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Physiological profiles of Zebu steers during transport and pre-slaughter¤

Perfiles fisiológicos de novillos Cebú durante el transporte y pre-sacrificio

Perfis fisiológicos de novilhos Zebu durante o transporte e pré-sacrifício

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Summary

Background: pre-slaughter handling causes stress in cattle that may alter numerous physiological variables. **Objective:** to determine whether in-farm handling of steers, road transport by truck, or slaughterhouse lairage affect blood stress indicators. **Methods:** a total of 65 castrated Zebu steers were randomly selected and transported during 4 h in the same truck, under similar handling conditions. Blood samples were taken by jugular or coccygeal venipuncture at the farm, at the slaughterhouse, and during exsanguination to measure plasma cortisol, glucose, lactate, creatine kinase (CK), ß-hydroxybutyrate (ßHB), creatinine, total protein, urea, packed cell volume (PCV) values, white blood cells (WBC) and neutrophil:lymphocytes ratio (N/L). **Results:** pre-slaughter handling did not have a negative influence on protein metabolism nor did it cause dehydration. ß-hydroxybutyrate and lactate values did not change ($p>0.05$). Transportation increased cortisol, glucose, creatine kinase concentrations and N/L ratio ($p<0.05$). **Conclusion:** pre-slaughter was a stress-generating event that moderately affected animal welfare and increased physiological variables within a range considered normal for steers.

Keywords: *animal welfare, cattle, physiological variables, stress.*

Resumen

Antecedentes: el manejo presacrificio causa estrés en el ganado que puede alterar variables fisiológicas. **Objetivo:** determinar si el manejo en la granja, el transporte terrestre o la estadía en la planta de bovinos cebú sacrificados en una planta localizada en una zona productiva tienen efecto sobre los indicadores sanguíneos de estrés. **Métodos:** se seleccionaron aleatoriamente 65 novillos Cebú, los cuales fueron transportados durante 4 h, bajo las mismas condiciones de manejo. El ganado fue muestreado por venopunción de la vena

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yugular o coccígea en la granja, en la planta de sacrificio y durante el desangrado para medir los niveles de cortisol, glucosa, lactato, creatinquinasa (CK), betahidroxibutirato (ßHB), creatinina, proteína total, urea, volumen celular acumulado (PCV), conteo de células blancas (WBC) y la relación neutrófilos/linfocitos (N/L). **Resultados:** el manejo presacrificio no influyó negativamente sobre el metabolismo proteico, ni causó deshidratación. El ß-hidroxibutirato y el lactato no presentaron cambios (p>0,05). El transporte incrementó las concentraciones de cortisol, glucosa, creatinquinasa y la relación N/L (p<0,05). **Conclusiones:** el presacrificio afectó moderadamente el bienestar animal e incrementó algunas variables fisiológicas, las cuales se encontraron dentro de los rangos considerados como normales para la especie bovina.

Palabras clave: *bienestar animal, estrés, ganado, variables fisiológicas.*

Resumo

Antecedentes: o manejo pré-abate causa estresse no gado, o que pode alterar numerosas variáveis fisiológicas. **Objetivo:** determinar se o manejo na fazenda, o transporte terrestre ou a permanência no frigorífico de bovinos zebuínos abatidos em um abatedouro instalado numa região produtiva, tem efeito nos indicadores sanguíneos do estresse. **Métodos:** foram selecionados aleatoriamente 65 novilhos zebu, os quais foram transportados durante 4 h, sob as mesmas condições de manejo. O gado foi monitorado por venopunção na fazenda, no frigorífico e durante a sangria para medir os níveis de cortisol, glucosa, lactato, creatinoquinase (CK), beta hidroxibutirato (ßHB), creatinina, proteína total, uréia, volume celular acumulado (PCV), contagem de células brancas (WBC) e a relação neutrófilos/linfócitos (N/L). **Resultados:** o manejo pré-abate não influenciou negativamente o metabolismo proteico, nem provocou desidratação. O ß-hidroxibutirato e o lactato não apresentaram mudanças (p>0,05). O transporte elevou as concentrações de cortisol, glicose, creatina-quinase e a relação N/L (p<0,05). **Conclusões:** o pré-abate afetou moderadamente o bem-estar animal e incrementou algumas variáveis fisiológicas avaliadas, as quais encontraram-se dentro de intervalos considerados como normais para a espécie bovina.

Palavras chave: *bem-estar animal, estresse, gado, variáveis fisiológicas.*

Introduction

Most cattle in South America are transported by truck from farms to slaughterhouses, which generally requires extended periods of time because farms are located far away from the places of consumption (Paranhos da Costa *et al.*, 2012). Handling and transportation are stress-generating events that affect animal welfare (Ljungberg *et al.*, 2007; Paranhos da Costa y Tarazona*,* 2011). In Colombia, cattle farming presents a high geographic dispersion. Approximately 70% of the livestock is slaughtered in plants located in consumption centers with the participation of a high number of intermediaries, which involves longer transport times, loading and unloading, multiple handling procedures, and likely mixing with unfamiliar animals (Estévez-Moreno *et al.*, 2009; Romero y Sánchez, 2011a). Transportation is conducted through mountainous roads with precarious infrastructure and times exceeding 10 h, aggravated by improper handling combined with a lack of consideration for ruminant welfare (Romero *et al.*, 2011b, 2012a). One strategy proposed to strengthen the competitiveness of the cattle meat production chain is the modernization and regionalization of the slaughterhouses with the intention of increasing the installed slaughter capacity, decreasing the intermediaries, avoiding extra transport time, and improving animal management, among others (Estévez-Moreno *et al.*, 2009).

Several blood variables, which are influenced by pre-slaughter conditions, can be used as stress indicators. They include the concentration of serum cortisol, glucose, ß-hydroxybutyrate (ßHB), lactate, creatine phospho-kinase (CK), urea, creatinine, and total protein levels (Tadich *et al.*, 2009). Stress may produce physical and neuroendocrine changes that can be used to evaluate the pre-slaughter influence on cattle welfare (Miranda-de la Lama *et al.*, 2012). Nevertheless, in Colombia there is little information available regarding the impact of the short-term transportation and pre-slaughter operations on animal welfare, especially for slaughterhouses located in production areas. The information obtained can help to establish the biological benefits of having

regional slaughterhouses located in the principal production areas. The objective of this study was to evaluate the pre-slaughter physiological response of Zebu steers transported and handled in Cordoba province (Northern Colombia) in accordance with the requirements of health and safety legislation of the country.

Material and methods

The animals were reared and slaughtered in compliance with national regulations applied in research and commercial slaughter, and the Ethics Committee of the University of Caldas approved the study. Slaughtering was conducted at the Monteria city Slaughterhouse (8º 45′ 0″ N, 75º 53′ 0″ W; Cordoba) in July, 2011. This slaughterhouse complies with Decree 1500 (Ministerio Colombiano de la Protección Social, 2007). Monteria city is located in a tropical dry forest region with a mean annual rainfall of 1,156 mm and mean annual temperature of 28 ºC. It is situated at approximately 18 m above sea level along the Sinú river valley.

Study description

A total of 65 castrated steers (*Brahman* commercial crossbreeds) aged 18 to 24 mo, live weight = 445.4 ± 0.7 kg, from the Ayapel area (Cordoba) were evaluated. They were fattened by a single farmer under similar conditions, mainly on improved natural pasture (*Brachiaria plantaginea* and *Dichanthium aristatum*) and fed mineral salt supplements. The steers were randomly selected and arranged into groups of 14 (n = 5 journeys), and individually transported under the same climate and driving conditions. Before loading at the farm, all steers were herded through a cattle race and bled from the coccygeal vein. Two blood samples were taken: one with sodium fluoride (NaF) (for glucose and lactate measurements) and the other with anticoagulant (ethylenediaminetetraacetic acid, EDTA). The steers were individually identified with numbers on the skin using a marker and were loaded into the truck without the use of electric prods. They were transported for 4 h using the same truck and driver. The average speed was 60 km/h. The distance covered was 140 km (30 km of unpaved road and 110 km of paved road). Transport of the animals occurred during different days and under similar handling conditions. Animals did not receive any food or water during transportation. The slaughterhouse employees unloaded the animals with the help of palm fronds and did not use any other persuasive elements. Blood samples were taken from each steer via jugular venipuncture in a clamping bracket immediately after unloading. A third blood sample was collected during exsanguination.

Transport conditions and slaughterhouse

The truck used during the study complied with Colombian standards for cattle transport. This type of truck is commonly used in Colombia for this purpose. Its load capacity is 10-tons (14 to 15 animals), and it has two axles, a rigid chassis (combined wood and steel), passive ventilation and a canvas roof. The slaughter plant assessed in our study operates from Monday to Saturday (0600 to 1400 hours) and has a slaughtering capacity of 500 to 600 animals/day at a rate of 40 to 45 animals/hour. The concrete unloading ramps have nonslip floors that are about as wide as the livestock trailers. The ramps are connected by a series of corridors leading to a lairage area that has 260 m^2 of pens (3.65 m wide × 20 m long) with high-density polyethylene screen and nonslip concrete floors. The steers were individually weighed. Animals arriving in different trucks are not mixed at the plant and each group is housed in separate pens. Water is freely available, but there is no access to food. A concrete curved passageway leads from the lairage area to a stunning box (1.80 m high × 0.90 m wide × 2.53 m long) with a head fixation system. Access to the box is through a guillotine door and a revolving iron exit door. After being stunned by a non-penetrating captive bolt, cattle are slaughtered, suspended by a hind leg, bled, and transferred to the production line.

Blood analysis

Packed cell volume (PCV) values were obtained using the microhaematocrit technique. Leukocyte profile (WBC) and neutrophil:lymphocytes ratio (N/L) were performed during the microscopic observation of blood sample slides stained with Wright's stain. Serum cortisol concentrations (µg/dl) were determined by radioimmunoassay (RIA). The inter-assay variation coefficient was 9.31%. The levels

of glucose (mmol/L), urea (mmol/L), total protein (g/L), creatinine (mmol/L), β-hydroxybutyrate (ßHB, mmol/L), lactate (mmol/L), cortisol (µg/dl), and creatine kinase (CK, U/L) plasma concentrations were determined.

Statistical analysis

The data were analyzed using Stata 12.0 program (College Station, Texas, USA). A normal distribution of variable indicators of stress was established by means of residual analysis. The activity of CK, lactate, βHB, urea, creatinine and glucose did not present normal distributions, thus, values were transformed to their natural logarithm and the PCV values to their squared value—transformed values were used in all subsequent analysis. Three observations were used from each animal. Mixed linear models were used to correct the correlation between the repeated observations in each animal with several covariance structures. In addition to the physiological variables, the journey effect was also included in the model. Correlations between physiological variables were analyzed by Pearson's correlation coefficient. The average (±SE) of the stress indicators was reported, and probability values of $p<0.05$ were considered to be statistically significant.

Results

In the present study a journey of less than 4 hours did not affect protein metabolism as indicated by normal values of creatinine, urea, total protein and albumin plasma concentrations. In spite of being increased when compared to basal values obtained at the farm, these values were within reference values. Nevertheless, there were significant changes in the concentrations of cortisol, glucose, and CK (Table 1). No changes were evident in βHB plasma concentrations among the three stages of sampling. During exsanguination, blood levels of cortisol, PCV, lactate, and total protein increased, while creatinine plasma concentration decreased compared to the values obtained after unloading at the slaughterhouse.

The steers had significantly higher values ($p<0.05$) of cortisol after unloading and at exsanguination. The same tendency was observed for lactate (Figure 1). There were significant differences in positive correlations between cortisol and lactate levels ($p<0.05$).

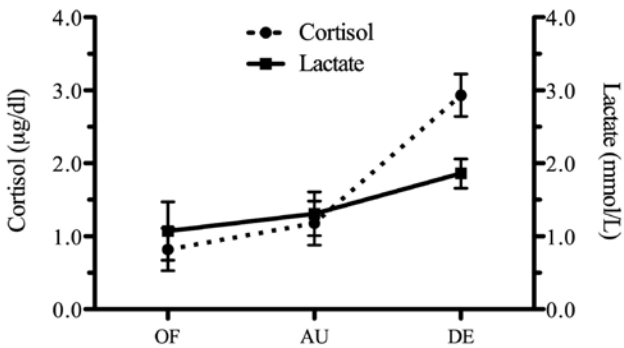

Figure 1. Mean (SEM) cortisol and lactate levels of Zebu steers at different blood sampling times. OF: on farm; AU: after unloading; DE: during exsanguination.

Table 1. Blood parameters in Zebu steers at different sampling times (means ± SE).

Biochemical parameters	On farm	After unloading	During exsanguination
Cortisol (µg/dl)	0.82 ± 0.1[a]	1.8 ± 0.15[b]	2.93 ± 0.12[c]
Glucose (mmol/L)	4.18 ± 0.81[a]	5.99 ± 0.35[b]	6.17 ± 0.45[b,c]
CK (U/L)	172.43 ± 8.5[a]	354.25 ± 10.5[b]	437.03 ± 9.56[b,c]
Creatinine (mmol/L)	102.5 ± 0.58[a]	117.9 ± 0.75[b]	106.7 ± 0.58[a,c]
Urea (mmol/L)	3.78 ± 1.07[a]	3.97 ± 0.92[a]	11.02 ± 1.5[c]
Albumin (g/L)	25.91 ± 1.15[a]	28.45 ± 0.98[b]	28.94 ± 0.59[b]
Total protein (g/L)	78.33 ± 2.94[a]	80.93 ± 1.6[a]	83.15 ± 1.15[a,b]
PCV (%)	30.4 ± 1.2[a]	34.4 ± 3.5[b]	37.4 ± 5.1[c]
βHB (mmol/L)	0.34 ± 0.02[a]	0.31 ± 0.02[a]	0.33 ± 0.05

[a,b,c] Means with a different superscript within the same row are statistically different ($p<0.05$). SE: Standard error of the mean.

Leukocyte recounts did not show a consistent pattern; results were high after unloading at the slaughterhouse and then decreased at exsanguination ($p<0.05$). The same pattern was observed for the N/L ratio (Figure 2). The coefficient of correlation (rho) indicates that a small correlation exists between blood variables evaluated at the farm, upon arrival to the slaughterhouse, and during exsanguination (Table 2).

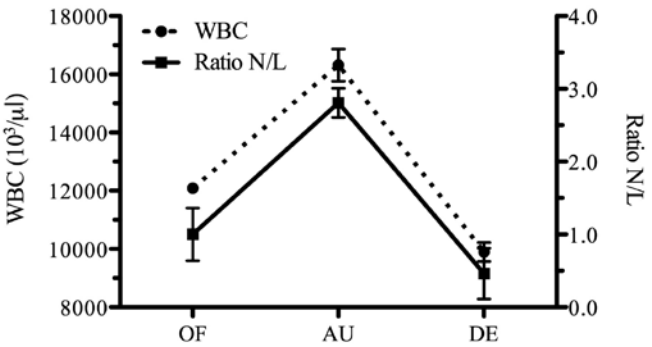

Figure 2. Mean (±SE) WBC and N/L ratio of Zebu steers at different blood sampling times. OF: on farm; AU: after unloading; DE: during exsanguination.

Table 2. Blood parameters in Zebu steers (linear mixed model).

Biochemical parameters	On farm	After unloading	SEM	rho
	Regression coefficients			
Cortisol (µ/dl)	0.97[a]	2.1 [b]	0.19	0.14
Glucose (mmol/L)	0.36[a]	0.39[b]	0.04	0.008
Lactate (mmol/L)	0.24[a]	0.79[b]	0.079	0.06
CK (U/L)	0.71[a]	0.93[b]	0.13	0.16
Creatinine (mmol/L)	0.13[a]	0.03[b]	0.02	0.20
Urea (mmol/L)	0.05[a]	1.11[b]	0.07	0.34
Albumin (g/L)	0.09[a]	0.11[b]	0.03	0
Total protein (g/L)	2.6	4.8	2.04	0.07
PCV (%)	258.4[a]	448.9[b]	56.4	0.12
βHB (mmol/L)	-0.058	-0.028	0.06	0.13
WBC (10^3/µ)	-0.0007[a]	0.0010[b]	0.0002	0.16

[a,b,c] Values with a different superscript within the same row are statistically different ($p<0.05$). SEM: Standard error of the mean. rho: Pearson correlation coefficient.

Discussion

Increase in circulating levels of glucocorticoids, such as cortisol, is a neuroendocrine indicator of acute stress (Odore *et al.*, 2011; Miranda de-la Lama *et al.,* 2011). The initial sampling of the animals in the farm prior to loading showed that the cortisol concentrations were within reference values (0.61 ± 0.07 µg/dl), which suggests that blood sampling was not a stressor for the animals given that it was done quickly and steers were sampled in small groups (14 animals). Furthermore, the blood cortisol increased even higher and was significantly different immediately following unloading and during exsanguination, surpassing the initial basal values of the stressor. Studies by Tadich *et al.* (2003), Van de Water *et al.* (2003) and Odore *et al.* (2011) suggest that short transport time is a stress factor for cattle, causing a wide range of physiological changes such as increase of cortisol concentrations, especially during herding, loading, and initial stages of this process. Furthermore, the road connecting the farm with the main roadways was unpaved. During transport on unpaved roads, floor vibrations can be transmitted to the animal and may create discomfort, displacing the animal's center of gravity and may generate stress (Miranda-de la Lama *et al.,* 2011). Nonetheless, other existing factors related to transport could influence the increase of cortisol serum concentrations, such as unloading, handling, a new environment, and blood sampling procedures that could disguise the true stress agent in the study (Tadich *et al.*, 2005).

Hyperglycemia, observed immediately after unloading and exsanguination, could be a secondary effect of cortisol production. It could also be a result of increased liver glucose production (gluconeogenesis) due to greater sympatho-adrenal activity by adrenaline-mediated pre-slaughter stress (Minka and Ayo, 2009; Ekis *et al.*, 2012). Cortisol favors the synthesis of enzymes required for converting amino acids, glycerol, and lactate into glucose, thereby increasing amino acids mobility from the muscle (Muchenje *et al.*, 2009). As previously described, after short and long-term transport stress, glucose level increases indicating metabolic depletion associated with mobilization of energy reserves (Miranda-de la Lama *et al.*, 2011). Hyperglycemia during exsanguination could also be related to a peak

in adrenaline and glucagon production at the moment of stunning, both of which are regulators of hepatic and muscular glycogenolysis (Tadich *et al.*, 2009).

Plasma lactate is an indicator of acute stress related to handling conditions, especially physical exercise, agitation and muscular damage (Hambrecht *et al.*, 2005). In this study, average lactate plasma levels were different between animals and statistically different at the three samplings, although they were all within normal ranges for cattle (Kaneko, 2008). Additionally, positive correlation between lactate and cortisol levels after transport and during exsanguination were observed. Cortisol secretion was related to anaerobic activity, suggesting that lactate may stimulate cortisol concentrations (Nogueira *et al.*, 2002).

The CK activity is a sensible indicator of muscular damage, increased physical activity, and muscular fatigue, as reported in pigs (Hambrecht *et al.*, 2005), cattle (Early *et al.*, 2012), lambs (Miranda-de la Lama *et al.*, 2012), and goats (Ekiz *et al.*, 2012). In our study, CK activity drastically increased during the three blood samplings, surpassing the average reference values (Kaneko, 2008). The high CK values at the farm could have been related to cattle handling during truck loading or transportation. These activities may have increased membrane permeability or harm muscle cells due to cattles' efforts to maintain balance and avoid being thrown against the truck walls during the trip (Romero *et al.*, 2013). Conversely, besides absence of social structure ruptures at the slaughterhouse, it is possible that the presence of antagonistic meetings between steers (Strappini *et al.*, 2013) could have caused damage to the skeletal muscles and subsequent enzymatic excretion (Kannan *et al.*, 2003).

The results obtained indicate that stress during transport to a slaughterhouse located in the same production area did not considerably affect metabolism, in spite of the fact that changes in PCV, urea, creatinine and albumin plasma concentrations were observed, though they were within normal ranges (Buckham Sporer *et al.*, 2008). Total protein values were slightly greater than the normal values for steers (Kaneko, 2008). PCV allows evaluating electrolytes and fluids alterations, indicating transport stress (Tadich *et al.*, 2003). Transport, fasting, and insufficient water ingestion increase PCV (Tadich *et al.*, 2005). Significant increases in PCV in each sampling were evident, yet they remained within values considered normal for cattle. This is possibly due to stressors exposure during pre-slaughter, inducing a rapid and brief response that causes neuronal hypothalamus activation and adrenaline and noradrenaline excretion (Herskin *et al.*, 2004). Catecholamines place cattle in a state of alert, producing tachycardia, peripheral vasoconstriction, and hyperglycemia (Borell, 2001). PCV increase observed during exsanguination could be due to erythrocytes mobilization via splenic contraction in response to catecholamines excreted during stunning and exsanguination (Möstl and Palme, 2002; Ekis *et al.,* 2012). This generates great stress in animals as evidenced in Colombian slaughterhouses where the lack of stunning efficiency causes many of the animals to regain consciousness during exsanguination, which negatively impacts animal welfare (Romero *et al.*, 2012b; Romero *et al.,* 2012c). Dehydration was not evident after an average lairage of 20.2 ± 0.21 h, reinforcing the importance of adequate infrastructure and optimal lairage conditions.

Blood cell constituents maintain the physiological balance in response to environmental conditions by restoring normal homeostasis, and are sensitive indicators of the physiological response to stress (Miranda de-la Lama *et al.,* 2011). The N/L ratio has been used as a complementary measurement and is useful for evaluating transport stress (Gupta *et al*., 2007). Upon arrival to the slaughterhouse, average leukocytes were greater than the normal range (4 to 12.000 $10^3/\mu l$). Neutrophilia was also detected, suggesting an immune suppression, which indicates a cumulative effect of factors associated with transport handling, unloading, and blood sampling, thus increasing cortisol levels, elapsing a leukocyte and and N/L ratio increase (Blanco *et al.*, 2009). Declines in leukocytes to basal values and N/L ratio during exsanguination indicate that lairage had a beneficial effect on welfare. At present, Colombian legislation states a minimum of 12 h pre-slaughter lairage for cattle (Romero *et al.*, 2011a). Colombian researchers have recently found that cattle usually remain 20 h or more in lairage, in agreement with our results (Romero *et al.*, 2011b; Romero *et al.*, 2013). Some researchers consider 12 h pre-slaughter lairage for cattle being too long because stressful conditions

(noise, fear, novelty, thirst, hunger, etc.) may increase with time spent at the slaughterhouse, which may lead to a decrease in meat quality. Hence, lairage should be reduced to the minimum necessary to allow antemortem inspection (Tadich *et al.*, 2005). The differences found by several researches vary with the factors studied, such as the effect of previous handling (particularly if it has been relatively stress-free), the physiological state of the steers upon arrival to the slaughterhouse, environmental factors that encourage cattle to rest, and the recommended holding times, among others (Warriss, 2003). These aspects need to be clarified and evaluated in further research.

This study confirmed that βHB is not a good indicator of acute stress in cattle because it is used as an energy source for external tissues when glucose levels and glycogen reserves are reduced (McCue, 2010). Glucose, ketones and free fatty acids are completely exhausted during exercise. Following this process, muscle ketone oxidation decreases and βHB increases, indicating prolonged food deprivation (Knowles and Warriss, 2006). The lack of differences between βHB at the farm, upon slaughterhouse arrival, and during exsanguination indicate that food deprivation for a short time period was counteracted by βHB provided by the rumen, where multiple days are required for cattle to reach a physiological fasting state (Tadich *et al.*, 2003).

Our results showed that 4-hour transportation caused an increase in plasma cortisol, CK, WBC and N/L ratio, and it was a stress-generating event that moderately affected animal welfare. Transport and resting at the slaughterhouse did not alter protein metabolism and dehydration was not present in the steers. Changes in the studied variables indicate that 20 h of lairage with adequate infrastructure and optimal lairage conditions have a beneficial effect on welfare of Zebu steers.

These results highlight the importance of modernization and regionalization of Colombian slaughterhouses, allowing shorter transport periods and quick adaptation to lairage conditions. Slaughterhouses should be modern facilities to improve animal welfare. They must offer fresh water, optimal lairage conditions, good handling practices, and adequate transportation infrastructure, among others. It is necessary to develop tools and protocols to minimize cattle stress, considering that sanitary legislation permits extensive holding times and lairage.

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Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

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