Effect of heat treatment of whole cottonseed on *in vitro, in situ* and *in vivo* ruminant digestion characteristics

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The *in vitro* ammonia production and ruminal *in situ* disappearance of dry matter, organic matter, protein and ether extract of heat-treated whole cottonseed were determined. Abomasal flow of nitrogen and ether extract and terminal ileal flow of lysine and methionine of heat-treated whole cottonseed were also evaluated. Whole cottonseed was treated with microwaves at 85, 115, 130, 144 and 155 °C for 5, 10, 15, 20 and 30 min. Heat treatment at 144 °C and 155 °C reduced *in vitro* ammonia production for all treatment durations. The effective ruminal disappearance of dry matter, organic matter, protein and ether extract was also decreased by heat treatment. Protein degradation was decreased from 54.2% to 29.5% at a fractional rumen outflow rate of 0.08/h while ruminal *in situ* ether extract disappearance was decreased from 47.8% to 28% with heat treatment at 155 °C for 20 min. Abomasal flow of nitrogen increased by 15% with heat treatment. Lysine and methionine flow in the terminal ileum was not influenced by heat treatment. Whole cottonseed treated at 155 °C for 20 min apparently gave the best protection for protein against rumen degradation and for increasing the proportion of rumen inert fat.

Die *in vitro*-ammoniakproduksie en ruminale *in situ*-verdwyning van droë materiaal, organiese materiaal, proteïen en eterekstrak van hittebehandelde heel katoensaad is bepaal. Abomasale vloei van stikstof en eterekstrak en die terminale ileale vloei van lisien en metionien van die hittebehandelde heel katoensaad is ook geëvalueer. Heel katoensaad is met mikrogolwe teen 85, 115, 130, 144 en 155 °C vir 5, 10, 15, 20 en 30 min behandel. Hittebehandeling teen 144 °C en 155 °C het *in vitro*-amoniakproduksie vir alle behandelings verlaag. Die effektiewe ruminale verdwyning van droë materiaal, organiese materiaal, proteïen en eterekstrak is ooreenkomstig deur die hittebehandeling verlaag. Hittebehandeling teen 155 °C vir 20 min het proteïendegradeerbaarheid, teen 'n fraksionele rumenuitvloeitempo van 0.08/h, vanaf 54.2% tot 29.5% verlaag, terwyl die *in situ*-verdwyning van vet vanaf 47.8% tot 28% verlaag is. Abomasale vloei van stikstof is met 15% deur hittebehandeling verhoog. Lisien- en metionienvloei in die terminale ileum is nie deur die hittebehandeling beïnvloed nie. Heel katoensaad wat teen 155 °C vir 20 min met mikrogolwe behandel is bied waarskynlik die beste moontlikheid vir proteïenbeskerming teen rumendegradering en vir die verhoging van die proporsie rumeninerte vet.

Keywords: Amino acid flow, heat treatment, protein degradation, whole cottonseed.

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Introduction

High-producing dairy cows need large quantities of energy and protein to fulfil their requirements. Feed proteins may be utilized more efficiently if larger quantities of the protein are protected against degradation in the rumen. It has been shown that heat treatment of oilseeds effectively decreases the rumen degradability of crude protein. Kenelly & de Boer (1986) found that the 'effective' crude protein disappearance of canola seed decreased from 75.0 to 34.7% with jet-sploding at 117 °C while Coomer & Stiles (1989) found that the application of dry heat reduced the *in situ* nitrogen disappearance of whole soybeans from 97.1 to 32.6%. In most studies where heat-treated soybeans were compared with raw soybeans, it was found that milk production increased significantly (Owen *et al.*, 1985; Faldet & Satter, 1989) while milk fat percentage also increased in some of the studies.

The energy requirements of high-producing dairy cows have increased to such an extent that it cannot be satisfied by increasing the grain content of the diet alone. Incorporating additional fat in the diet offers several opportunities for enhancing the efficiency of energy utilization for milk production (Smith, 1988a). The feeding of fat to dairy cows has been reviewed by several authors in recent years (Moore & Christie, 1984; Palmquist, 1984; Smith, 1991) while the feeding of whole cottonseed to ruminants has been reviewed by Coppock *et al.* (1987).

It is important to note that, although the mechanism by which unprotected fat affects rumen fermentation has not been clearly established, several studies concluded that the rumen micro-organisms can only tolerate a maximum of 5% unprotected dietary fat (Devendra & Lewis, 1974; Palmquist & Jenkins, 1980; Palmquist, 1984). The negative effect of more than 5% unprotected dietary fat on rumen metabolism is probably due to the coating of the fibrous portion of the diet with lipids, thus preventing microbial enzyme activity (Devendra & Lewis, 1974). Too many lipids also modify the rumen population by inhibiting the growth of certain micro-organisms and by reducing the absorption of calcium and magnesium owing to complexing with fatty acids (Devendra & Lewis, 1974; Chalupa *et al.*, 1984).

According to Palmquist (1987) most studies suggest maximal energetic efficiency of ruminant animals with 15 to 20% [5 to 8% fat in diet dry matter (DM)] of metabolizable or net energy being derived from dietary fatty acids. If more than 5 to 6% fats should be included in the diet DM inert fats may be used to provide an additional 2 to 3% fat in the diet (Palmquist, 1988).

The objectives of this trial were:

(1) to determine *in vitro* ammonia production and *in situ* DM, organic matter (OM), nitrogen and fat disappearance from polyester bags as indicators of protein protection against rumen degradation and futhermore to determine the possibility of increasing the proportion of rumen inert fat, by heating whole cottonseed with microwaves at different temperatures and for different heating times, and

(2) to compare amino acid flow and absorption in the small intestine of the most effective heat-treated cottonseed with untreated cottonseed.

Materials and Methods

Whole cottonseed (500 g) was heated at 85, 115, 130, 144 and 155 °C in a household microwave oven. These temperatures were dictated by the specific oven used and were measured between seeds with a needle thermometer immediately after each treatment. The duration of heat treatments was 5, 10, 15, 20 and 30 min. After heat treatment the cottonseed was spread to cool, frozen in liquid nitrogen and then ground to pass through a 2-mm screen of a laboratory mill. Dry ice was used to cool the mill during grinding to ensure that all the oil passed through the screen.

Ammonia production was measured with the *in vitro* rumen technique (Raab, 1980; Smith, 1988b). Ammonia production was determined with 3-h intervals over a total incubation period of 12 h. Test tubes were removed after a specific incubation time, alkalized with MgO, distilled and titrated. Ammonia production was expressed as mg NH_3-N per 100 mg N (as whole cottonseed) added to each tube and corrected for NH_3-N in sample at time 0. Ammonia production is directly related to protein degradation (Raab, 1980). Linear regressions were fitted to the data and regression slopes were compared according to *t*-test procedures (Snedecor & Cochran, 1967). Each data point on the different graphs was represented by six analyses.

The most promising treatment conditions identified by means of *in vitro* ammonia production as indicated by difference in slopes (especially different temperatures at a treatment duration of 20 min) were also tested by the *in situ* technique. Protein, OM and fat disappearance from polyester bags in the rumen were determined for incubation periods of 2.25, 4.50, 9, 18 and 36 h. One bag each of the test samples was placed in the rumen of 3 Holstein cows receiving a total mixed diet for high-producing dairy cows (30 l+) (NRC, 1989). The procedure was repeated for a second period giving a total of 6 observations (3 animals × 2 periods) for every variable studied (Mehrez & Ørskov, 1977). Polyester bags were made and treated according to standard procedures (Ørskov, 1982; Erasmus *et al.*, 1988). The bags were made of polyester cloth (Rhologan Engineering, P.O. Box 84158, Greenside, 2034 Republic of South Africa) with a pore size of 53 µm. After incubation, bags were rinsed under running tap water untill the water was clear (3 min per bag). The extent of degradation of the protein and OM in the rumen and the proportion of rumen active fat were estimated according to Ørskov & McDonald (1979). A fractional rumen outflow rate of 0.08/h, representing complete dairy diets (ARC, 1984), was used in the calculations.

Amino acid flow and absorption in the small intestine of diets containing untreated and heat-treated cottonseed were determined with four comparable wethers fitted with abomasal and terminal ileal cannulae. Four pelleted complete diets containing 30% whole cottonseed [(i) control; (ii) heat treated at 130 °C for 20 min; (iii) heat treated at 144 °C for 20 min, and (iv) heat treated at 155 °C for 20 min] were fed to the four wethers according to switch-over design no. 5 of Patterson & Lucas (1962) (Figure 1). Ytterbium acetate (Yb acetate) and chromium ethylenediaminetetraacetic acid (Cr EDTA) were used in the feed as dual phase markers for the particulate and liquid phases to estimate 'true' abomasal and duodenal DM flow (Faichney, 1980; Siddons *et al.*, 1985). The wethers received 12 equal meals of 100 g each (every 2 h) per day to assure a constant intake of markers and feed. Wethers were adapted for 21 days on each treatment before sampling started. Six composite samples of abomasal and terminal ileal digesta were obtained from each wether during each collection period. The samples were collected every 2 h from 08:00 until 18:00, spread over an eight-day period. All digesta were frozen until analysed. After thawing, reconstituted samples were analysed for nitrogen and ether extract according to standard AOAC procedures (AOAC, 1980) and for lysine and methionine according to the procedures used by Spackman *et al.* (1958). Comparisons were made between the control and treatments and also among treatment means according to tests of designed comparisons among means as described by Snedecor & Cochran (1967).

		Wethers				Heat treatments of
		A	B	C	D	whole cottonseed
PERIODS	(i)	1*	2	3	4	1 - Control
	(ii)	2	4	1	3	2 - 130°C for 20 min
	(iii)	3	1	4	2	3 - 144°C for 20 min
	(iv)	4	3	2	1	4 - 155°C for 20 min

* Treatments

Figure 1 Treatments and experimental design according to Patterson & Lucas (1962).

Results and Discussion

Data from all treatment combinations were analysed for linear, quadratic and cubic effects of incubation time on ammonia production. In all cases it was found that the regression of ammonia production on incubation time could conveniently be described by a straight line. Consequently, a simple straight line was fitted to the data of each of the 15 temperature by duration treatment combinations (Figure 2).

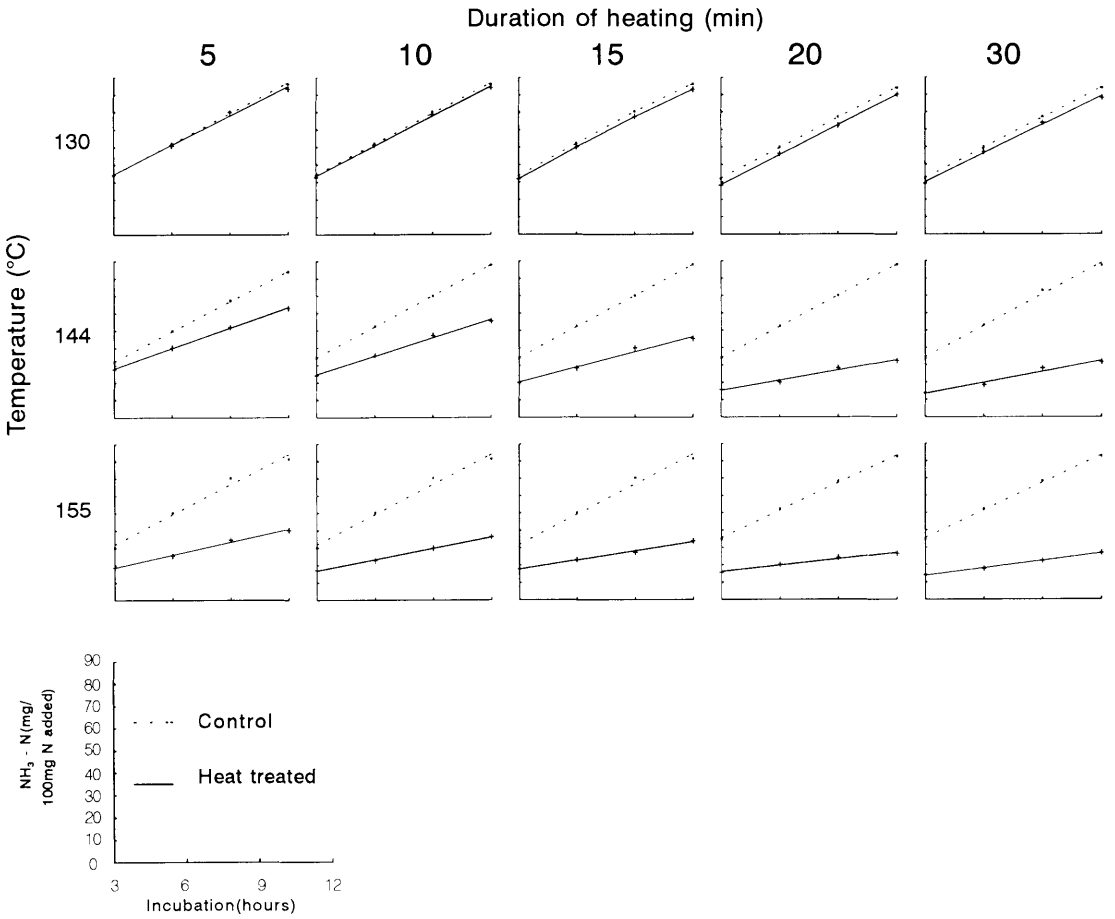

Figure 2 Effect of heat treatment (microwaves) of whole cottonseed on *in vitro* ammonia production.

The estimated difference between the parameters of the regression in the 15 temperature by duration treatment combinations and those of the controls, are given in Table 1.

The conclusion offered by Table 1 is that microwaving at 130 °C is virtually indistinguishable from no microwaving at all (the controls), almost regardless of the duration of microwaving. There is a hint that microwaving for a longer period (120 min or more) does have an effect since the intercept is different when the duration is 20 min (5.10 ± 2.10) and the slope is different when the duration is 30 min (0.34 ± 0.174). Similarly, when the cottonseed is microwaved for only a short period at 144 °C there seems to be little or no effect on the intercept. However, the slope is clearly decreased in these circumstances, indicating a decreased ammonia production. In the remaining cases both the intercept and the slope are decreased. All this information is displayed graphically in Figure 2. The dotted line in each graph is the fitted linear regression common to the seven controls.

Tagari *et al.* (1986) found that a small amount of heat through autoclaving increased *in vitro* degradation of cottonseed protein. This finding is supported by the general acceptance that modest heating or denaturation of protein can improve digestibility and availability of the protein to proteolytic enzymes (Fennema, 1976). In this study, however, the lower heat treatments (85 to 115 °C) did not increase *in vitro* ammonia production from cottonseed protein.

The effect of heating time was clearly shown with cottonseed held at 144 and 155 °C (Figure 2) for any length of time with the best results being achieved for a heat duration of 20 to 30 min. Tagari *et al.* (1986) also found the lowest NH_3

Table 1 Comparison of the intercepts and slopes of the ammonia production of the different temperature by duration treatment combinations to those of the controls

Temperature	Duration of microwaving (min)									
(°C)	5		10		15		20		30	
130	−1.6[1]	0.21[2]	0.1	0.04	0.8	0.10	5.1	0.06	2.5	0.34
144	−0.3	1.89	2.7	2.18	4.5	2.90	6.5	3.83	9.0	3.68
155	5.0	3.31	6.0	3.61	3.8	4.04	3.8	4.57	6.6	4.37

[1] $a_{control} - a$; [2] $b_{control} - b$.

SEs: $a_{control} - a \pm 2.10$; $b_{control} - b \pm 0.174$.

release at treatment temperatures above 140 °C but had to use longer treatment times (40 to 90 min). In this study treatment times of longer than 30 min at 144 or 155 °C resulted in some cottonseed starting to scorch.

Because no practical difference was found between *in vitro* ammonia production of cottonseed treated at 144 or 155 °C for 20 or 30 min, and because treatment times of 30 min and longer resulted in scorching of some cottonseed, it was concluded that heat treatments at 144 or 155 °C for 20 min represented the best potential treatments for decreasing rumen protein degradation.

Data on the influence of heat treatment on the ruminal disappearance of DM, OM, protein and ether extract from polyester bags calculated at a ruminal outflow rate of 0.08/h, were analysed by analysis of variance as shown for DM in Table 2. With the exception of OM, the influence of temperature on component disappearance was clearly curvilinear in nature. These regressions are shown in Figure 3. The disappearance of DM and ether extract tended to slow down with increasing temperatures, while protein tended to disappear at an increasing rate when temperature treatments above 110 °C were applied. Treatment temperatures of 155 °C for 20 min decreased DM disappearance with 42.3% and OM disappearance with 42.6%.

The percentage degradation of protein in untreated cottonseed calculated at a ruminal outflow rate of 0.02/h was 80.6 ± 3.23% (*SE* of the mean). The same value was also found by Ørskov (1982). At a ruminal outflow rate of 0.08/h the result of the present study was 54.2 ± 0.75% which is in good agreement with the 62.7% found by Ørskov (1982). These values are lower than the values of 82.1% and 76.5% found by Erasmus *et al.* (1988) and Arieli *et al.* (1989), respectively. Degradability values in this study were considerably lower than those found by Tagari *et al.* (1986) for corresponding temperatures in a forced draft oven. One possible explanation for this phenomenon is that the temperature of the whole cottonseed was measured between seeds while the mid-kernel temperature could have been higher. Another possibility is that microwave treatment could be more efficient than dry heat for the protection of protein against rumen degradation. Results from the *in situ* incubation are in close agreement with those reported by Deacon *et al.* (1986). In the present study effective protein degradability values of 31.1% and 29.5% were found for cottonseed treated at 144 and 155 °C for 20 min while Deacon *et al.* (1986) found 35.9% for rapeseed that was jet-sploded at 177 °C. It is also clear from Figure 3 that temperature had a larger effect than

Table 2 ANOVA of the influence of heat treatment on *in sacco* dry matter disappearance

Source of variation	df	SS	MS	F ratio	SL
Cows	2	48.139			
Periods	1	6.144			
Cows × periods	2	4.167			
Treatments	9	4447.110			
Control vs. rest	1	2220.4167			
Temperature	4	2130.7733			
Linear	1	1924.1184			
Quadratic	1	190.3893	190.3893	11.63	0.0014
Cubic	1	3.0696			
Quartic	1	13.1961	13.1961		
Duration	2	66.26			
Linear	1	52.2150	52.2150	3.19	0.0809
Quadratic	1	14.0450	14.0450		
Temp. x duration	2	29.6600	14.8300		
Error	45	736.9100	16.3758		
Total (corrected)	59	5242.4700			

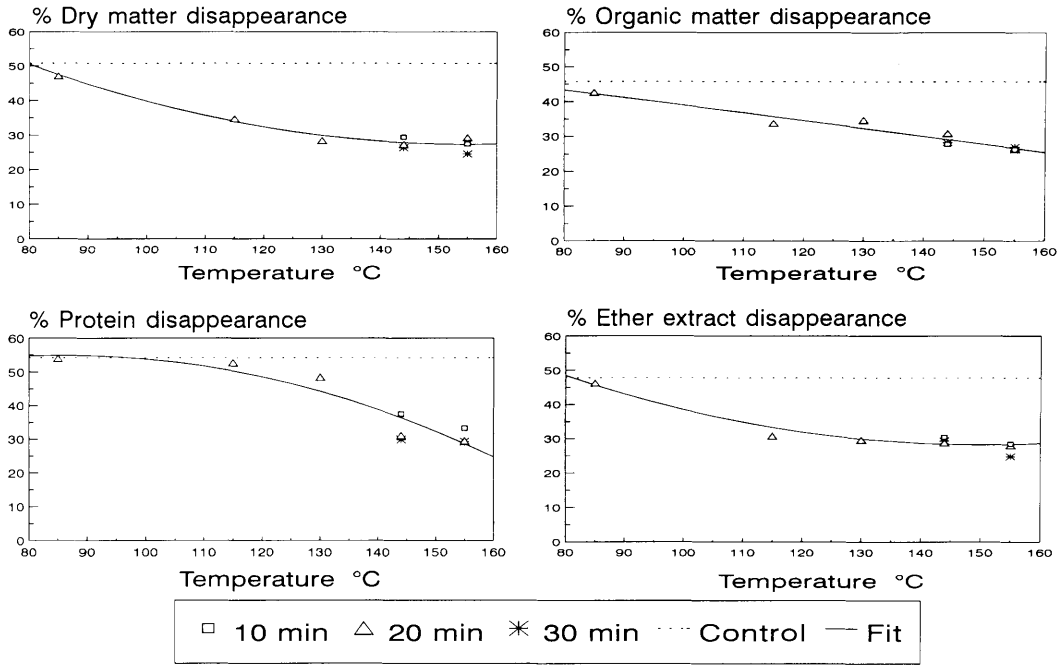

Figure 3 Influence of heat treatment of whole cottonseed on the average *in situ* disappearance of dry matter, organic matter, protein and ether extract.

duration of treatment. The *in situ* results of samples treated at 144 or 155 °C for 20 to 30 min were very similar and corresponded to the pattern obtained with the *in vitro* ammonia production results.

The heat treatment of cottonseed at 115 °C had a large effect on ether extract disappearance when compared with cottonseed treated at 85 °C, while higher temperatures than 115 °C had little further effect (Figure 3). There was also very little difference in *in situ* fat disappearance for the different durations of treatment. The results clearly demonstrate that heat treatment was successful in diminishing the portion of rumen-active fat in the whole cottonseed. This was probably due to the fact that fat globules were protected by surrounding protein which resisted rumen breakdown because of the heat treatment (Pena *et al.*, 1983; Deacon *et al.*, 1986).

The inclusion of 30% heat-treated whole conttonseed in complete diets, contributing 45% of the total dietary nitrogen, increased the daily flow of nitrogen in the abomasum of sheep significantly (Table 3) from 18.6 g to 21.2 g. Pena *et al.* (1983) found that extruded and roasted cottonseed, providing 62% of the protein in a dairy concentrate, resulted in a 18% higher nitrogen flow to the duodenum. Several other workers also reported higher N flow to the duodenum when protein sources were heat treated (Oldham & Taminga, 1980; Stern *et al.*, 1985). There were no differences in abomasal flow of ether extract between treatments (average: 42.8 g/sheep/day). This is in agreement with the general acceptance that, although lipids can be changed in the rumen through the processes of lipolysis and biohydrogenation, total fat entering and leaving the rumen is normally constant or could even be enlarged through biosynthesis of fatty acids in the rumen (Harfoot, 1981).

Table 3 ANOVA for the daily flow of nitrogen (g) in the abomasum of sheep

Source of variation	df	SS	MS	F-ratio[a]	SL[a]
Sheep	3	1.977725	0.659242		
Periods	3	5.518475	1.839492		
Treatments	3	21.928275	7.309425	5.21	
Control vs. rest	1	20.988075	20.988075	14.70	0.0086
Linear	1	0.016994	0.016994	0.01	
Quadratic	1	0.923206	0.923206	0.65	
Carry over	3	4.356565	1.452188		
Error	3	4.207735	1.402578		
Total (corrected)	15	37.988775			

[a] Using error mean square of (4.35 + 4.21)/(3 + 3) = 1.427383.

The different heat treatments of whole cottonseed had no effect on the lysine (average: 944 mg/sheep/day) or methionine (average: 65 mg/sheep/day) flow in the terminal ileum, probably because the heat treatment did not decrease amino acid digestion or absorption in the lower digestion tract.

Conclusions

Heat treatment of whole cottonseed with microwaves at 144 and 155 °C for 5, 10, 20 or 30 min reduced *in vitro* ammonia production. Treatment temperature had a bigger effect than duration of treatment at a specific temperature. Because carbonization occurred at treatment times of 30 min and longer, it was concluded that heat treatments at 144 or 155 °C for 20 min probably had the best possibilities as a means of reducing microbial protein degradation.

The effective *in situ* ruminal disappearance of DM, OM, protein and ether extract was also successfully reduced with microwave heating at temperatures varying from 115 to 155 °C and treatment times varying from 10 to 30 min. Temperature again had a larger effect than duration of treatment and it was concluded that whole cottonseed, treated at 130, 144 and 155 °C for 20 min, offered the best possibilities for reducing rumen protein degradability and for increasing the proportion of rumen inert fat.

Abomasal flow of nitrogen was increased up to 15% in wethers receiving diets containing 30% cottonseed heat treated at 130, 144 and 155 °C for 20 min. Terminal ileal flow of lysine and methionine was not influenced by heat treatment and it was concluded that the heat treatments used probably had no effect on amino acid digestibility and absorption.

It was concluded that heat treatment of whole cottonseed with microwaves at 155 °C for 20 min gave maximum protection to protein and fat against rumen degradation.

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