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EXTENDED ABSTRACT
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Impact of Rearing System on Various Stress Indexes in Pregnant Does and Ewes During Winter

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Small ruminants are primarily raised outdoors, regardless of season and weather conditions in the southeast United States. When there is a fluctuation in ambient temperature, the thermoregulatory mechanism of animals is disrupted. Animals can maintain their body temperature when the ambient temperature is within the thermoneutral zone (TNZ). Ambient temperatures below the lower limit of TNZ cause cold stress in animals. Cold stress activates the hypothalamus-pituitary-adrenal (HPA) axis which stimulates the adrenal gland to increase the production of stress hormones, mainly cortisol. Increased cortisol production over the long term poses a risk of physiological disorders, particularly in pregnant animals, which can compromise their performance and health. Although indoor facilities may offer a solution to mitigate the negative impact of cold stress, that potential has not been thoroughly evaluated. We hypothesized that pregnant does and ewes would show less stress when raised indoors vs. outdoors. The objective of this study was to evaluate the stress status of pregnant does and ewes when raised indoors and outdoors during winter.

This study was conducted for 69 days (late January to early April 2023) using sixteen pregnant Kiko does (44-45 months of age) and eighteen pregnant Katahdin-St. Croix crossbred ewes (51-52 months of age). Both species were divided uniformly into indoor and outdoor groups based on their performance, and the group division was random. The indoor group was kept in individual pens. Each pen was 1.2 m long and 1.1 m wide and contained a slatted plastic floor to facilitate animal excreta passing underneath the floor. Separate feeders for hay, minerals, concentrate feed, and water were installed in each pen. Outdoor does and ewes were kept in a fenced plot (1 acre) containing mobile shelters (2), mineral feeder, hay and grain feeder, water lines, and water troughs throughout the study period. Both groups were fed a whole corn and whole soybean (mixed in 3:2 ratio) at the rate of 0.8% of their live weight and *ad libitum* hay. Both groups had free access to mineral mix (Purina goat and sheep mineral) and clean water throughout the study period.

Temperature and relative humidity (RH) data for the outdoor site was collected from a nearby weather station, and that of the indoor site was collected with a smart thermo-hygrometer. The temperature-humidity index (THI) was calculated using the formula: $THI = 0.8 * T + RH * (T-14.4) + 46.4$. Animal performance data (live weight, body condition score (BCS), and FAMACHA score) were collected on Day 1, weekly throughout the study, and at the end of the study. Blood and hair samples were collected on Days 1, 34, and 69. Blood samples were collected from the jugular vein into three different tubes: a tube with EDTA, an untreated tube, and a lithium heparin (LH) tube. Blood samples collected in EDTA tube were analyzed for red blood cells (RBC), hemoglobin, hematocrit, neutrophils, lymphocyte, and neutrophil-to-lymphocyte ratio using the ProCyte Dx (www.idexx.com). Catalyst One (www.idexx.com) was used to analyze glucose from blood samples collected in untreated tubes. Blood samples collected in the LH tube were analyzed for plasma cortisol using an ELISA kit after plasma separation. Hair samples were collected from the loin region from a 15 cm x 15 cm area on Day 1, and subsequent hair samples were collected from the same area on Days 34 and 69 and analyzed for hair cortisol using an ELISA kit. Data were analyzed in SAS 9.4 (α=95%). Data sets on weather parameters, animal performance, and blood parameters were analyzed using the GLM procedure with MANOVA option. Plasma and hair cortisol data were analyzed using the Mixed procedure. Log transformation and square root transformation were done for plasma and hair cortisol data, respectively, to fulfill the assumption of normal distribution. Least-square means were back-transformed for presentation, and upper and lower limits were presented as measures of dispersion.

Indoor temperature was higher by 16-22% ($p<0.01$), RH was lower by 5-10% ($p<0.05$), and THI was higher by 4-7.5% ($p<0.01$) compared to outdoor conditions. Indoor ewes had higher BCS by 5% during overall observation and an 11% higher BCS in the fourth week of the study ($p<0.05$) (Figure 1). RBC was lower in indoor does (15%, $p<0.05$) on Day 34 compared to outdoor does. Plasma cortisol was higher by 105% ($p<0.05$) on Day 34, and hair cortisol was higher by 125% and 140% ($p<0.01$) on Days 34 and 69, respectively, in outdoor compared to indoor does (Table 1). Outdoor ewes had higher hair cortisol by 280% ($p<0.0001$) on Day 34 (Table 1). The results showed that pregnant does and ewes raised indoors were less stressed compared to their counterparts raised outdoors. Hair cortisol was found to be a better indicator than plasma cortisol for long-term cold stress in pregnant animals, as this parameter differed between groups, coinciding with the difference observed in weather parameters indoors and outdoors. However, such difference between the groups was

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not revealed in plasma cortisol. Securing animals in indoor facilities or providing shelters for animals kept outdoors is recommended to protect pregnant animals during extreme cold.

Table 1. Hair cortisol of pregnant Kiko does and St. Croix-Katahdin-cross ewes kept indoor and outdoor during the study period (January – April 2023), Tuskegee University, Tuskegee, Alabama, USA.

Observation day	LSMean (pg/mg)	Lower limit	Upper limit	LSMean (pg/mg)	Lower limit	Upper limit
	Indoor does			Outdoor does		
1	0.056	0.033	0.084	0.041	0.021	0.066
34	0.038^{b}	0.020	0.062	0.086^{a*}	0.057	0.121
69	0.045^{b}	0.024	0.071	0.108^{a**}	0.075	0.147
	Indoor ewes			Outdoor ewes		
1	0.02	0.01	0.04	0.02	0.01	0.04
34	0.05^{b}	0.03	0.07	0.19^{a****}	0.16	0.23
69	0.13	0.10	0.16	0.17	0.14	0.21

[ab]LSMean in the same row with different superscripts differ (*p<0.05, **p<0.01, ****p<0.0001).

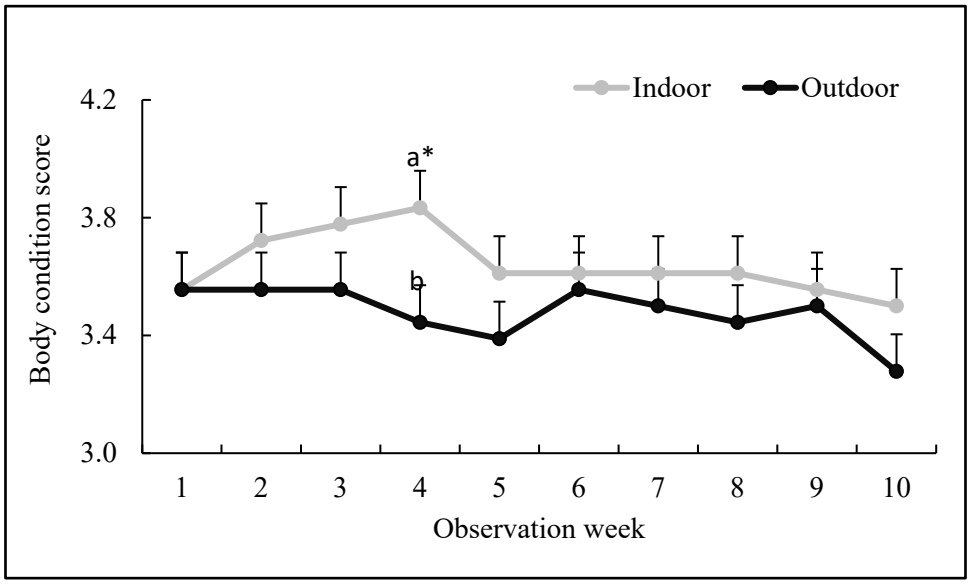

Figure 1. Body condition score (LS mean ± SE) of pregnant St. Croix-Katahdin-cross ewes kept indoor and outdoor, January – April 2023, Tuskegee University, Tuskegee, Alabama, USA (*p<0.05)

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