	0.348	0.175
NDF intake, kg.d ⁻¹	6.05 ^b	6.61 ^a	6.63 ^a	5.88 ^b	0.110	0.0001
Production (kg.d ⁻¹)						
Milk	28.68	28.30	28.81	27.37	0.569	0.218
% 4 FCM ²	25.00	26.43	25.92	24.64	0.907	0.498
ECM ³	25.95	27.08	26.88	25.48	0.690	0.332
SCM ⁴	28.82	30.05	30.12	28.30	0.775	0.262
Protein	0.88	0.85	0.91	0.86	0.042	0.882
Fat	0.9	0.97	0.96	0.91	0.056	0.667
Lactose	1.41	1.44	1.48	1.36	0.064	0.624
SNF ⁵	2.37	2.39	2.46	2.28	0.105	0.717
TS ⁶	3.23	3.34	3.37	3.15	0.093	0.319
Energy ⁷	90.41	94.30	94.51	88.78	2.368	0.262
Composition (g.kg ⁻¹)						
Protein	31.00	30.30	31.60	31.20	1.32	0.908
Fat	31.40	34.40	33.60	33.40	2.001	0.767
Lactose	49.20	50.10	51.50	49.80	1.650	0.791
SNF	82.70	83.00	85.60	83.60	2.820	0.881
TS	112.70	115.8	117.70	115.50	1.860	0.319
Milk yield per unit DM	1.48	1.47	1.45	1.470	0.037	0.973
FCM yield per unit DM	1.29	1.34	1.310	1.330	0.054	0.909
FE ⁸ , ECM/DMI	1.34	1.37	1.36	1.37	0.046	0.928
N eff ⁹ , Protein yield/CP intake	0.205	0.20	0.207	0.211	0.013	0.947

¹BG = barley grain, SBP = sugar beet pulp, WB = wheat bran, DCP = dried citrus pulp; ²Fat corrected milk 4 % = $0.4 \times$ milk yield (kg) + $15 \times$ fat yield (kg) (NRC, 2001); ³Energy corrected milk = $0.3246 \times$ milk yield (kg) + $12.86 \times$ fat yield (kg) + $7.04 \times$ protein yield (kg). (Orth, 1992); ⁴Solid corrected milk = $12.3 \times$ fat yield (kg) + 6.56 SNF yield (kg) + 0.0752 milk yield (kg). (Tyrrell and Reid, 1965); ⁵Solid not fat; ⁶Total solid of milk; ⁷Milk energy (MJ) = SCM yield (kg) \times $0.75 \times$ 4.184 . (Tyrrell and Reid, 1965); ⁸Feed efficiency = ECM (kg.d⁻¹)/DMI (kg.d⁻¹); ⁹Nitrogen efficiency (Milk protein, kg.CP⁻¹ intake, kg).

error. Least squares mean procedure (LSMEANS) was used to detect the difference between dietary treatments.

Data were analyzed using the following statistical model:

$$Y_{ijk} = \mu + C_i + P_j + T_k + e_{ijk}$$

where: Y_{ijk} is the dependent variable, μ overall mean, C_i the random effect of a cow ($i = 1$ to 8), P_j the fixed effect of the period ($j = 1$ to 4), T_k the fixed effect of treatment ($k = 1$ to 4) and e_{ijk} the residual error.

RESULTS

Performance data are presented in Table 2. There were no differences ($P > 0.05$) in DMI ($\text{kg}\cdot\text{d}^{-1}$) among treatments. The NDFI ranged from 5.88 to 6.63 $\text{kg}\cdot\text{d}^{-1}$ and was higher for diets containing SBP and WB than that for DCP and BG ($P < 0.05$). There was no treatment effect on milk production and composition ($P > 0.05$). Similarly, feed and nitrogen efficiency did not differ significantly among groups ($P > 0.05$).

Analysis of variance revealed that there was no significant difference between the treatments on ruminal pH and blood metabolites ($P > 0.05$) (Table 3). The data showed that there were significant effects among treatments for rumen N ammonia ($P < 0.05$)

and the diet containing WB and DCP produced the highest N ammonia in the rumen.

Chewing activities are presented in Table 4. Time spent for eating ranged from 6.21 to 6.51 $\text{h}\cdot\text{d}^{-1}$. That eating time (min/d and min/kg of DMI) was not different among treatments ($P > 0.05$), but eating time as min/kg of NDFI was affected by the treatment, and it was higher for the diet containing DCP than for other treatments ($P < 0.05$). Time spent for ruminating ranged from 7.6 to 8.5 $\text{h}\cdot\text{d}^{-1}$, and rumination time per day was significantly higher for a diet containing BG than other diets ($P < 0.05$). Chewing time ranged from 13.73 to 14.85 $\text{h}\cdot\text{d}^{-1}$, and it was significantly higher for the diet containing BG than other diets ($P < 0.05$).

DISCUSSION

As the Table 2 demonstrates, pectin feedstuffs, which are used in this study, can support milk yield similar to barley grain, when these pectin sources were substituted for up to 10 % of diet DM for barley grain (as a starch source) without any adverse effects on milk composition, body weight changes and blood metabolites. These results were in accordance with previous studies. Fegeros *et al.* (1995) studied the nutritive value of DCP and its

Table 3. Effect of treatments on blood metabolites and ruminal parameters of dairy cows

Item	Treatments ¹				SEM	P-value
	BG	SBP	WB	DCP		
Blood metabolites						
Glucose ($\text{mg}\cdot\text{dl}^{-1}$)	55.80	53.80	54.10	53.50	2.902	0.722
Blood urea nitrogen ($\text{mg}\cdot\text{dl}^{-1}$)	24.37	25.62	25.87	26.25	0.786	0.387
Cholesterol ($\text{mg}\cdot\text{dl}^{-1}$)	186.25	186.88	193.88	185.63	8.142	0.879
Triglyceride ($\text{mg}\cdot\text{dl}^{-1}$)	9.25	10.63	10.63	12.87	2.690	0.818
Alkaline phosphatase ($\text{U}\cdot\text{L}^{-1}$)	68.25	75.50	69.25	71.87	4.212	0.629
Albumin ($\text{g}\cdot\text{dl}^{-1}$)	4.23	4.25	4.28	4.22	0.047	0.810
Ruminal parameters						
Ruminal pH	6.25	6.36	6.30	6.26	0.071	0.683
Rumen N ammonia ($\text{mg}\cdot\text{dl}^{-1}$)	18.71 ^b	20.86 ^b	26.10 ^a	23.30 ^{ab}	1.494	0.018

¹BG = barley grain, SBP = sugar beet pulp, WB = wheat bran, DCP = dried citrus pulp.

Means in the same rows with different superscripts a, b, c are significantly different with ($P < 0.05$).

effect on milk yield and the composition of 26 lactating ewes fed alfalfa hay (700 g.d⁻¹), wheat straw (300 g.d⁻¹) and one of two concentrates. The DCP concentrate was DCP (300 g.kg⁻¹) in partial replacement of corn grain, barley grain, wheat middlings and sugar beet meal. The NEI, DM, CP and crude fat intakes, milk yield and milk fat, protein and lactose contents were unaffected by the diet. Lopez *et al.* (2014) carried out a trial to assess the effect of DCP in the replacement of corn grain in lactating goat diet. They recorded no significant differences in milk production among treatments. Ahooei *et al.* (2011) examined the effect of DCP on the performance of fattening male calves and reported that the experimental diets had no significant effect on feed intake, rumen pH and blood metabolites. Also, Ashraf (2015) examined the impact of DCP as an alternative energy source on fattening lambs. They added 10, 20, 30 or 40 % of DCP to the concentrate and showed a non-significant effect of various levels of DCP on nutrient intake, digestibility, nitrogen metabolism, weight gain, feed efficiency and blood metabolites. Solomon *et al.* (2000) also studied the effect of the TMR non-structural carbohydrate source, being high starch (corn grain) or high pectin (DCP), on lactating dairy cow performance and milk composition. Milk yield and fat content were not influenced by the non-structural carbohydrate source but the milk fatty acid profile was affected. Several studies showed that replacing cereal grain with SBP, WB (Dann *et al.*, 2014), or a mixture of SBP and WB (Ertl *et al.*, 2016) did not affect ruminal fermentation, milk yield and milk composition.

Ruminal pH was 6.25 – 6.36 and treatments had no significant effect on this parameter. Leiva *et al.* (2000) investigated the impact of feeding DCP or different products of corn grain on fermentational properties of dairy cows. They found no significant differences among treatments for ruminal pH. In another study, Voelker (2002) showed that replacing 6 %, 12 % or 24 % of corn grain by sugar beet pulp had no significant effect on the ruminal pH of lactating dairy cows.

Ruminal nitrogen ammonia ranged from 18.71 to 26.10 (mg.dl⁻¹) and it was significantly different among treatments (Table 3). Ruminal N ammonia was higher for diets containing pectin feedstuffs, except SBP, than the diet containing

barley grain. Broderick *et al.* (2002) indicated that a DCP-contained diet increased concentrations of nitrogen ammonia in rumen liquid from 128 to 152 mg.l⁻¹ compared with a diet without DCP. Ahooei *et al.* (2011), by feeding 12 % DCP to fattening male calves, showed that rumen nitrogen ammonia was higher in calves fed a diet containing DCP. Rouzbehan *et al.* (1994) reported that the rumen of ewes that supplied by a diet containing SBP had a higher concentration of N ammonia.

Our data indicate that there was no dietary effect of eating time, but there were significant differences among treatments for times spent ruminating and chewing (Table 4). Cows fed pectin feedstuffs had shorter ruminating and chewing times (min.kg.d⁻¹). It can be related to the fact that pectin (NDSF) was digested faster than starch (Barrios-Urdaneta *et al.* 2003) and, therefore, less stimulated chewing and rumination. While pectinolytic bacteria prevent a decline in pH, these bacteria, containing starch, can increase the digestibility of cellulose and hemicelluloses (Marounek and Duskova, 1999). Furthermore, Voelker (2002) found that pectin can increase the fermentation rate of carbohydrates after feed intake. It can improve the digestion of cellulose and hemicelluloses. Therefore, as the data show (Table 4), by enhancing the digestion of cellulose and hemicellulose in the rumen, the feed particle size in the rumen will become smaller in a shorter time, and the retention time of feeds in the rumen will decrease. After that, the passage rate of feed will increase, so rumination and chewing times can decrease. Bayat Kohsar *et al.* (2010) used DCP as a replacement for barley grain to 5, 10, and 15 % of DM in the diet of lactating dairy cows and reported that there were no differences among treatments for rumination and chewing times.

Clark and Armentano (1997) studied the effect of SBP and corn grain on rumination time and found that these feedstuffs had a similar impact on rumination time. Swain and Armentano (1994) compared the effects of SBP and corn grain with chewing time (min/kg of DMI) and reported that chewing time was higher for a diet containing SBP than corn grain. Previous studies showed that partial replacement of cereal grain with SBP and WB (Dann *et al.*, 2014) or complete substitution of cereal grains with a mixture of SBP and WB (Ertl *et al.*, 2016) had no effect on chewing behaviour. Ghadami

Table 4. Effects of treatments on the 24-h eating behaviour of lactating dairy cows

Item	Treatments ¹				SEM	P-value
	BG	SBP	WB	DCP		
Eating						
min.d ⁻¹	380.63	390.63	373.13	378.13	11.205	0.733
min.kg ⁻¹ of DMI	19.75	19.89	18.92	20.58	0.731	0.475
min.kg ⁻¹ of NDF intake	63.10 ^{ab}	59.04 ^{ab}	56.31 ^b	64.34 ^a	2.300	0.053
Rumination						
min.d ⁻¹	510.63 ^a	460.00 ^b	452.50 ^{ab}	490.00 ^b	16.121	0.069
min.kg ⁻¹ of DMI	26.36 ^a	23.46 ^b	22.97 ^b	26.28 ^a	0.803	0.010
min.kg ⁻¹ of NDF intake	84.23 ^a	69.61 ^b	68.36 ^b	83.44 ^a	2.449	0.0001
Chewing						
min.d ⁻¹	891.25 ^a	850.63 ^{ab}	824.38 ^b	857.50 ^{ab}	15.998	0.059
min.kg ⁻¹ of DMI	46.11 ^a	43.35 ^{ab}	41.82 ^b	46.32 ^a	1.05	0.018
min.kg ⁻¹ of NDF intake	147.32 ^a	128.65 ^b	124.48 ^b	147.04 ^a	3.267	<0.0001

¹BG = barley grain, SBP = sugar beet pulp, WB = wheat bran, DCP = dried citrus pulp.

Means in the same rows with different superscripts a, b, c are significantly different with ($P < 0.05$).

Kohestani *et al.* (2011) examined the impact of SBP in the replacement of barley grain on pre- and post-partum performance of ewes. Data showed that partial replacement of SBP increased feed intake and milk production, but milk composition was not affected by treatments. Mojtahedi and Danesh Mesgaran (2011) conducted a study on the effect of the inclusion of SBP in a low-forage diet on the performance of Holstein steers. They suggested that partial replacement of barley grain with SBP at low moderate inclusion rates might improve the chewing behaviour, ruminal environment and nutrient digestibility of Holstein steers fed low-forage diet.

Afghahi and Esteghamat (2015) reported that the use of 20 % of wheat bran caused a significant improvement in weight gain and feed conversion ratio (FCR) in experimental lambs. Their study indicated that wheat bran at the rate of 20 % proved to be a suitable substitution of other valuable grains.

CONCLUSION

These data suggest that feeding these by-products for up to 10 % of diet DM to dairy cows, instead of barley grain, had no any adverse effects

on DMI, milk production, milk composition, chewing behaviour and blood metabolites in Holstein dairy cows.

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