PHYSIOLOGICAL PARAMETERS OF THREE SOUTH AFRICAN ANGORA GOAT CYP17 GENOTYPES SUBJECTED TO COLD STRESS

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INTRODUCTION

High mortality rates of Angora goats during periodic cold weather cause considerable annual monetary loss in the mohair industry. According to Mohair South Africa, an estimated number of 60 000 to 70 000 goats died from exposure to cold conditions over a ten year period from 1997 to 2007. This implies a financial loss of R14 million in terms of mohair production at the producer level, and a direct loss of R21 million in terms of animals. Physiological and endocrinological reactions to cold stress have been discussed in detail by various studies (Thompson & Thomson, 1977; Bianca & Kunz, 1978; Fregly, 1982; Feistkorn et al., 1983; Fregly, 1989; Oda et al., 1995; Sano et al., 1997; Al-Tamimi, 2006; Al-Tamimi, 2007).

During investigations done into the probable cause of the susceptibility of Angora goats to stress, two CYP17 genes were identified in the South African Angora goat (Storbeck et al., 2007). Subsequently three CYP17 genotypes (named H_e, H_o and H_u) were identified and shown to differ with regard to their cortisol production efficiency (Storbeck et al., 2008; Storbeck et al., 2009). In this study, the ability of these three genotypes to withstand cold stress conditions was evaluated under simulated cold and wet conditions.

MATERIALS AND METHODS

The GADI ethical committee approved the project procedures (Approval number GVE/AP5/28), with the provision that goats whose rectal temperature drop below 32 °C were removed from the trial.

The study was conducted at the Grootfontein Agricultural Development Institute (GADI) during July and August 2011. Sixty 10-month-old Angora goat ewe kids were genotyped for the CYP17 locus using an ARMS-qPCR (amplification refractory mutation system qPCR) assay (Snyman et al., 2016). Thirty-six animals (12 per genotype) were selected to be used for the trial.

On 18 July 2011, temperature data loggers were implanted under the skin on the back between the shoulder blades of all animals. Each data logger was encapsulated in a silicone capsule that would

prevent any reaction from the body. The data loggers were programmed to start hourly temperature recordings on 18 July 2011 at 18:00. All animals were shorn on 23 July 2011.

A cold stress trial was carried out on 27 July 2011. The night before the trial the goats were moved to a shelter in order to prevent cold stress from the inclement weather. For the trial the goats were divided into three groups, with each group consisting of four goats from each genotype. This was done to facilitate easier data and sample collection during the trial period. The animals were put into three small pens in a shaded area, exposed to the wind.

Ambient temperature over the trial period was also recorded with one of the data loggers. The ambient temperature was 4.2 °C at the start of the trial at 09:00 when the pre-rain treatment (0 minute) readings were taken, and increased to 8.72 °C at 13:00 when the trial ended. After the first round of data recording, the animals were subjected to an equivalent of 5 mm of rain every 60 minutes for a four-hour period. Thus the animals received 20 mm rain over the trial period. Rain treatment was applied by sprinkling the animals with water from a hose pipe. During the five-minute rain application, the goats were moved around the pen to ensure that all goats received the same amount of rain. Lucerne hay and water was freely available during the trial period.

Rectal temperatures and blood samples were recorded during the four-hour trial period at 0 minutes (before applying rain treatment) and again at 60, 120, 150, 180 and 240 minutes after applying the first rain treatment. The sequence of events were as follow: Took 0 minute measurements, applied 5 minute rain treatment, took measurements 60 minutes after the previous recording, applied 5 minute rain treatment, took measurements 60 minutes after the previous recording, applied 5 minute rain treatment, etc.

Blood samples were collected from the animals *via* veni-puncture in the left jugular vein into 5 ml lavender top EDTA plastic vacutainer blood collection tubes. The animals were sampled in the same order at each sampling interval. Animals were caught and restrained in a standing position by one person, while the blood sample was taken. Immediately after this, the digital rectal thermometer was inserted into the rectum. After one minute, the rectal thermometer was removed and the temperature recorded.

Plasma cortisol and cortisone were extracted from whole blood and quantified by ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) as described by Schloms et al. (2014).

After the cold stress trial the goats, fitted with the subcutaneous temperature loggers, were maintained

under field conditions until 7 October 2011.

The SAS statistical package (PROC GLM) was used to determine differences in physiological parameters of goats of the three CYP17 genotypes (SAS, 2009).

The following model was applied for all traits recorded:

$Y_{ijk} = \mu + t_i + k_j + (tk)_{ij} + b_1BW + e_{ijk}$

Where

Y_{ijk} = trait of the k'th animal of the j'th pen of the i'th genotype,

μ = overall mean,

t_i = fixed effect of the i'th genotype (H_e, H_u, H_o),

k_j = fixed effect of the j'th pen (1, 2, 3),

$(tk)_{ij}$ = effect of the interaction between the i'th genotype and the j'th pen,

b_1 = linear regression coefficient of the appropriate deviation from the mean of body weight at the start of the trial (BW),

e_{ijk} = random error with zero mean and variance $I\sigma^2_e$.

RESULTS AND DISCUSSION

Rectal temperatures of the animals of the three genotypes recorded over the trial period are presented in Table 1 and illustrated in Figure 1. Subcutaneous temperatures as well as ambient temperature recorded with the temperature loggers over the trial period are presented in Table 2 and depicted in Figure 2. Subcutaneous temperatures when the animals were run under veld conditions are illustrated in Figures 3 and 4 for the three genotypes for two periods when ambient temperature dropped to below 0 °C at night (average minimum temperature -4.6 °C; average maximum temperature 20.5 °C) and for a warmer period (average minimum temperature 0.2 °C; average maximum temperature 24.6 °C) respectively.

During the cold stress trial period, significant differences (P <0.05) in rectal temperature were recorded among the three genotypes. Animals from the H_u genotype already had a lower rectal temperature than the H_e goats at the start of the trial. From 60 minutes onwards, until the end of the trial period, the H_u animals had lower rectal temperatures than the H_e and H_o animals (P <0.05), which did not differ from each other. Over the four-hour trial period the rectal temperature of the H_u genotype goats dropped by 5.58 °C, compared to 3.85 °C and 3.58 °C for the H_e and H_o genotypes, respectively.

At 150 minutes, the first H_u goat had to be removed from the trial as its rectal temperature dropped below 32 °C. At 180 minutes a second H_u goat had to be removed and revived, while at the end of the trial, three more H_u goats whose rectal temperatures dropped below 32 °C had to be revived. None of

the H_e or H_o goats had to be removed from the trial due to a rectal temperature dropping below 32 °C. Animals whose rectal temperature dropped below 32 °C were removed from the pens and placed in a heated room for recovery. These goats also received an isotonic glucose solution intra-peritonically, administered by a veterinarian.

Table 1. Rectal temperature (°C ± s.e.) of the three genotypes over the cold stress trial period

Minutes after rain treatment started	H_e	H_o	H_u
0	$39.17^{a} \pm 0.12$	$39.08^{ab} \pm 0.12$	$38.76^{b} \pm 0.12$
60	$39.07^{a} \pm 0.17$	$39.08^{a} \pm 0.17$	$38.31^{b} \pm 0.17$
120	$37.81^{a} \pm 0.33$	$37.89^{a} \pm 0.33$	$36.45^{b} \pm 0.33$
150	$37.17^{a} \pm 0.38$	$37.18^{a} \pm 0.38$	$35.30^{b} \pm 0.38$
180	$36.36^{a} \pm 0.46$	$36.44^{a} \pm 0.46$	$34.16^{b} \pm 0.46$
240	$35.32^{a} \pm 0.54$	$35.51^{a} \pm 0.54$	$33.18^{b} \pm 0.54$
Difference between start and end values	$-3.85^{a} \pm 0.55$	$-3.58^{a} \pm 0.55$	$-5.58^{b} \pm 0.55$

[a, b] Values with different superscripts differed significantly (P <0.05)

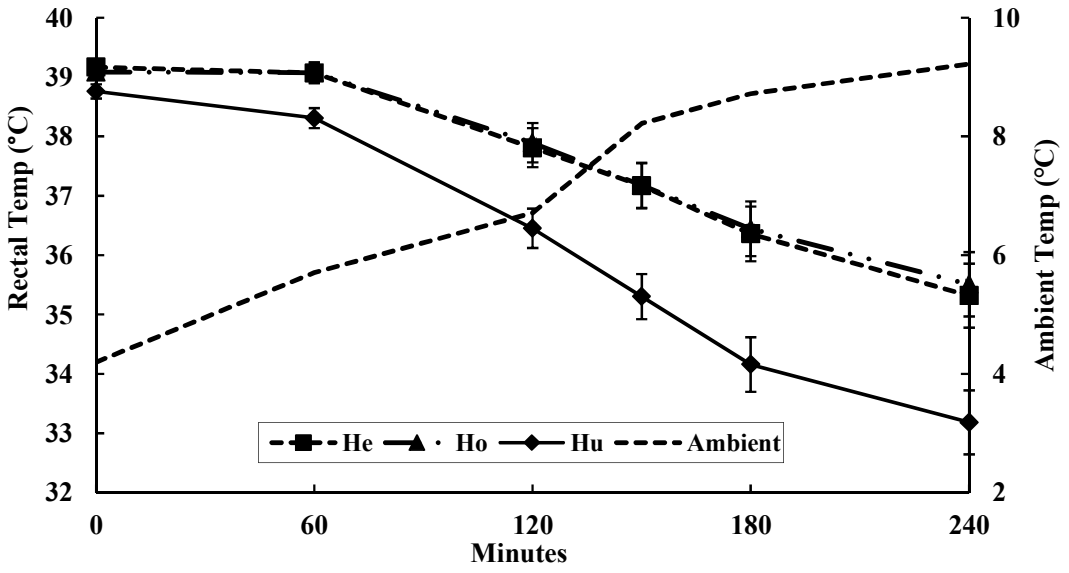

Figure 1. Rectal temperature of the three genotypes over the cold stress trial period

The H_u genotype had lower subcutaneous temperatures at 180 and 240 minutes, but this was not significant (P >0.05). The H_u genotype animals also recorded a higher subcutaneous temperature loss (-7.82 °C) than the H_e (-6.26 °C; P =0.2655) and H_o (-5.80 °C; P =0.1354) genotype animals by the end of the trial (240 minutes), but this was again not significant, due to the large variation among the animals within the genotypes.

Table 2. Subcutaneous temperature (°C ± s.e.) of the three genotypes over the cold stress trial period

Minutes after rain treatment started	H_e	H_o	H_u
0	37.98 ± 0.19	37.82 ± 0.17	37.64 ± 0.19
60	38.70 ± 0.24	38.72 ± 0.22	38.25 ± 0.24
120	36.76 ± 0.61	37.50 ± 0.55	36.76 ± 0.61
180	33.38 ± 0.98	34.37 ± 0.89	31.98 ± 0.98
240	31.72 ± 1.02	32.01 ± 0.92	29.82 ± 1.02
300	30.22 ± 0.94	30.78 ± 0.85	29.88 ± 0.94
360	33.49 ± 0.91	34.32 ± 0.83	33.21 ± 0.91
420	35.88 ± 0.97	36.64 ± 0.88	34.59 ± 0.97
480	37.54 ± 0.79	38.27 ± 0.72	36.81 ± 0.79
540	37.98 ± 0.55	38.63 ±0.50	37.48 ± 0.55
600	38.65 ± 0.41	39.03 ± 0.37	38.36 ± 0.41
Difference between start and 240 min values	-6.26 ± 0.31	-5.80 ± 0.27	-7.82 ± 0.31

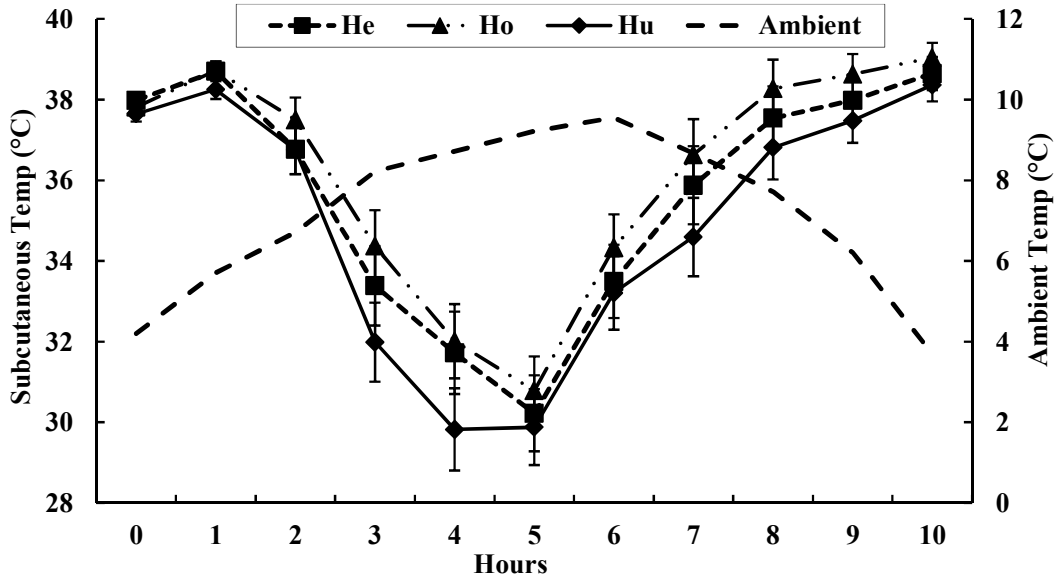

Figure 2. Subcutaneous temperature of the three genotypes over the cold stress trial period

At the start of the cold stress trial, subcutaneous temperatures of all animals were, on average, 1.2 °C lower than rectal temperatures. This difference increased to 3.5 °C at the end of the trial (240 minutes).

When exposed to cold conditions, the body loses heat through the skin at a more rapid rate than under higher ambient temperature conditions (McCullough & Arora, 2004). This increased heat loss causes the hypothalamus to activate the temperature regulation mechanisms (Kaiser, 2010). The primary objective of the hypothalamus is to maintain the vital organs at an acceptable temperature, thus all the thermoregulation mechanisms are designed to protect the core. The core includes all the vital organs such as heart, lungs, liver, kidneys and the brain. Blood vessels in the skin constrict to prevent excessive heat loss and muscles shiver to produce heat. The body will begin to shift blood flow from the extremities and skin to the core. This allows exposed skin and the extremities to cool more rapidly until the periphery reaches the same temperature as the surrounding environment, and then the heat flow stops and body heat is preserved. The bigger drop in subcutaneous temperature compared to rectal temperature of the trial animals is consistent with this mechanism.

Wentzel et al. (1979), Al-Tamimi (2006) and Al-Tamimi (2007) also reported a lowering in core body temperature, as well as in subcutaneous (Al-Tamimi, 2006) and skin temperatures (Bianca & Kunz, 1978) in goats exposed to cold conditions. Similar results have also been shown for various sheep breeds (Slee & Sykes, 1967; Panaretto & Vickery, 1971; Bennett, 1972).

From Figures 3 and 4 it can be seen that animals from the H_u genotype tended to have lower subcutaneous temperatures, especially during the period of relatively warmer ambient temperatures (Figure 4). Diurnal fluctuations in subcutaneous temperatures were also less in goats of all genotypes during the warmer period.

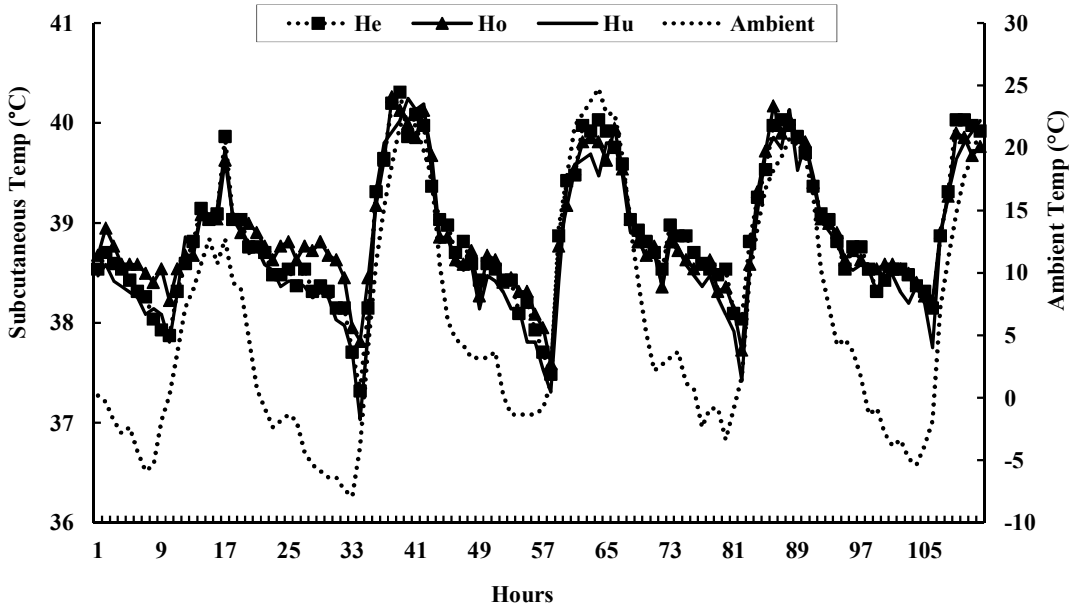

Figure 3. Subcutaneous temperature of the three genotypes from 30 July until 3 August 2011

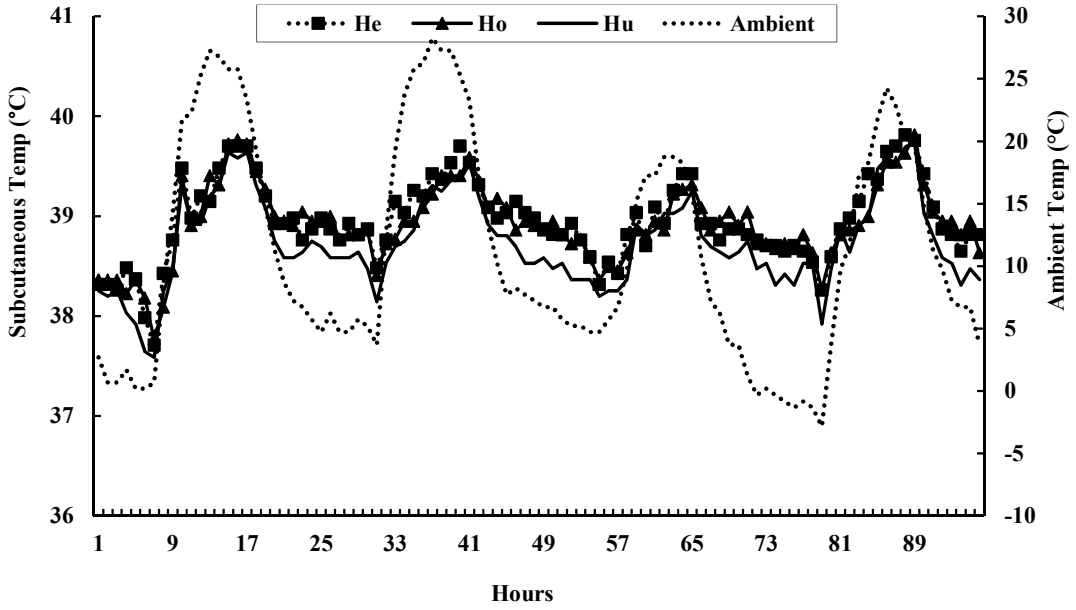

Figure 4. Subcutaneous temperature of the three genotypes from 25 August until 28 August 2011

From both the rectal and subcutaneous temperature data it is clear that the H_u genotype was worse at coping with the cold stress than the other genotypes. Indeed, five of the H_u goats had to be administered glucose to aid with their recovery from the cold stress trial.

Plasma cortisol concentrations of the three genotypes over the trial period are depicted in Figure 5, while the plasma cortisol/cortisone ratios are illustrated in Figure 6. No significant differences were recorded in plasma cortisol concentrations among the genotypes over the cold stress trial period. However, the H_o genotype was the poorest performer, especially after 60 minutes, where its cortisol/cortisone ratio was significantly decreased. Cortisone is the inactive form of cortisol and the cortisol/cortisone ratio thus gives an indication of availability of the active form. Nonetheless, the observation that no significant differences were observed in absolute cortisol values concentrations were surprising considering that an insulin-induced stress trial previously showed that the H_o genotype was the least effective cortisol producer (Storbeck et al., 2008).

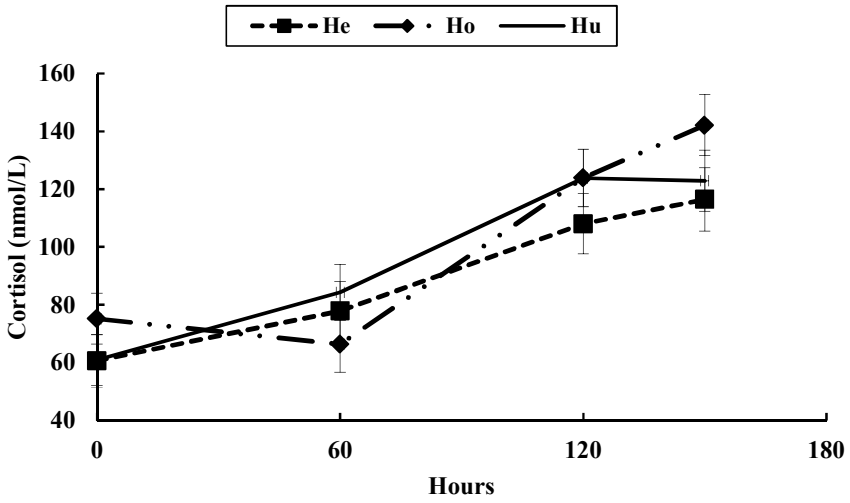

Figure 5. Plasma cortisol concentration (nmol/L) of the three genotypes over the trial period

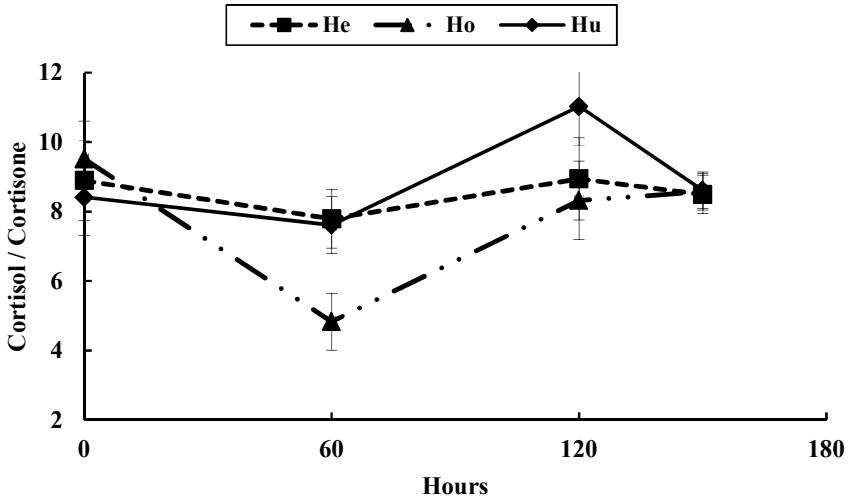

Figure 6. Plasma cortisol/cortisone ratio of the three genotypes over the trial period

Cold exposure has previously been shown to significantly decrease insulin secretion in response to a variety of stimuli in goats (Oda et al., 1995). Whole-body blood glucose turnover rate was also found to be lower during mild cold exposure (Sano et al., 1997). In another cold stress trial (Wentzel et al., 1979), blood glucose concentration of cold stressed goats showed an initial increase from 4.7 ng/ml to 8.2 ng/ml, after which it decreased rapidly to a minimum of 2.2 ng/ml at the point of collapse.

The difference in cortisol production between the insulin-induced stress test and the cold stress test may be due to the different types of stress. The insulin-induced stress test can be considered to be acute stress. The stressor (insulin) caused a decrease in glucose levels that resulted in the production of cortisol, which in turn acted to restore glucose levels. The cold stress test could, however, be considered

to be more like a chronic stress as the stressor (cold) is continuous. Cortisol production can only act to counteract the effects of the stressor, but the stressor itself (cold) remains constant. The observation that the H_u genotype was the least effective at coping with the cold stress, but produced similar levels of cortisol to the H_e goats, suggests that other factors besides cortisol may play a role.

CONCLUSIONS

This cold stress trial suggested that the H_u genotype is the most susceptible to cold stress. In both the *in vivo* studies to date (insulin-induced stress test and cold stress test) the H_e genotype was the best performer. It is therefore suggested that this genotype would likely be the hardiest.

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