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Effect of Formaldehyde Treated Rape Seed Oil Cake Based Diet Supplemented with Molasses on Growth Rate and Histopathological Changes in Goats

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ABSTRACT: An experiment with twenty crossbred goat kids (male) of 2-3 months old, weighing about 12 kg was conducted to study the effect of feeding formaldehyde treated rape seed oil cake based diet supplemented with molasses on growth rate and histopathological changes of different organs. Goats were randomly divided into four groups of 5 animals each and were individually fed for a period of 120 days. The animals in group I (URC) and II (URCM) were fed concentrate mixture (CM-I) containing untreated rape seed oil cake (30%) while, the animals in group III (TRC) and IV (TRCM) were offered concentrate mixture (CM-II) containing formaldehyde treated rape seed oil cake. Further, molasses as energy source was additionally supplemented with the concentrate mixture at the rate of 8% of concentrate mixture on dry matter basis to animals in group II and IV. All the animals were maintained on roughage (Berseem hay:wheat straw = 2:1) and concentrate in 50:50 ratio. Average daily gain (g/d) of animals in group IV was significantly (p<0.05) higher than that in group I but at par with group II and III. Feed conversion efficiency was also significantly (p<0.05) higher in group IV (10.14) than group I and II but at par with group III. The growth rate however increased by 50.2% in group IV showing more consistency in maintaining highest growth rate due to better balance of nutrients. At the end of four months of feeding trial, two animals from each group were sacrificed for histopathological study of different organs. Significant histopathological changes in liver, heart, lungs tissue of animals fed untreated rape seed oil cake diet were recorded which were totally absent in the organ of animals fed formaldehyde treated cake. The liver tissue of goats receiving control diet (containing untreated rape seed oil cake) were found to be associated with engorged central vein and blood vessels. Hepatocytes were swollen, pale and degenerated with cellular infiltration and fibrosis of portal areas. The muscles of heart were found to have intermyofibral edema. Emphysema accompanied by dilated and ruptured alveoli was also recorded in lung tissue. However, histopathological examination of various tissues of goats fed formaldehyde treated cake diet did not exhibit any degenerative changes. Additional supplementation of molasses with or without treated cake diet, apparently did not have any significant effect on ameliorating the above degenerative changes. (Key Words: Rape Seed Oil Cake, Formaldehyde, Molasses, Growth, Histopathology, Goat)

INTRODUCTION

In tropical countries oilseed cakes are used as the major protein sources in the ruminant ration. India is the second largest producer of rape seed mustard in the world, contributing to one-fifth of the world's rapeseed mustard production (Kiresur, 1999). Rapeseed mustard cake is the most commonly used protein supplement for livestock in India. It is one of the cheaper protein supplements, rich in some of the essential aminoacids such as methionine and

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lysine. However, high rumen degradability of rapeseed oil cake reduces its nutritive value. Formaldehyde treatment of cake is the common used method for protection of protein from microbial degradation (Kanjana pruthipong et al., 2002) supplying better aminoacid profile at post ruminal tract. The supplementation of molasses as additional source of energy along with formaldehyde treated cake may provide better balance of energy and aminoacids for tissue utilization (Maiga and Schingoethe, 1997). Rapeseed mustard cake contains a non nutrient substance glucosinolate which is hydrolyzed in to a variety of products such as thiocyanate, isothiocyanate and nitrile by the endogenous enzyme myrosinase of plant and microbial origin (Wink, 1993). Some research workers have reported that glucosinolate metabolites have deleterious effect on different internal organs damaging the functional integrity

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Component	CM-I	CM-II	Berseem hay	Wheat straw	Molasses
OM	87.83	88.12	81.72	88.43	90.00
CP	21.63	21.54	17.78	4.26	3.00
EE	6.19	6.11	4.14	0.98	0.55
NDF	33.24	32.54	53.83	81.84	-
ТСНО	60.01	60.47	59.80	83.19	86.45
Ash	12.17	11.88	18.28	11.57	10.00
CP (kg/100 kg DM)	21.63	21.54	-	-	-
RDP	15.89	12.62	-	-	-
UDP	5.74	8.92	-	-	-
RDP/UDP ratio (calculated)	73.47:26.53	58.59:41.41	-	-	-

Table 1. Chemical composition of experimental feed and fodder (% DM basis)

of tissues. Formaldehyde treatment may be useful in protecting the glucosinolate degradation which could be beneficial in preventing the adverse effect of glucosinolate and subsequently the histopathological changes of different organs. The present results describe the effects of feeding formaldehyde treated rape seed oil cake along with supplementation of molasses on growth rate and pathological alterations in goats.

MATERIALS AND METHODS

Formaldehyde treatment of rape seed oil cake

Rape seed oil cake procured from a local market was ground to pass 1.0 mm sieve size and subjected to crude protein (CP) estimation by Kjeldahl's technique. Formaldehyde treatment of rape seed oil cake was conducted by spraying 1.2 g of formaldehyde per 100 g crude protein or 11 ml of 40% formaldehyde solution per 100 g cake. The treatment was commenced in the feed mill and the formalin was poured over the crushed cake from the molasses tank. The treated cake samples were mixed thoroughly and stored in tightly sealed plastic bags for 7 days at room temperature (25°C) to form protected protein. The protein was then tested for degree of protection and effective protein degradability using nylon bag technique (Orskov and Mc Donald, 1979).

Experimental design and management

Twenty healthy growing male goats of 2 to 3 months old weighing 12 kg were randomly assigned into four groups such as I (URC), II (URCM), III (TRC) and IV (TRCM) comprising each of five animals. The animals were fed four different diets satisfying the requirement of NRC (2001). Two types of concentrate mixture (CM-I and CM-II) consisting of 42% Maize, 17% Wheat bran, 30% rape seed oil cake, 8% Groundnut cake, 2% Mineral mixture and 1% Salt were prepared. CM-I containing untreated rape seed oil cake was fed to animals in group 1 and 2. CM-II fed to animals in group 3 and 4 was similar to CM-I except that untreated cake was replaced by formaldehyde treated rape

seed oil cake. Both the concentrate mixtures were isonitrogenous and isocaloric in nature with only difference in RDP:UDP ratio (Table 1). Energy in the form of molasses was additionally supplied at the rate of 8% of concentrate mixture on DM basis to the groups 2 and 4. Further, all the animals were offered concentrate and roughage (Berseem:wheat straw = 2:1) in 50:50 ratio. Concentrate mixture was offered at 9.30 h. Berseem in the form of hay was offered at 14.30 h and wheat straw was made available from 17 h onwards for the rest of the day. Fresh water was provided to all the animals twice a day at 10.30 h and 16.00 h, the animals were housed in a well ventilated shed having cemented floor with individual feeding and watering arrangement throughout the experimental period. The animals were dewormed before starting the experiment and good managemental practices were followed in the shed.

Growth trial

A growth trial of 120 days was conducted. Feed intake of the animals was measured at least twice a week for two consecutive days. Body weights were recorded for two consecutive days once weekly throughout the trial. A metabolism trial of 7 days period for individual animals was conducted at the end of the trial in which the goats were placed in metabolic cages with facilities for separate collection of faeces and urine. Following a 3 days adaptation period, measurements of daily feeds offered and residues were recorded along with the 24 h interval output of faeces and urine. Aliquots of faeces from each animal equal to 1/10th part of total faeces was dried in a hot air oven (100±5°C) for dry matter. The dried samples of the trial period were pooled and kept for analysis of proximate principles, except N. For analysis of N, another aliquot of 1/40th part of total faeces voided by each animal were preserved with dilute sulfuric acid (1:4) in wide mouth air tight weighed bottles. At the end of collection period, the bottles were weighed. The composited and weighed samples were mixed thoroughly and suitable aliquots were taken for N estimation.

Table 2. Mean intake and digestibility of nutrients of experimental goats during metabolism trial

Attributes					
	I (URC)	II (URCM)	III (TRC)	IV (TRCM)	SEM
DM (g/kg $W^{0.75}/d$)	83.5	90.3	86.3	87.7	1.80
$CP \left(g/kg W^{0.75}/d \right)$	15.3	16.1	15.7	15.5	0.31
TDN (g/kg $W^{0.75}/d$)	52.5	58.6	53.6	57.0	1.36
Digestibility coefficient (%)					
DM	65.9	67.6	66.3	68.8	0.49
OM	67.4	69.6	67.9	70.6	0.47
CP	60.8	63.2	61.2	63.8	0.67
EE**	83.6 ^{ab}	87.1 ^b	77.6 ^a	82.1 ^{ab}	1.13
NDF	51.0	52.9	53.9	54.1	0.77
Total carbohydrate*	68.1 ^a	70.0^{b}	68.2 ^a	70.9^{b}	0.52

URC = Control group (I) fed concentrate mixture containing untreated rape seed oil cake.

Chemical analysis

Dried samples of feed offered, residues and faeces were reduced to a particle size of 1 mm by a hammer mill. The DM content of feed and faeces were determined by oven drying at 100±5°C overnight, while OM was determined by ashing in muffle furnace for 3 h at 550°C. Representative samples of feed (offer and residues) and faeces were analyzed for CP, EE by the standard methods (AOAC, 1995) and NDF as per methods of Goering and Vansoest, 1970. Total carbohydrate (TCHO) and TDN percent were estimated as follows:

$$TCHO(\%) = OM(\%)-[CP(\%)+EE(\%)]$$

$$TDN (\%) = DCP (\%) + DTCHO (\%) + [DEE(\%) \times 2.25]$$

Total glucosinolates and thiocyanate content in rape seed oil cake

Total glucosinolates content in mustard cake was estimated using the method of McGhee et al. (1965) in which 10 g of ground rape seed oil cake was deactivated in 200 ml of boiling water for 5 min in a 250 ml flask followed by filtration on Buchner funnel. The residue was washed with 50 ml of hot water. The volume of total filtrate was made up to 500 ml. An aliquot of 250 ml was taken in a conical flask to which 10 ml 0.1 N AgNO3 and 25 ml ethanol (95%) were added. The contents were refluxed on a water bath for 45 minutes cooled to room temperature, volume was made to 100 ml with distilled water and filtered. An aliquot of 25 ml of supernatant was taken in a125 ml flask containing 2 ml of 6 N-HNO3 and 6 ml of 8% w/v ferric ammonium sulphate solution. The homogenous mixture was titrated against 0.01 N potassium thicyanate till a pale salmon colour was obtained. A blank was also run each determination.

% Glucosinolates (GSL)

(Blank-titration)×4×0.01×Mol wt. of GSL×Total volume

 $1.000 \times 2 \times \text{sample wt.} \times 25$

Thiocyanate content in *in vitro* rumen incubation medium was estimated as per procedure of Bowler (1944) in which one g of ground rape seed oil cake was taken in a conical flask. 2.5 ml of 20% trichloroacetic acid and 6.5 ml of fresh rumen liquor was added to it. After thorough mixing it was allowed to stand for 12 h and 24 h separately in incubation medium (pH 6.7). The solutions were filtered through whatman no 40. Five ml of aliquot from filtrate of solution at 0, 12 and 24 h incubation was taken in test tubes followed by addition of 5 ml of ferric nitrate-nitric acid. The O.D. at 460 nm was taken within 15 minutes in spectrophotometer and concentration of thiocyanate was measured in comparison with with the standard curve.

Histopathological study

At the end of the growth trial two goats from each group were sacrificed after keeping the experimental animals fasting for 24 h, providing water only prior to sacrifice. The internal organs were removed and examined grossly for any changes. Representative tissue pieces of liver, heart, intestine, lungs etc. were collected and fixed in 10 percent formal saline. The tissue pieces after fixation were sliced in to 2 mm thick pieces, washed thoroughly under running tap water for several hours, dehydrated with ascending grades of ethyl alcohol, cleared in turpentine oil and embedded with melted paraffin (at 60°C), paraffin block were prepared, sections were cut at 5 μ thickness, stained routinely with haematoxylin and eosin stain for histopathological examination.

URCM = Group II fed concentrate mixture containing untreated rape seed oil cake and molasses.

TRC = Group III fed concentrate mixture containing formaldehyde treated rape seed oil cake.

TRCM = Group IV fed concentrate mixture containing formaldehyde treated rape seed oil cake and molasses.

^{a, b, ab} Means bearing different letters differ significantly.

^{*} p<0.05. ** p<0.01.

Statistical analysis

The data were subjected to 2×2 factorial completely randomized design and analyzed by analysis of variance (ANOVA) techniques (Snedecor and Cochran (1989) to compare the differences between treatments by Duncan multiple range test using General Linear Model procedures of SPSS (version 7.5).

RESULTS AND DISCUSSION

Growth and ntrient utilization

The chemical composition along with RDP and UDP value of concentrate/diets is presented in Table 1. The two concentrate mixtures (CM-I and CM-II) were similar in protein and energy concentration. Difference was only in RDP/UDP ratio due to replacement of untreated rape seed oil cake by formaldehyde treated rape seed oil cake in CM II. The additional supplementation of energy in the form of molasses at the rate of 8 g per 100 g concentrate mixture was thoroughly mixed with offered concentrate mixture and fed to group II and IV to observe the associative effect of energy with undegradable protein at tissue level. The mean nutrient intake and digestibility of nutrient were shown in Table 2 which showed DM, CP and TDN intake (Table 2) on metabolic body size basis were similar among the different groups with similar digestibilities except ether extract and total carbohydrate. Higher fat outgo in faeces of group III might be the cause for lower fat digestibility. Supplementation of molasses in the diet of group II and IV increased (p<0.05) total carbohydrate digestibility which might be due to better fermentation ability of molasses. Similar digestibility of DM, OM, CP and NDF in all the four groups revealed that formaldehyde treatment of rape seed oil cake and also the energy supplementation through molasses had no adverse effect on digestibility of nutrients. Similar results were reported by several workers on feeding of formaldehyde treated cakes to animals (Kuldip et al., 1998; Mathur et al., 1998; Devant et al., 2000). Although total CP and TDN intake were significantly different among the four groups, the values of per kg W^{0.75} basis were similar which was due to similar CP and DCP percent of all

the four isonitrogenous diets (Table 2). The difference in the protein content was not quantitative rather qualitative as in the diets of group III and IV in which undegradable protein having better amino acid profile was incorporated. The data in Table 3 revealed that average total DMI (g/d) during the 17 weeks growth trial were similar in group II and IV which were higher (p<0.01) than group I but at par with group III. Higher intake in group II and IV might be due to additional intake through molasses improving the palatability of diet (Bethard et al., 1997). However, DM intake (g/d) on percent body weight basis was higher (p<0.01) in group II than III and IV but at par with I. The results revealed that feeding formaldehyde treated cake has no significant effect on DM intake on percent body weight basis though DM intake was lower in treated groups (III and IV) which is supported by various workers (Pratihar and Walli,1995 and Chatterjee and Walli, 2003). Contrary to the above findings, Tomilson et al. (1997) reported that the DM intake (kg/d) linearly decreased (p<0.05) from 6 to 4.4 as UDP percent in diet increased from 31 to 55 percent. Feeding of formaldehyde treated cake improved growth rate than untreated groups but the growth rate was only significant (p<0.05) when molasses was supplemented along with treated rape seed cake in group IV. The higher growth rate in animals were also reported by feeding formaldehyde treated cakes (Cho et al., 1990; Tomilson et al., 1997; Chatterjee and Walli, 1998). Additional supplementation of molasses along with treated cakes resulted in maintaining highest growth rate. Feed conversion efficiency was also found to be higher (p<0.05) in group IV fed formaldehyde treated cakes along with supplemented molasses than group I and II but at par with III (Table 3). Feeding of formaldehyde treated soyabean meal, sunflower seed meal and cotton seed meal did not improve efficiency of feed utilization, and lamb performance (Abdullah and Awawdeh, 2004). Similar digestibility of CP with better productive performance in groups fed formaldehyde treated cakes (III and IV) is a clear indication of availability of better form of N at tissue level which was used with a better efficiency. This may be because of the more supply of essential amino acids (Methionine, Lysine) from treated rape seed oil cake and

Table 3. Growth rate and feed efficiency in goats during growth trial

Attributes	Group					
	I (URC)	II (URCM)	III (TRC)	IV (TRCM)	SEM	
						Initial body wt. (kg)
Final body wt.(kg)*	17.43 ^a	18.35 ^{ab}	19.40 bc	20.64 ^c	0.60	
Avg. live wt. gain (g/d)*	47.56 a	52.27 ab	62.94 ab	71.43 ^b	3.85	
Avg. DM intake (g/d)**	651.4 ^a	697.7 bc	673.0 ^b	702.4 ^c	5.31	
DM intake on percent	4.62 ab	4.87 ^b	4.55 ^a	4.35 ^a	0.05	
body wt. basis (kg)**						
Feed conversion efficiency (%)*	7.28 ^a	7.47 ^a	9.35 ^{ab}	10.14 ^b	0.44	

a, b, ab, bc, c Means bearing different letters differ significantly.

^{*} p<0.05. ** p<0.01.

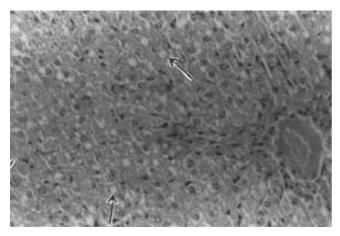


Figure 1. Liver tissue of goat receiving control diet (URC)containing untreated rape seed cake: Engorgement of central vein, hepatocytes swelling with occasional fatty vacuoles (arrow) (H & E, $\times 250$).

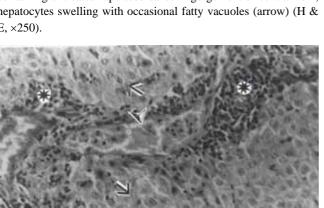


Figure 2. Liver tissue of goat receiving control diet (URC): Hepatocytes swollen, pale and degenerated (arrow), engorgement of blood vessels (arrow head) and cellular infiltration (star) in portal area (H & E, ×250).

better balance of protein and energy for optimum tissue protein synthesis (Kung and Rhode, 1996).

Effect of formaldehyde treatment ruminal degradation of glucosinolates

Formaldehyde treatment of rape seed oil cake cake had only a negligible effect on glucosinolate content, the value being 6.81 and 6.72 percent in untreated and treated cake, respectively. Major glucosinolate content of rape seed mustard cake was glucanapin which is hydrolyzed into a variety of products by myrosinase enzyme in the plant and rumen, out of which thiocyanate is the major metabolite (Wink, 1993). These products are toxic and are responsible for lower palatability of rape seed cake which ultimately affect the productivity of animals (Das et al., 2003). The thiocyanate concentration (mg/100 ml) in incubation medium after 24 h of incubation (in vitro) was significantly (p<0.05) reduced from 4.50 to 3.51 µg per ml which may

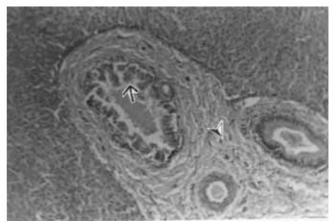


Figure 3. Liver tissue of goat receiving control diet (URC): Bile duct epithelial hyperplasia (arrow) and increased fibrosis in portal area (arrow head) (H & E, ×250).

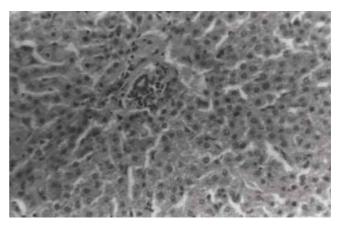


Figure 4. Liver tissue of goat receiving formaldehyde treated rape seed cake diet (TRC): Normal (H & E, ×250).

possibly be due to the binding of formaldehyde with glucosinolates to make it a lesser degradable product inside the rumen.

Histopathological study

The haematoxylin and eosin stained tissue sections of various vital organs such as liver, heart, intestine, lungs, kidney and skin were examined microscopically for any histopathological alterations. The histopathological alterations observed in liver tissue of goats receiving untreated rape seed cake diet (URC and URCM) was more prominent than other organs which included dilation and engorgement of central vein (Figure 1) and portal vein (Figure 2) with red blood corpuscles. The bile duct epithelium showed variable degree of proliferation which desquamated at places (Figure 3). There was also moderate ductular proliferation and increased connective tissue (fibrosis) in the portal area (Figure 3). Hepatocytes were swollen with pale granular cytoplasm and variably degeneration particularly in the centrilobular region with

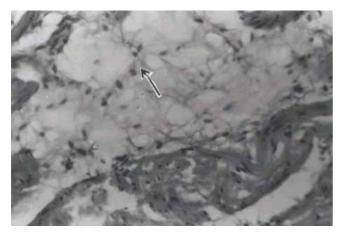


Figure 5. Heart tissue of goat receiving untreated rape seed cake diet (URC): Section of heart showing intermyofibral edema (H & E. $\times 250$).

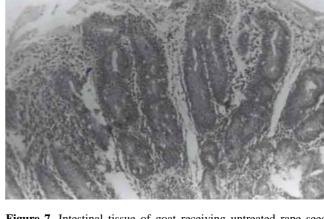


Figure 7. Intestinal tissue of goat receiving untreated rape seed cake (URC): Long and thin villi and submucosal edema (H & E, $\times 100$).

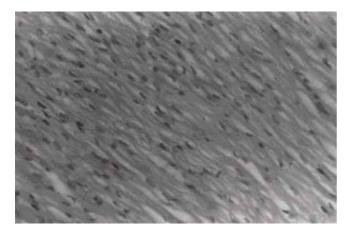


Figure 6. Heart tissue of goat receiving formaldehyde treated cake diet (TRC): Normal.

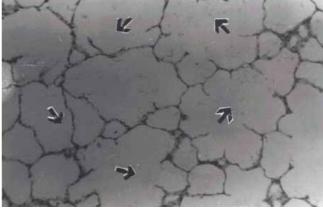


Figure 8. Lung tissue of goat receiving untreated rape seed cake (URC): Lung emphysema exhibited by dilated and ruptured alveoli forming large air spaces (arrow) (H & E, ×100).

occasional fatty vacuoles in their cytoplasm (Figure 1). Heart tissue of goat receiving untreated cake diet showed increased intermyofibral space with edema particularly in subepicardial muscle fibres (Figure 5). Intestinal tissue of goat receiving untreated diet revealed long mucosa with thin villi (Figure 7). Congestion of blood vessels was evident in the lamina propria and there was mild mononuclear cell infiltration. Sub epithelial tissue was loose particularly at the tip of villi. There was an increased goblet cells activity in between the enterocytes and submucosa showed sparse, loose tissue with edema of milder degree. Histopathology of lungs in goats fed untreated cake diet revealed dilated alveoli which ruptured at some places forming large air spaces (emphysema) (Figure 8). The inter alveolar space were thin and stretched and the blood capillaries were engorged.

Liver tissue of goat (Figure 4) receiving formaldehyde treated rape seed cake (TRC) and heart tissue of goat (Figure 6) receiving formaldehyde treated rape seed cake (TRC) were found to be normal. The kidney tissue of goat

receiving untreated cake diet did not show significant changes except some vascular congestion. Similarly skin tissue of control group showed mild to moderate edema in the dermis. The epidermis showed folds with increased keratinization and the hair follicles were normal in appearance. However, the tissues of different organs of goats receiving formaldehyde treated cake diet (TRC and TRCM) were practically normal in appearance. Additional supplementation of molasses irrespective of the treatment of cake did not have any significant improvement in the pathological changes. The type of information generated in the present study with respect to histopathological changes taking place in different organs on feeding untreated and formaldehyde treated rape seed oil cake to growing kids could not be traced on perusal of available literature. There is scanty information regarding the effect of glucosinolate derivatives on different tissues. Van etten et al. (1969) observed liver and bile duct hyperplasia with fibrosis and megalocytosis of hepatocytes in rats fed 10% crambe meal. There was mild hyperplasia of thyroid follicular cells and lymphocytic infiltration in the thyroid glands of kids fed on 40% mustard cake (Pailan, 1995).

IMPLICATIONS

From the above findings it may be concluded that inclusion of formaldehyde treated rape seed oil cake in the concentrate mixture improved 32.3 percent increase in growth rate of goats over the control (untreated) group. Further supplementation of additional molasses in concentrate mixture increased growth rate by 50.2% over control. Feeding of formaldehyde treated rape seed oil cake appears to be safe for ruminants, as the histopathological examination of various tissues of goats fed treated cake did not exhibit any degenerative changes. In fact, the degenerative changes were observed in the tissues of goats fed untreated rape seed oil cake, possibly due to degradation of glucosinolates to thiocyanate in rumen.

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