Rearing System Influenced the Performance and Health Status of Pregnant Ewes During Winter

Sadiksha Lamsal [1, *], Uma Karki [1], Santoshi Chaudhari [1,2], Durga P. Dhakal [1] and Bidur Paneur [1,3]

[1]*Department of Agricultural and Environmental Sciences, Tuskegee University, Tuskegee, AL 36088, USA*
[2]*Currently at the Department of Veterinary and Biomedical Sciences, Pennsylvania State University, University Park, PA 16802, USA*
[3]*Currently at the Department of Poultry Science, University of Georgia, Athens, GA 30602, USA*

MANUSCRIPT INFO

Article history:
Received: 23 September 2024
Revised: 16 January 2025
Accepted: 18 February 2025

Keywords:
Body condition score
Immunological Parameters
Reticulocytes
Serum chloride

ABSTRACT

Small ruminants are raised outdoors in the southeast US irrespective of seasons and associated weather conditions. Severe weather in winter can affect the performance of pregnant animals and compromise their health. Keeping animals indoors may provide comfort and minimize the adverse weather impact; however, such potential has not been assessed. This study tested the hypothesis that the performance and health status of pregnant ewes would improve when raised indoor versus outdoor during winter. The objective of this study was to evaluate the performance and health status of pregnant ewes when raised indoor versus outdoor during winter. Eighteen pregnant Katahdin-St. Croix cross ewes were divided into indoor and outdoor groups and fed corn-soybean mix (3:2) (0.8% of live weight) and *ad libitum* hay. Temperature and relative humidity were measured, and temperature-humidity index (THI) was calculated for both sites. Animal performance data (live weight, body condition score (BCS), and FAMACHA score) and fecal samples were collected on Day 1, weekly during the study, and at the end of the study. Blood samples were collected on Days 1, 34, and 69, and analyzed for hematological, biochemical, and immunological parameters. Indoor temperature was higher (7%, $p<0.01$), humidity was lower (8%, $p<0.05$), and THI was higher (5%, $p<0.05$) vs. outdoor. Indoor ewes had higher BCS (5%, $p<0.05$), EPG for GI nematodes (56%, $p<0.05$), reticulocytes (75%, $p<0.05$), mean platelet volume (8%, $p<0.01$), globulin (10%, 0.01), and serum chloride (12%, $p<0.01$) compared to outdoor ewes. Indoor rearing of pregnant ewes improved performance and impacted a few health parameters.

Citation:
Lamsal, S., Karki, U., Chaudhary, S., Dhakal, D.P., & Paneru, B. (2025). Rearing system influenced the performance and health status of pregnant ewes during winter. *Global Journal of Agricultural and Allied Sciences, e*1-10.

1. Introduction

In the southeastern United Staes, sheep are predominantly raised on pastures throughout the year (Karki, 2020), irrespective of weather conditions such as rainstorms, high humidity, extreme temperatures, and chilled winds (Jin et al., 2019). Stress caused by fluctuations in environmental temperature is one of the major concerns affecting animal production (Bhimte et al., 2018). When the ambient temperature is too high or too low, the thermoregulatory mechanism of animals is disrupted, resulting in thermal stress. Animals maintain their body temperature and perform satisfactorily when they are in the thermoneutral zone (TNZ) (Shi et al., 2022). When the ambient temperature is below the lower critical temperature of TNZ, heat loss is greater than heat production, thereby disrupting the thermal equilibrium and resulting in cold stress in animals (Wang et al., 2023). In such instances, the animals will require more energy to maintain their body temperature. The TNZ for sheep is approximately between 5°C and 25°C; however, if the ambient temperatures fall below 7°C during windy and wet conditions, the sheep will enter a state of cold stress (Piirsalu et al., 2020; USDA, 1997). The winter of the southeastern United States is mild; however, the weather data for the past ten years show the minimum winter temperature can reach below 2^0C exposing animals to cold stress, which potentially affects their performance, immune function, and health (Bhimte et al., 2018).

Indoor raising of small ruminants during winter can be one of the options to minimize the adverse effects of cold stress, especially when they are pregnant, as they require additional nutrients for fetal development (Jin et al., 2019; Zhang et al., 2016). The restricted capacity of pregnant animals to consume and digest enough food due to the limited space available in the digestive tract as a result of the expanded uterus with the developing fetus(es) makes them vulnerable to adverse weather conditions. A previous study found better live weight and higher white blood cell counts in ewes housed indoors compared to grazing ewes during a cold winter (Jin et al., 2019). Another study reported the preference of ewes for indoors vs. outdoors at a low temperature (-2°C) when they were given free access to both indoor and outdoor facilities (Piirsalu et al., 2020). However, very

* *Corresponding author.* E-mail address: slamsal3101@tuskegee.edu

https://doi.org/10.35251/gjaas.2025.008

little is known about the physiological and immune response of pregnant ewes when raised indoors compared to outdoors during winter.

During cold stress, the neuroendocrine pathway is activated and releases the stress hormone, cortisol, into the circulation. Cortisol promotes various biochemical reactions such as gluconeogenesis (production of glucose from fat and protein) and glycogenolysis (breakdown of stored glycogen into glucose) and increases glucose levels in the blood circulation (Young, 1981). Blood glucose is the readily available source of energy to produce metabolic heat to maintain body temperature (Berian et al., 2019; Bhimte et al., 2018; Liu et al., 2019; Shida et al., 2020). Increased utilization of stored fat and protein to maintain homeostasis impairs animal weight and other performance parameters (Adam et al., 2010). In stressed animals, the catabolism of protein into amino acids can increase amino acid concentration in the blood circulation until amino acids are used for gluconeogenesis. Cold stress is also known to affect various hematological parameters such as red blood cells (RBC), hematocrit (HCT), and hemoglobin (Hb) levels. In response to cold stress, the sympathetic nervous system is activated, and cardiac output and redistribution of blood flow from skin and extremities to vital organs are increased (Sawasaki et al., 2001). Moreover, RBC, Hb, and HCT levels are increased to improve oxygen-carrying capacity to tissues and vital organs which is essential for meeting the elevated energy demands of cold-stressed animals to maintain body temperature during the prolonged cold stress. Studies have found elevated levels of RBC, HCT, and Hb in cold-stressed rats (Gordon, 2012). The levels of Hb and hematocrit were increased in lactating goats exposed to cold temperatures (-3˚C to 6˚C) compared to goats kept at the thermoneutral temperature (15˚C to 20˚C) (Coloma-García et al., 2020). A similar response can be expected from pregnant ewes when kept in different environmental conditions; however, this response is not well documented.

Besides hematological parameters, prolonged exposure of animals to cold conditions may impair the immune system due to endocrine and metabolic changes. The immune system is important to protect animals against pathogens and infectious agents and keep animals in good health. Immune cells rely heavily on glucose utilization for activation and maintenance (Delmastro-Greenwood & Piganelli, 2013). A study done in rats reported that stress reduced lymphocytes (26%, $p<0.05$) but increased neutrophils (9%, $p<0.05$) (Dhabhar et al., 1994). In response to the elevated level of cortisol, lymphocytes adhere to the endothelial lining and undergo transmigration from circulation into various tissues, such as lymph nodes, spleen, bone marrow, and skin. In contrast, stress hormones induce the entry of neutrophils from bone marrow into the bloodstream but reduce the exit of neutrophils from blood into other tissues and organs where neutrophils are needed. As a result, the neutrophil-to-lymphocyte ratio (N: L) is also increased in stressed animals (Davis et al., 2008). However, in a study done on cold-stressed cattle, there was no difference in total leucocyte and lymphocyte count (Kim et al., 2023). Due to these inconsistent results, it is necessary to understand the effect of the rearing system on different types of blood leucocyte dynamics in pregnant ewes during winter. Cold exposure can also diminish the levels of plasma proteins required for antibody synthesis. Long-term stress also decreases B lymphocytes, subsequently reducing the production of antibodies (Dhabhar, 2009; Wu et al., 2022). Guo et al. (2021) found decreased IgG in cold-exposed sheep with high wind (9%, $p<0.05$) compared to sheep exposed to low wind. Similarly, Olson et al. (1980) found decreased IgG in cold-exposed calves.

When long-term stress reduces the immune response, this can increase the vulnerability of animals to parasitic infection (Brown & Fuller, 2006; Romeo et al., 2020). The major immune cells produced against gastrointestinal (GI) parasites are eosinophils (Huang & Appleton, 2016). An increased stress hormone in mice has been reported to be associated with the decreased production of eosinophils (Louch et al., 1953). Davis et al. (2008) suggested that cortisol could reduce the eosinophil level, resulting in decreased resiliency of animals against parasitic infection. A study done in rodents has reported an association between high fecal cortisol metabolites and high fecal egg count ($p<0.05$) of *Strongyloides* spp., suggesting that physiological stress may result in high parasitic infestation (Romeo et al., 2020). However, other studies suggest that the association between stress and parasitic infection is highly dependent on the specific host-parasite system under study (Hammond et al., 2019; Lindsay et al., 2016).

This study tested the hypothesis that the performance and health status of pregnant ewes would improve when raised indoors versus outdoors during winter. The objective of this study was to evaluate the performance and health status of pregnant ewes when raised indoors or outdoors during winter.

2. Methods

2.1. Study Site

This study was conducted from late January to early April 2023 for 69 days using the indoor facility (Fig. 1a) and the outdoor facility (Fig. 1b) at the Browse Research and Demonstration Site. The facilities were located at the Caprine Research and Education Unit (CREU), George Washington Carver Agricultural Experiment Station, Tuskegee University, Tuskegee, Alabama, USA. The indoor facility was at latitude 32°44’17.4” N, longitude 85°73’96.4” W, and the outdoor facility was at latitude 32°43’41.0” N, longitude 85°71’56.9” W. The two sites were approximately two kilometers apart.

2.2. Study Animals

Eighteen Katahdin-St. Croix crossbred pregnant ewes (51-52 months of age) were used for the study. Based on the initial liveweight, body condition score (BCS), FAMACHA© score, and gastrointestinal parasite fecal egg count (EPG - parasite eggs per gram of animal feces), ewes were divided into two uniform groups: indoor and outdoor. The average EPG was less than 260 in both groups. Before the study began, ewes with egg per gram (EPG) of feces greater than 1000 were dewormed. Indoor ewes were kept in individual pens in the small ruminant barn of CREU, which was enclosed from all sides with the provision of natural sunlight from east and west and enough ventilation. Each pen was 1.2 m long and 1.1 m wide and contained a slatted plastic floor to facilitate the passing of animal excreta underneath the floor. Separate feeders for hay, minerals, concentrate feed, and water were installed in each pen. Outdoor ewes were kept in a fenced plot (0.4 hectare) with dormant forage during winter, containing mobile shelters made of galvanized steel (2), mineral feeder, hay and grain feeder, water lines, and water troughs throughout the study period. Both indoor and outdoor ewes were supplemented with whole corn and whole soybean mixed at a 3:2 ratio, fed at the rate of 0.8% of live weight, and given *ad libitum* hay. The same type of hay was fed to both groups. Both groups had free access to mineral mix (Purina sheep mineral) and clean water throughout the study period. Indoor pens were cleaned twice daily, in the morning and evening. Animals were monitored and cared for as per the

protocol approved by the Animal Care and Use Committee of Tuskegee University (TUACUC no: RTD11-2022-7).

Figure 1. *Indoor ewes in individual pens at the Caprine Research and Education Unit; each pen contained hay feeder, mineral feeder, and water trough (a), and outdoor ewes resting on study plot at the Browse Research and Demonstration Site (b), January-April 2023, Tuskegee University, Tuskegee, Alabama, USA*.

2.3. Data Collection

2.3.1. Weather Parameters

Hourly daily ambient temperature (T) and relative humidity (RH) data from the indoor site were collected using the Govee-smart thermo-hygrometer. Outdoor weather data were downloaded from a secondary source, a nearby weather station, TUSKEGEE AL US SCAN, which was located approximately 4.8 km west of the study site (https://www.visualcrossing.com/weather-history/Tuskegee/metric/2023-01-31/2023-04-9).

2.3.2. Animal Performance

FAMACHA score, body condition score (BCS), and live weight were taken on Day 1, every week during the study, and at the end of the study. FAMACHA scoring was done by using the FAMACHA card and following the guidelines for the FAMACHA system (Petersson, 2014). The color of animals' conjunctiva of the lower eyelid on both eyes was compared with the color on the FAMACHA card and scored with the most matching color. The BCS was evaluated by feeling the muscles and fat tissues over the backbone, ribs, and brisket bone, and scores were provided ranging from 1 to 5 (1 extremely thin, 5 obese). Both FAMACHA score and BCS were evaluated by a single trained person (the first author) throughout the study period to avoid potential individual bias. Live weight was measured using a digital weighing scale installed at both study sites.

2.4. Sample Collection and Analysis to Determine the Health Status of Animals

2.4.1. Fecal Samples

Fecal samples were collected and analyzed to determine the prevalence of gastrointestinal nematodes. Fecal samples were collected on the very first day just before putting ewes into their respective study sites, then weekly during the study, and on the very last day of the study. Fresh feces were collected directly from the rectum and kept individually in airtight Ziploc plastic bags. Fecal samples were kept in a refrigerator until analyzed on the same day of collection for the type and quantity of GI nematodes. The number of nematode eggs per gram (EPG) of ewe feces was determined by using the McMaster technique (sensitivity: 1 egg = 50 EPG) and nematode egg identification was based on parasite egg chart (Merck Animal Health, 2014).

2.4.2. Blood Samples

To assess the health status of pregnant ewes, blood samples were collected and analyzed for various hematological, biochemical, and immunological parameters. The first set of blood samples was collected on the first day of the study before allocating ewes to their respective sites. The subsequent sets of blood samples were collected on Day 34 and Day 69 on the last day of the study. Two sets of blood samples per ewe were collected directly from the jugular vein. The first set included 2 ml of blood collected in EDTA (ethylenediaminetetraacetic acid, purple cap) Vacutainer® tube and inverted gently a couple of times immediately after collection. This sample was used for hematological analyses (fifteen parameters). Another set consisted of 3.5 ml of whole blood collected into an untreated (red cap) Vacutainer® tube, from which serum was separated later for analyzing biochemical parameters (sixteen parameters) and immunoglobulins (IgG, IgE, and IgA). Hematological parameter values were assessed using the IDEXX ProCyte Dx® Hematology Analyzer and biochemical parameter values were assessed by using the IDEXX Catalyst One® Blood Chemistry Analyzer.

For analyzing each type of serum immunoglobulin (IgG, IgA, and IgE) in ewes, a separate set of sheep ELISA kit (96-well plate) from antibodies.com, which was coated with mouse monoclonal antibody, was used. Stored serum samples (-20^{0}C) were kept at the room temperature for about 30 minutes and diluted with the sample dilution solution. For IgG and IgA, samples were diluted at 1:10000, and for IgE at 1:2. For all IgG, IgA, and IgE, standards and samples were added into the wells and immunoglobulins present in each sample allowed to bind to the wells. The wells were washed, and biotin HRP-conjugated streptavidin was added into the wells. After another wash, TMB substrate solution was pipetted to the wells. As the TMB substrate reacted, blue color developed in the wells in proportion of the immunoglobulins bound into the well. A stop solution was

then added, changing the color from blue to yellow. The intensity of the color was evaluated at 450 nm in a microplate reader (Varioskan Lux ver. 1.00.38) using SkanIt software. The coefficient of variance (CV) for each sample for the sheep ELISA was read, and samples with greater than 20% CV were reanalyzed until CV was 20% or less. The first set of serum samples was used as baseline for immunoglobulins level.

2.5. Data Analysis

All data sets were analyzed in SAS 9.4, and significance level (alpha) was set at 0.05 for the hypothesis test. Weather parameters (temperature and relative humidity), animal performance (live weight, BCS, and FAMACHA score), and hematological, biochemical, and immunological parameters were analyzed using the Generalized Linear Model (GLM) with Multivariate Analysis of Variance (MANOVA) option. The neutrophils-to-lymphocyte ratio (N: L) was calculated and analyzed using the Mixed Model. Temperature-humidity index (THI) was also calculated from ambient temperature and relative humidity for every week using the formula: THI = T – 0.55 (1 – RH) (T – 58), where "T" is ambient temperature and "RH" is relative humidity, and the THI data were analyzed using the GLM procedure. The EPG data were analyzed by using the Wilcoxon Rank-Sum test.

i. The model used to analyze the weather parameters:

$Y_{ij} = \mu + \alpha_i + (\alpha\beta)_{ij} + e_{ij}$

MANOVA h = Ewe group and the interaction of research site and observation date

Where, Y_{ij}= weather variables from i^{th} study site and j^{th} observation date, μ = grand mean, α_i = effect of study site, $(\alpha\beta)_{ij}$ = interaction of i^{th} site and j^{th} observation date, e_{ij} = error associated with the i^{th} site and j^{th} observation date.

ii. The model used to analyze the animal performance data:

$Y_{(1\text{-}3)\,ij} = \mu + \alpha_i + (\alpha\beta)_{ij} + e_{ij}$

MANOVA h = Ewe group and the interaction of ewe group and observation date

Repeated factor = Individual ewe

Where, $Y_{(1\text{-}3)\,ij}$ = performance variables of animals from i^{th} group (indoor and outdoor) and j^{th} observation date, μ = grand mean, α_i = group effect, $(\alpha\beta)_{ij}$ = interaction of i^{th} group and j^{th} observation date, e_{ij} = error associated with the i^{th} group and j^{th} observation date.

iii. The statistical model for hematological and biochemical parameters:

$Y_{(1\text{-}33)\,ij} = \mu + \alpha_i + (\alpha\beta)ij + e_i$

Where, $Y_{(1\text{-}33)\,ij}$ = blood parameters for the i^{th} group (indoor and outdoor) and j^{th} observation date, μ = grand mean, α_i = main effect of i^{th} group, $(\alpha\beta)ij$ = interaction of i^{th} group and j^{th} observation date, e_{ij} = error associated with the i^{th} group and j^{th} observation date.

iv. The statistical model for immunoglobulins:

$Y\ (1\text{-}3)\ ij = \mu + \alpha i + (\alpha\beta)ij + eij$

Where, $Y_{(1\text{-}3)\,ij}$ = immunoglobulins for i^{th} group (indoor and outdoor) and j^{th} observation date, μ = grand mean, α_i = main effect of the i^{th} group, $(\alpha\beta)_{ij}$ = interaction of i^{th} group and j^{th} observation date, e_{ij} = error associated with the i^{th} group and j^{th} observation date

3. Results

3.1. Weather Parameters

Indoor temperature was higher than outdoor temperature regardless of the observation week (Table 1). Overall, average indoor ambient temperature was higher by 7% ($p<0.01$) and RH was lower by 8% ($p<0.05$) compared to outdoor temperature and RH. In the seventh week of the study, the outdoor site had the lowest temperature which was 8.8°C. The maximum temperature difference between indoor and outdoor sites was 2.9°C in the sixth week in early March and minimum temperature difference was 1.6°C in the second week of the study in early February. Indoor THI was higher every week compared to outdoor THI (Table 1). Overall, average indoor THI was higher by 5% ($p<0.01$) compared to outdoor.

3.2. Animal Performance

Overall, indoor ewes had higher body condition scores by 5% ($p<0.05$) compared to outdoor ewes. In the fourth week of the study, BCS was higher in indoor ewes by 11% ($p<0.05$) (Figure **1**); however, no difference was observed in other weeks. Although the difference was seen only in week 4, the BCS values of indoor ewes tended to be higher around other dates as well. BCS for indoor ewes ranged from 3.5 to 3.8 and outdoor ewes ranged from 3.3 to 3.7 (Figure 1). No difference was found between ewe groups in their live weight and FAMACHA scores.

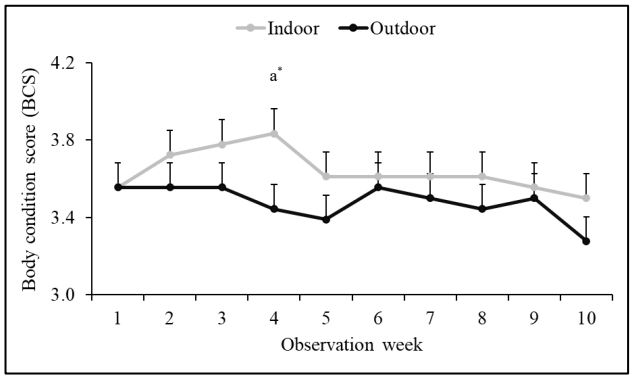

Figure 1. *Body condition score (LS mean ± SE) of St. Croix-Katahdin cross pregnant ewes kept indoors and outdoors in winter, January–April 2023, Tuskegee University, Tuskegee, Alabama, USA (*p<0.05).*

3.3. Health Parameters

3.3.1. Prevalence of Gastrointestinal Nematodes

Haemonchus contortus was the major GI nematode found in both indoor and outdoor groups. Indoor ewes had higher EPG for GI nematodes by 76% - 129% ($p<0.05$) from the sixth to last week (tenth) during the study period compared to outdoor ewes. There was no difference in EPG between ewe groups for the first five weeks (Figure 2).

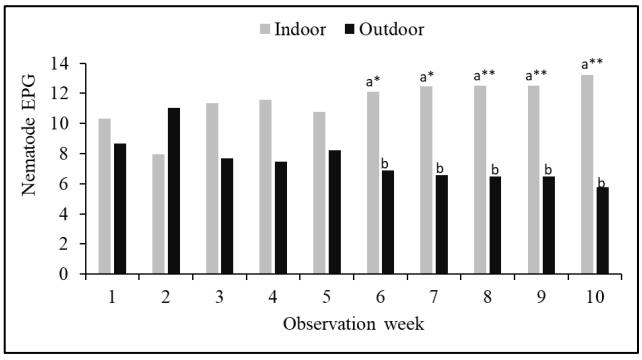

Figure 2. *Mean score of GI nematodes EPG in St. Croix-Katahdin cross pregnant ewes kept indoors and outdoors during winter, January–April 2023, Tuskegee University, Tuskegee, Alabama, USA (*p<0.05, **p<0.01).*

3.3.2. Hematological Parameter

In indoor ewes, hemoglobin (Hb) was higher by 11% on Day 1 (Table 3); however, no difference was observed for Hb between the two groups in later observations during the study. Reticulocyte was higher by 243% ($p < .05$) and MPV was higher by 22% ($p < .01$) in indoor ewes at the end of the study (Table 4). All hematological parameters were within the normal range for both indoor and outdoor ewes, except mean corpuscular volume (MCV), which was higher than normal, and mean corpuscular hemoglobin concentration (MCHC), which was lower than normal in both groups. However, this was the case for MCV and MCHC since the beginning of the study.

3.3.3. Biochemical Parameters

For biochemical parameters, glucose was higher in outdoor ewes (12%, $p<0.05$) and phosphorus was higher in indoor ewes (3%, $p<0.01$) at Day 1 (Table 5); however, no difference was observed for these parameters between the two groups at later observation dates. Globulin was higher by 17% ($p<0.05$) and serum chloride was higher by 45% ($p<0.001$) (Table 6) in indoor vs. outdoor ewes at the end of the study. Other biochemical parameters were similar in both groups. Serum globulin was above the normal range in indoor ewes on Days 34 and 69 but was within the normal range in the outdoor group. Additionally, cholesterol was above the normal range in both groups at the end of the study. Calcium was lower than normal in both groups at the end of the study, alkaline aminotransferase (ALT) was higher than normal at the first and second observations but was normal in both groups at the end, and GGT was higher than normal in both groups however, this was the case since the beginning of the study. All other biochemical parameters analyzed were within the normal range.

3.3.4. Immunological Parameters

Among the immune cells, no difference in lymphocyte, eosinophils, monocytes, and basophils count (Table 4) and N:L ratio was observed between indoor and outdoor ewes at any point of the study or for the overall observation. Lymphocytes were lower than the reference level in both groups at the end of the study period. There was no difference in immunoglobulins (IgG, IgA, and IgE). IgA was higher in indoor ewes before the study began, but this difference subsided during the study (Table 7).

Table 2**.** *Weather parameters in indoor and outdoor sites during the study period (January–April 2023), Tuskegee University, Tuskegee, AL, USA.*

Obs. week	Temperature (0C)		RH (%)		THI	
	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
	LSMean ± SE					
1	$13.1 \pm 0.74^{a****}$	10.5 ± 0.74^{b}	61.1 ± 1.49^{b}	$67.9 \pm 1.48^{a**}$	$55.9 \pm 0.19^{a****}$	52.0 ± 0.19^{b}
	$13.9 \pm 0.74^{a**}$	12.3 ± 0.74^{b}	69.1 ± 1.48	72.5 ± 1.48	$56.7 \pm 0.58^{a**}$	54.1 ± 0.58^{b}
3	$15.6 \pm 0.74^{a**}$	13.8 ± 0.74^{b}	66.9 ± 1.48^{b}	$72.5 \pm 1.48^{a**}$	$60.0 \pm 0.58^{a***}$	57.2 ± 0.58^{b}
4	$22.6 \pm 0.74^{a***}$	20.5 ± 0.74^{b}	72.7 ± 1.48^{b}	$79.5 \pm 1.48^{a**}$	$70.2 \pm 0.58^{a***}$	67.3 ± 0.58^{b}
5	$21.6 \pm 0.74^{a****}$	19.1 ± 0.74^{b}	65.3 ± 1.48^{b}	$71.9 \pm 1.48^{a**}$	$68.1 \pm 0.58^{a****}$	64.6 ± 0.58^{b}
6	$16.4 \pm 0.74^{a****}$	13.5 ± 0.74^{b}	53.9 ± 1.48^{b}	$59.4 \pm 1.48^{a*}$	$60.6 \pm 0.58^{a****}$	56.4 ± 0.58^{b}
7	$11.4 \pm 0.74^{a****}$	8.8 ± 0.74^{b}	48.4 ± 1.48^{b}	$52.6 \pm 1.48^{a*}$	$53.8 \pm 0.58^{a****}$	50.3 ± 0.58^{b}
8	$21.8 \pm 0.74^{a***}$	19.7 ± 0.74^{b}	68.3 ± 1.48^{b}	$74.2 \pm 1.48^{a**}$	$68.6 \pm 0.58^{a***}$	65.8 ± 0.58^{b}
9	$19.4 \pm 0.74^{a**}$	17.6 ± 0.74^{b}	60.7 ± 1.50	63.8 ± 1.48	$64.6 \pm 0.58^{a**}$	62.3 ± 0.58^{b}
10	$20.9 \pm 0.91^{a***}$	18.3 ± 0.91^{b}	73.1 ± 1.82^{b}	$80.5 \pm 1.82^{a**}$	$67.0 \pm 0.58^{a**}$	63.4 ± 0.58^{b}

[ab]LSMean in the same row of Temperature, RH, and THI with different superscripts differ (*p<0.05, **p<0.01, ***p<0.001, **** p<0.0001).

Table 3. *Hematological parameters of pregnant St. Croix-Katahdin cross ewes on Day 1 when raised indoors and outdoors in winter during the study period (January - April 2023), Tuskegee University, Tuskegee, Alabama, USA.*

Hematological parameters	Indoor	Outdoor	Normal range
	Day 1		
	LSMean ± SE		
Red blood cell (M/µL)	11.9 ± 0.69	11.0 ± 0.74	9.49 – 15.12
Hemoglobin (g/dL)	$12.3 \pm 0.79^{a*}$	10.1 ± 0.64^{b}	10.00 – 14.90
Hematocrit (%)	40.2 ± 1.92	36.0 ± 1.92	27.00 – 42.00
Mean corpuscular volume (fL)	33.9 ± 1.69	33.7 ± 1.91	24.40 – 32.50
Mean corpuscular hemoglobin (g/dl)	10.4 ± 0.24	10.3 ± 0.27	8.50 – 11.8
Mean corpuscular hemoglobin concentration (g/dl)	30.9 ± 0.80	30.5 ± 0.90	32.30 – 42.00
Reticulocytes (K/µL)	2.5 ± 0.99	2.7 ± 1.13	0.00 – 15.00
White blood cell (K/µL)	6.9 ± 0.64	6.2 ± 0.72	5.06 – 14.12
Neutrophil (K/µL)	2.3 ± 0.40	1.8 ± 0.45	1.72 – 10.61
Lymphocyte (K/µL)	3.2 ± 0.32	3.1 ± 0.38	2.68 – 11.54
Monocyte (K/µL)	0.1 ± 0.06	0.2 ± 0.07	0.06 – 0.89
Eosinophil (K/µL)	1.2 ± 0.16	1.1 ± 0.18	0.03 – 1.29
Basophil (K/µL)	0.1 ± 0.04	0.05 ± 0.04	0.00 – 0.24
Platelet (K/µL)	268.6 ± 40.85	272.7 ± 43.67	NA
Mean platelet volume (K/µL)	$9.0 \pm 0.24^{a**}$	8.9 ± 0.28^{b}	NA

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

Table 4. *Hematological parameters of pregnant St. Croix-Katahdin cross ewes on Day 34 and Day 69 when raised indoors and outdoors in winter during the study period (January - April 2023), Tuskegee University, Tuskegee, Alabama, USA.*

Hematological parameters	Indoor	Outdoor	Indoor	Outdoor
	Day 34		Day 69	
	LSMean ± SE			
Red blood cell (M/µL)	10.7 ± 0.69	9.5 ± 0.69	11.1 ± 1.37	9.8 ± 1.12
Hemoglobin (g/dL)	11.2 ± 0.39	10.5 ± 0.39	12.3 ± 0.79	10.9 ± 0.65
Hematocrit (%)	36.6 ± 1.92	33.7 ± 2.04	37.4 ± 1.92	35.2 ± 2.04
Mean corpuscular volume (fL)	33.3 ± 1.79	35.3 ± 1.79	35.2 ± 3.58	36.4 ± 2.93
Mean corpuscular hemoglobin (g/dl)	10.4 ± 0.25	11.0 ± 0.25	11.0 ± 0.50	11.1 ± 0.41
Mean corpuscular hemoglobin concentration (g/dl)	31.3 ± 0.84	31.1 ± 0.84	31.3 ± 1.69	30.5 ± 1.38
Reticulocytes (K/µL)	6.7 ± 1.05	4.8 ± 1.05	7.9 ± 2.11[a*]	2.3 ± 1.72[b]
White blood cell (K/µL)	7.0 ± 0.68	5.6 ± 0.68	7.1 ± 1.35	5.9 ± 1.10
Neutrophil (K/µL)	2.4 ± 0.42	1.6 ± 0.42	3.7 ± 0.84	2.2 ± 0.68
Lymphocyte (K/µL)	3.5 ± 0.34	3.0 ± 0.34	2.5 ± 0.68	2.4 ± 0.56
Monocyte (K/µL)	0.2 ± 0.07	0.3 ± 0.07	0.4 ± 0.14	0.3 ± 0.11
Eosinophil (K/µL)	0.8 ± 0.17	0.7 ± 0.17	0.4 ± 0.33	0.9 ± 0.27
Basophil (K/µL)	0.04 ± 0.03	0.03 ± 0.03	0.06 ± 0.077	0.02 ± 0.063
Platelet (K/µL)	319.5 ± 40.85	262.9 ± 40.85	354.5 ± 81.69	286.5 ± 66.71
Mean platelet volume (K/µL)	9.5 ± 0.26	9.4 ± 0.26	11.2 ± 0.51[a**]	9.2 ± 0.42[b]

[ab]LSMean in a row under the same day with different superscripts differ (*p<0.05, **p<0.01). Normal range for the hematological parameters is presented in Table 3.

Table 5. *Biochemical parameters of pregnant St. Croix-Katahdin cross ewes on Day 1 when raised indoors and outdoors in winter during the study period (January – April 2023), Tuskegee University, Tuskegee, Alabama, USA.*

Biochemical parameters	Indoor	Outdoor	Normal range
	LSMean ± SE		
	Day 1		
Glucose (mg/dl)	48.1 ± 1.75b	53.8 ± 1.98[a*]	50.00 – 80.00
Creatinine (mg/dl)	0.9 ± 0.03	0.9 ± 0.03	0.60 – 1.50
Blood urea nitrogen (mg/dl)	18.7 ± 1.28	16.7 ± 1.45	5.00 – 20.00
Phosphorus (mg/dl)	5.3 ± 0.29[a**]	4.1 ± 0.33[b]	4.00 – 8.90
Calcium (mg/dl)	9.0 ± 0.12	8.8 ± 0.14	9.10 – 10.80
Albumin (g/dl)	3.1 ± 0.05	3.0 ± 0.06	2.40 – 3.70
Globulin (g/dl)	4.0 ± 0.11	3.8 ± 0.12	3.20 – 4.10
Alanine Amino Transferase (U/L)	24.5 ± 1.77	20.3 ± 2.02	5.0 – 17.00
Alkaline Phosphate (U/L)	56.4 ± 6.69	54.7 ± 7.58	50.00 – 228.00
Gamma Glutamyl Transferase (U/L)	63.6 ± 5.38	55.4 ± 6.09	33.00 – 55.00
Total Bilirubin (mg/dl)	0.3 ± 0.03	0.3 ± 0.04	0.10 – 0.40
Cholesterol (mg/dl)	78.8 ± 4.57	71.4 ± 5.18	44.00 – 82.00
Amylase (U/L)	27.6 ± 1.67	24.5 ± 1.89	1.00 – 30.00
Lipase (U/L)	237.5 ± 17.54	223.1 ± 19.88	1.00 – 71.00
Sodium (mmol/L)	149.8 ± 0.51	149.8 ± 0.57	NA
Potassium (mmol/L)	4.7 ± 0.11	4.7 ± 0.13	NA
Chlorine (mmol/L)	111.4 ± 3.35	111.0 ± 3.80	NA

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

Table 6**.** *Biochemical parameters of pregnant St. Croix-Katahdin cross ewes on Day 34 and Day 69 when raised indoors and outdoors in winter during the study period (January–April 2023), Tuskegee University, Tuskegee, Alabama, USA.*

Biochemical parameters	Indoor	Outdoor	Indoor	Outdoor
	Day 34		Day 69	
	LSMean ± SE			
Glucose (mg/dl)	52.5 ± 1.85	51.2 ± 1.85	52.5 ± 3.72	51.0 ± 3.03
Creatinine (mg/dl)	0.7 ± 0.03	0.8 ± 0.03	0.8 ± 0.06	0.9 ± 0.05
Blood urea nitrogen (mg/dl)	13.1 ± 1.35	14.5 ± 1.35	15.5 ± 2.72	18.3 ± 2.22
Phosphorus (mg/dl)	4.6 ± 0.31	4.7 ± 0.31	4.6 ± 0.61	4.1 ± 0.51
Calcium (mg/dl)	9.3 ± 0.13	9.4 ± 0.13	9.0 ± 0.27	8.7 ± 0.22
Albumin (g/dl)	2.9 ± 0.06	2.9 ± 0.06	2.9 ± 0.12	2.7 ± 0.10
Globulin (g/dl)	4.3 ± 0.11	4.0 ± 0.12	4.6 ± 0.23[a*]	3.9 ± 0.19[b]
Alanine Amino Transferase (U/L)	24.3 ± 1.88	20.4 ± 1.88	13.0 ± 3.77	18.7 ± 3.08
Alkaline Phosphate (U/L)	58.1 ± 7.09	68.5 ± 7.09	53.0 ± 14.19	65.7 ± 11.59
Gamma Glutamyl Transferase (U/L)	81.0 ± 5.70	66.7 ± 5.70	96.5 ± 11.41	70.7 ± 9.32
Total Bilirubin (mg/dl)	0.2 ± 0.03	0.3 ± 0.03	0.2 ± 0.06	0.3 ± 0.05
Cholesterol (mg/dl)	84.2 ± 4.85	80.8 ± 4.85	94.0 ± 9.71	95.0 ± 7.92
Amylase (U/L)	21.6 ± 1.77	17.0 ± 1.77	8.5 ± 3.54	9.6 ± 2.89
Lipase (U/L)	237.5 ± 18.60	223.1 ± 18.60	203.5 ± 37.20	177.3 ± 30.37
Sodium (mmol/L)	149.3 ± 0.53	150.1 ± 0.53	152.5 ± 1.07	152.3 ± 0.87
Potassium (mmol/L)	5.0 ± 0.12	5.1 ± 0.12	5.2 ± 0.24	5.0 ± 0.20
Chlorine (mmol/L)	110.4 ± 3.56	110.7 ± 3.56	111.0 ± 7.12[a***]	76.6 ± 5.81[b]

[ab]LSMean in a row within the same day with different superscripts differ (*p<0.05, ***p<0.001). Normal range for biochemical parameters is presented in Table 5.

Table 7. *Immunoglobulins (IgE, IgG, and IgA) of pregnant St. Croix-Katahdin cross ewes when kept indoors and outdoors in winter during the study period (January–April 2023), Tuskegee University, Tuskegee, Alabama, USA.*

Observation date	Indoor	Outdoor
	LSMean ± SE	
	IgE (ng/mL)	
1/31/23	24.1 ± 7.2	29.7 ± 6.8
3/5/23	36.0 ± 6.8	31.8 ± 7.2
4/9/23	38.4 ± 7.2	37.8 ± 6.8
	IgG (mg/mL)	
1/31/23	0.21 ± 0.05	0.22 ± 0.05
3/5/23	0.37 ± 0.05	0.44 ± 0.05
4/9/23	0.35 ± 0.05	0.35 ± 0.05
	IgA (ng/mL)	
1/31/23	24.9 ± 1.13[a**]	19.9 ± 1.13[b]
3/5/23	26.9 ± 1.13	30.1 ± 1.13
4/9/23	20.4 ± 1.13	20.1 ± 1.13

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

4. Discussion

4.1. Animal Performance

The hypothesis that the performance of pregnant ewes would be better when raised indoors vs. outdoors was partially accepted, as there was better BCS in indoor ewes; however, there was no difference in FAMACHA score and live weight between the two groups. Both groups of ewes were within the thermoneutral zone throughout the study period which might be the reason for no difference in live weight and FAMACHA score. Better BCS in indoor ewes in this study might be due to the reduced walking activity. Such physical exercise can account for 25-50% of daily energy requirements for outdoor animals but can be utilized for improved performance by indoor animals (Animut et al., 2005; Osuji, 1974). Also, ewes raised outdoors during winter are affected by cold temperatures and chilled wind increasing their energy requirement for maintaining body temperature. The indoor facility provides warmer temperatures for pregnant ewes during the cold season which would reduce the weight loss due to cold stress and improve the overall performance (Zhang et al., 2014). Zhang et al. (2016) found similar results where ewes kept in warm shed maintained higher live weight by 4% ($p<0.05$) and weight gain of lambs kept in warm shed increased by 12% ($p<0.05$) compared to traditional open sheds.

4.2. Health Parameters

The hypothesis that the health status of pregnant ewes raised indoors would be better compared to outdoor ewes during winter was rejected, as EPG for GI nematodes was higher in indoor-raised ewes and other indicators of health (hematological, biochemical, and immunological parameters) were inconclusive. Reticulocytes, MPV, globulin, and serum chloride were higher in indoor ewes vs. outdoor; however, these parameters were within the normal range in both indoor and outdoor groups throughout the study period. Both groups of ewes were within the TNZ during the study period which might be the reason for no difference in health parameters. Animals do not require extra energy when raised within TNZ during cold conditions, which helps to improve performance, immune function, and health (Bhimte et al., 2018). Further study during the extreme cold (below TNZ) would be worthwhile to find out the possible differences between animals kept indoors and outdoors.

GI nematodes such as *H. contortus* have evolved to survive cold temperatures within the host as arrested larvae through hypobiosis and are dependent on pregnant or lactating animals for their life cycle (Emery et al., 2016). During winter, adult *H. contortus* neither lay eggs nor cause damage to the host (Simpson, n.d.). GI nematodes such as *H. contortus* can continue their life cycle in warm temperatures. The warmer indoor temperature compared to the outdoor may have allowed the arrested larvae in the GI tract of indoor ewes to develop into adult worms and released eggs into the feces. In contrast, the colder outdoor temperature may have inhibited the maturation of the larvae into adult worms. This could be the reason for lower EPG in outdoor vs. indoor ewes in the current study. Similar findings were reported by So-In & Sunthamala (2023), who reported high EPG of GI nematodes in barn-raised goats - around 120% ($p < 0.001$) higher than goats in the semi-intensive system and about 85% ($p < 0.001$) higher than free-ranging goats during November and December. This result was contradictory to the findings of Badran et al. (2012) where a higher prevalence of *H. contortus* (41%, $p<0.01$) was found in grazing cattle compared to cattle raised intensively (zero grazing) for one year. This was expected since their study was conducted beyond the winter season as opposed to the current study.

Elevated reticulocytes and MPV found in indoor ewes at the end of the study may be due to the higher EPG found in them compared to the outdoor ewes. A threefold increase in the rate of erythropoiesis has been observed in sheep infected with *H. contortus* as a compensatory process to maintain blood hematocrit (Dargie & Allonby, 1975; Flay et al., 2022). However, no signs of anemia were observed in indoor ewes in the current study. There was a 141% ($p < 0.01$) higher level of reticulocytes in bison highly infected with GI nematodes compared to low-infected ones (Kołodziej-Sobocińska et al., 2016). High infection with GI nematodes can cause oxidative stress in animals thereby increasing free radicals such as reactive oxygen species (ROS), which are known to cause platelet activation and aggregation resulting in high MPV (Masselli et al., 2020). Better MPV (within normal range) helps in blood clotting to minimize blood loss (Aydınyılmaz et al., 2021). Higher MPV in indoor vs. outdoor ewes in the current study might be the response of the indoor ewes against the higher infection with GI nematodes to minimize the blood loss from their GI tract. Schmidt et al. (2021) found increased oxidative stress in lambs infected with higher GI parasite burden due to GI tract inflammation compared to low-infected lambs. MPV was 41% ($p < 0.05$) higher in sheep infected with GI nematodes compared to uninfected sheep (Ngetich et al., 2019).

Higher globulin in indoor ewes at the end of this study might be due to GI inflammation caused by GI nematodes. A type of globulin (alpha-globulin) acts as an acute-phase protein and its level increases during chronic inflammation (Chovanová et al., 2021). Similar findings were reported in a previous study where higher globulin (22%, $p<0.01$) was found in sheep exhibiting high GI nematode eggs compared to sheep with no eggs of GI nematodes (Nagy et al., 2020). A higher level of serum chloride found in indoor vs. outdoor ewes in the current study might be due to less physical activity of the former group (Kratz et al., 2002). As indoor ewes were confined in individual pens (1.2 m x 1.1 m) unlike outdoor ewes having access to a one-acre plot, the movement of the former group was restricted, which might have resulted in less chloride loss through urine vs. outdoor ewes. Previous studies have indicated the influence of physical activity on serum chloride levels. A study by Kratz et al. (2002) found lower serum chloride ($p<0.001$) in 24-hour post-marathon runners vs. pre-marathon runners. Similarly, a study in horses by Prado et al. (2019) reported a higher serum chloride level (5.5%; $p<0.05$) before physical exercise compared to one-hour post-exercise. However, the effect of long-term physical activity on serum chloride levels in pregnant ewes needs to be further investigated.

Higher than normal levels of blood cholesterol in both groups of ewes at the end of this study might be due to reduced sensitivity towards insulin because of their pregnancy stage. In late gestation, maternal tissues become less responsive to insulin to ensure a continuous supply of glucose for the growing fetus. This reduction in insulin sensitivity promotes adipose tissue breakdown, and lipid metabolism, leading to increased cholesterol production. (Varanis et al., 2021). Similar findings were reported in Iranian fat-tailed sheep with higher cholesterol level ($p <0.05$) at the end of pregnancy compared to early pregnancy (Nazifi et al., 2002). Decreased calcium level than normal in both groups of pregnant ewes at the end of this study might be due to the high calcium demands for fetal development and preparation for the ensuing lactation (Kovacs & Kronenberg, 1997). Variation in ALT activity during early, mid, and late pregnancy in both groups found in this study might be due to changes in energy metabolism and liver activity. Similar findings were reported in Holstein and Montbeliard cattle with decreased level of ALT from early to late pregnancy, however the levels were within the normal range in this study (Chikhaoui et al., 2023).

Similar immunological parameters found in indoor and outdoor pregnant ewes in the current study could be attributed to the temperature in both sites being within the thermoneutral zone. A similar result was reported by Lejeune et al. (2010) with similar WBC and lymphocyte count in cattle raised indoors and outdoors (pasture-raised) during winter. However, the same study reported lower eosinophils in indoor vs. outdoor cattle (12.5%; $p<0.05$). In contrast to this study, Guo et al. (2021) found decreased IgG in sheep exposed to cold temperatures with high wind (9%, $p<0.05$) compared to low wind. Shi et al. (2022) found 50% higher IgA ($p<0.001$) in an indoor heated group of lambs compared to the outdoor group; however, no difference in IgG was reported between the two groups. Hence, further investigations in pregnant ewes during colder winters with the ambient temperature below the critical point would be useful to understand the effect of cold stress on their immune response.

5. Conclusion

Indoor housed ewes had better performance in terms of BCS as it was greater during the fourth week of the study (11%; $p<0.05$) vs. outdoor ewes. However, indoor ewes had higher EPG (76% - 120%, $p<0.05$) for GI nematodes during the second half of the study. Higher levels of reticulocytes (243%, $p<0.05$), MPV (22%, $p<0.05$), globulin (17%, $p<0.01$), and serum chloride (45%, $p<0.001$) were found in indoor ewes compared to outdoor ewes, although these parameters were within the normal range in both groups. Both groups of ewes had satisfactory performance and health status throughout the study period. Results suggested that the rearing system affected the performance and health status of pregnant ewes when raised indoors and outdoors, although temperatures in both systems were in the thermoneutral zone. The provision of outdoor shelters or indoor facilities for pregnant ewes during the extreme cold seems beneficial to minimize performance loss. Further studies are recommended to evaluate the benefits of shelters or indoor facilities for pregnant ewes, especially during the extreme cold.

Acknowledgements

This study was funded by United States Department of Agriculture (USDA), National Institute of Food and Agriculture (NIFA), Capacity Building Grant Number 2019-38821-29041. Authors are thankful to Tuskegee University George Washington Carver Agricultural Experiment Station and Cooperative Extension for providing necessary support to conduct this research.

References

Adam, T. C., Hasson, R. E., Ventura, E. E., Toledo-Corral, C., Le, K. A., Mahurkar, S., Lane, C. J., Weigensberg, M. J., & Goran, M. I. (2010). Cortisol is negatively associated with insulin sensitivity in overweight Latino youth. Journal of Clinical Endocrinology and Metabolism, 95(10), 4729–4735. https://doi.org/10.1210/jc.2010-0322

Animut, G., Goetsch, A. L., Aiken, G. E., Puchala, R., Detweiler, G., Krehbiel, C. R., Merkel, R. C., Sahlu, T., Dawson, L. J., Johnson, Z. B., & Gipson, T. A. (2005). Grazing behavior and energy expenditure by sheep and goats co-grazing grass/forb pastures at three stocking rates. Small Ruminant Research, 59(2), 191–201. https://doi.org/https://doi.org/10.1016/j.smallrumres.2005.05.014

Aydınyılmaz, F., Aksakal, E., Pamukcu, H. E., Aydemir, S., Doğan, R., Saraç, İ., Aydın, S. Ş., Kalkan, K., Gülcü, O., & Tanboğa, İ. H. (2021). Significance of MPV, RDW and PDW with the severity and mortality of COVID-19 and effects of acetylsalicylic acid Use. Clinical and Applied Thrombosis/Hemostasis, 27. https://doi.org/10.1177/10760296211048808

Badran, I., Abuamsha, R., Aref, R., Alqisi, W., & Alumor, J. (2012). Prevalence and diversity of gastrointestinal parasites in small ruminants under two different rearing systems in Jenin district of Palestine. J. Res. (N. Sc.), 26, 1–18.

Berian, S., Gupta, S. K., Ali, S., Dua, S., Ganaie, I., & Kumar, A. (2019). Effect of cold stress on milk yield, physiological and hemato-biochemical profile of cross bred dairy cattle. Journal of Animal Research. https://doi.org/10.30954/2277-940x.02.2019.18

Bhimte, A., Thakur, N., Lakhani, N., Yadav, V., Khare, A., & Lakhani, P. (2018). Endocrine changes in livestock during heat and cold stress. Journal of Pharmacognosy and Phytochemistry, 7(4), 127–132.

Brown, T. T., & Fuller, C. A. (2006). Stress and parasitism of white-footed mice (Peromyscus leucopus) in dry and floodplain environments. Canadian Journal of Zoology, 84(12), 1833–1839. https://doi.org/10.1139/Z06-183

Chikhaoui, M., Smail Fadhèla, S., & Souad, A. (2023). Serum biochemical profile during early, mid, late pregnancy, and calving periods in dairy cows in Algeria. Folia Veterinaria, 67(4), 54–60. https://doi.org/10.2478/fv-2023-0038

Chovanová, F., Tóthová, C., Klein, R., & Nagy, O. (2021). Changes in the serum protein fractions in goats after treatment of natural gastrointestinal parasite infections. Veterinary Medicine International, 2021:9946519. https://doi.org/10.1155/2021/9946519

Coloma-García, W., Mehaba, N., Such, X., Caja, G., & Salama, A. A. K. (2020). Effects of cold exposure on some physiological, productive, and metabolic variables in lactating dairy goats. Animals, 10(12), 1–13. https://doi.org/10.3390/ani10122383

Dargie, J. D., & Allonby, E. W. (1975). Pathophysiology of single and challenge infections of Haemonchus contortus in Merino sheep: Studies on red cell kinetics and the "self-cure" phenomenon. International Journal for Parasitology, 5(2), 147–157. https://doi.org/https://doi.org/10.1016/0020-7519(75)90021-1

Davis, A. K., Maney, D. L., & Maerz, J. C. (2008). The use of leukocyte profiles to measure stress in vertebrates: A review for ecologists. Ecology, 22(5), 760–772. https://doi.org/10.1111/j.1365-2435.2008.01467.x

Delmastro-Greenwood, M. M., & Piganelli, J. D. (2013). Changing the energy of an immune response. Am J Clin Exp Immunol, 2(1), 30–54. www.ajcei.us/ISSN:2164-7712/AJCEI1211002

Dhabhar, F. S. (2009). Enhancing versus suppressive effects of stress on immune function: Implications for immunoprotection and immunopathology. In NeuroImmunoModulation (Vol. 16, Issue 5, pp. 300–317). https://doi.org/10.1159/000216188

Dhabhar, F. S., Miller, A. H., Stein, M., Mcewen, B. S., & Spencer, R. L. (1994). Diurnal and acute stress-induced changes in distribution of peripheral blood leukocyte subpopulations. Brain, Behavior, and Immunity, 8(1), 66–79. https://doi.org/10.1006/brbi.1994.1006

Emery, D. L., Hunt, P. W., & Le Jambre, L. F. (2016). Haemonchus contortus: The then and now, and where to from here? International Journal for Parasitology, 46(12), 755–769. https://doi.org/10.1016/j.ijpara.2016.07.001

Flay, K. J., Hill, F. I., & Muguiro, D. H. (2022). A Review: Haemonchus contortus infection in pasture-based sheep production systems, with a focus on the pathogenesis of anaemia and changes in haematological parameters. Animals, 12(10), 1238. https://doi.org/10.3390/ani12101238

Gordon, C. J. (2012). Thermal physiology of laboratory mice: Defining thermoneutrality. Journal of Thermal Biology, 37(8), 654–685. https://doi.org/10.1016/j.jtherbio.2012.08.004

Guo, H., Zhou, G., Tian, G., Liu, Y., Dong, N., Li, L., Zhang, S., Chai, H., Chen, Y., & Yang, Y. (2021). Changes in rumen microbiota affect metabolites, immune responses and antioxidant enzyme activities of sheep under cold stimulation. Animals, 11(3), 1–15. https://doi.org/10.3390/ani11030712

Hammond, T. T., Hendrickson, C. I., Maxwell, T. L., Petrosky, A. L., Palme, R., Pigage, J. C., & Pigage, H. K. (2019). Host biology and environmental variables differentially predict flea abundances for two rodent hosts in a plague-relevant system. International Journal for Parasitology: Parasites and Wildlife, 9, 174–183. https://doi.org/10.1016/j.ijppaw.2019.04.011

Huang, L., & Appleton, J. A. (2016). Eosinophils in helminth infection: Defenders and dupes. Trends in Parasitology, 32(10), 798–807. https://doi.org/10.1016/j.pt.2016.05.004

Jin, Y. M., Zhang, X. Q., Badgery, W. B., Li, P., & Wu, J. X. (2019). Effects of winter and spring housing on growth performance and blood metabolites of Pengbo semi-wool sheep in Tibet. Asian-Australasian Journal of Animal Sciences, 32(10), 1630–1639. https://doi.org/10.5713/ajas.18.0966

Karki, U. (2020). 178 Use of silvopastures and woodlands to expand the grazing opportunity for small ruminants. Journal of Animal Science, 98(Supplement_2), 67–68. https://doi.org/10.1093/jas/skz397.157

Kim, W.-S., Ghassemi Nejad, J., & Lee, H.-G. (2023). Impact of cold stress on physiological, endocrinological, immunological, metabolic, and behavioral changes of beef cattle at different stages of growth. Animals, 13(6), 1073. https://doi.org/10.3390/ani13061073

Kołodziej-Sobocińska, M., Demiaszkiewicz, A. W., Pyziel, A. M., Marczuk, B., & Kowalczyk, R. (2016). Does the blood-sucking nematode Ashworthius sidemi (Trichostrongylidae) cause deterioration of blood parameters in European bison (Bison bonasus)? European Journal of Wildlife Research, 62(6), 781–785. https://doi.org/10.1007/s10344-016-1037-6

Kovacs, C. S., & Kronenberg, H. M. (1997). Maternal-fetal calcium and bone metabolism during pregnancy, puerperium, and lactation. Endocrine Reviews, 18(6), 832–872. https://doi.org/10.1210/edrv.18.6.0319

Kratz, A., Lewandrowski, K. B., Siegel, A. J., Chun, K. Y., Flood, J. G., Van Cott, E. M., & Lee-Lewandrowski, E. (2002). Effect of marathon running on hematologic and biochemical laboratory parameters, including cardiac markers. Clinical Chemistry / LABORATORY RESULTS IN MARATHON RUNNERS 856 Am J Clin Pathol, 118, 856–863. https://doi.org/10.1092/14TY2TDJ1X0Y1V6V

Lejeune, A., Monahan, F. J., Moloney, A. P., Earley, B., Black, A. D., Campion, D. P., Englishby, T., Reilly, P., O'Doherty, J., & Sweeney, T. (2010). Peripheral and gastrointestinal immune systems of healthy cattle raised outdoors at pasture or indoors on a concentrate-based ration. BMC Veterinary Research, 6. https://doi.org/10.1186/1746-6148-6-19

Lindsay, W. R., Wapstra, E., Silverin, B., & Olsson, M. (2016). Corticosterone: A costly mediator of signal honesty in sand lizards. Ecology and Evolution, 6(20), 7451–7461. https://doi.org/10.1002/ece3.2318

Liu, P., Yao, R., Shi, H., Liu, Y., Lian, S., Yang, Y., Yang, H., & Li, S. (2019). Effects of cold-inducible RNA-binding protein (CIRP) on liver glycolysis during acute cold exposure in C57BL/6 mice. International Journal of Molecular Sciences, 20(6). https://doi.org/10.3390/ijms20061470

Louch, C., Meyer, R. K., & Emlen, J. T. (1953). Effect of stress on diurnal fluctuations in eosinophils of the laboratory mouse. Proceedings of the Society for Experimental Biology and Medicine, 82(4), 668–671. https://doi.org/10.3181/00379727-82-20213

Masselli, E., Pozzi, G., Vaccarezza, M., Mirandola, P., Galli, D., Vitale, M., Carubbi, C., & Gobbi, G. (2020). ROS in platelet biology: Functional aspects and methodological insights. International Journal of Molecular Sciences, 21(14), 1–35. https://doi.org/10.3390/ijms21144866

Merck Animal Health. (2014). Guide to internal parasites of ruminants.

Nagy, O., Tóthová, C., Klein, R., & Chovanová, F. (2020). The electrophoretic pattern of serum proteins in sheep with naturally acquired gastrointestinal nematode infections. Acta Veterinaria, 70(3), 316–328. https://doi.org/10.2478/acve-2020-0024

Nazifi, S., Saeb, M., & Ghavami, S. M. (2002). Serum lipid profile in Iranian fat-tailed sheep in late pregnancy, at parturition and during the post-parturition period. Journal of Veterinary Medicine Series A, 49, 9–12. https://doi.org/10.1046/j.1439-0442.2002.00405.x

Ngetich, E. C., Ngeiywa, M., Ng'wena, G. M., & Chemwolo, L. (2019). Haematological parameters by age and sex of asymptomatic indigenous cattle and sheep infected with gastrointestinal parasites in Kerio valley, Kenya. American Journal of Biomedical Research, 7(2), 44–50. https://doi.org/10.12691/ajbr-7-2-4

Olson, D. P., Papasian, C. J., & Ritter, R. C. (1980). The effects of cold stress on neonatal calves. II. Absorption of colostral immunoglobulins. Can. J. Comp. Med., 44(1), 19–23.

Osuji, P. O. (1974). The physiology of eating and the energy expenditure of the ruminant at pasture. Journal of Range Management, 27, 437–443. https://doi.org/10.2307/3896717

Petersson, K. (2014). Improving small ruminant parasite control in New England. https://www.youtube.com/watch?v=I5rcuvVG56Q.

Piirsalu, P., Kaart, T., Nutt, I., Marcone, G., & Arney, D. (2020). The effect of climate parameters on sheep preferences for outdoors or indoors at low

ambient temperatures. Animals, 10(6), 1–11. https://doi.org/10.3390/ani10061029

Prado, R. O. F., Morales, B. J. E., García, M. L. J., Molina-Ochoa, J., Valpuesta, S. G., Hernández, R. J. A., & García, C. A. C. (2019). Effects of exercise on cations/anions in blood serum of English Thoroughbred horses. Arquivo Brasileiro de Medicina Veterinaria e Zootecnia, 71(3), 909–916. https://doi.org/10.1590/1678-4162-10830

Romeo, C., Wauters, L. A., Santicchia, F., Dantzer, B., Palme, R., Martinoli, A., & Ferrari, N. (2020). Complex relationships between physiological stress and endoparasite infections in natural populations. Current Zoology, 66(5), 449–457. https://doi.org/10.1093/cz/zoaa029

Sawasaki, N., Iwase, S., & Mano, T. (2001). Effect of skin sympathetic response to local or systemic cold exposure on thermoregulatory functions in humans. Autonomic Neuroscience, 87(2), 274–281. https://doi.org/https://doi.org/10.1016/S1566-0702(00)00253-8

Schmidt, E. M. dos S., Fachiolli, D. F., de Oliveira, R. M., Almeida, F. A., Pariz, C. M., de Lima Meirelles, P. R., Costa, C., Tvarijonaviciute, A., Erel, O., Neselioglu, S., Ceron, J. J., & Rubio, C. P. (2021). Changes in serum thiol-disulphide homeostasis in sheep with gastrointestinal nematodes. Animals, 11(10). https://doi.org/10.3390/ani11102856

Shi, L., Xu, Y., Jin, X., Wang, Z., Mao, C., Guo, S., Yan, S., & Shi, B. (2022). Influence of cold environments on growth, antioxidant status, immunity and expression of related genes in lambs. Animals, 12(19), 2535. https://doi.org/10.3390/ani12192535

Shida, A., Ikeda, T., Tani, N., Morioka, F., Aoki, Y., Ikeda, K., Watanabe, M., & Ishikawa, T. (2020). Cortisol levels after cold exposure are independent of adrenocorticotropic hormone stimulation. PLoS ONE, 15(2). https://doi.org/10.1371/journal.pone.0218910

Simpson, J. (n.d.). A fact sheet for the Canadian sheep and goat industries Haemonchus contortus Fact Sheet.

So-In, C., & Sunthamala, N. (2023). Influence of goat management systems on hematological, oxidative stress profiles, and parasitic gastrointestinal infection. Veterinary World, 16(3), 483–490. https://doi.org/10.14202/vetworld.2023.483-490

USDA. (1997). Livestock Nutrition, Husbandry, and Behavior. In National range and pasture handbook.

Varanis, L. F. M., Oliveira, K. A., Araújo, C. M., da CRUZ, W. F. G., & Macedo Júnior, G. de L. (2021). Serum biochemical reference ranges for pregnant sheep. Bioscience Journal, 37. https://doi.org/10.14393/BJ-v37n0a2021-47695

Wang, S., Li, Q., Peng, J., & Niu, H. (2023). Effects of long-term cold stress on growth performance, behavior, physiological parameters, and energy metabolism in growing beef cattle. Animals, 13(10), 1619. https://doi.org/10.3390/ani13101619

Wu, J., Kensiski, A., & Li, L. (2022). Cold stress-regulated immune responses: Insights, challenges, and perspectives. Frigid Zone Medicine, 2(3), 135–137. https://doi.org/10.2478/fzm-2022-0019

Young, B. A. (1981). Cold stress as it affects animal production. Journal of Animal Science, 52(1), 154–163. https://doi.org/10.2527/jas1981.521154x

Zhang, X. Q., Kemp, D., Hou, X. Y., Langford, C. M., Wang, K., & Yan, W. H. (2016). Effects of shed modifications on ewe reproductive performance and lamb growth rate in Inner Mongolia. Rangeland Journal, 38(5), 479–487. https://doi.org/10.1071/RJ15127

Zhang, X. Q., Luo, H. L., Hou, X. Y., Badgery, W. B., Zhang, Y. J., & Jiang, C. (2014). Effect of restricted time at pasture and indoor supplementation on ingestive behaviour, dry matter intake and weight gain of growing lambs. Livestock Science, 167, 137–143. https://doi.org/https://doi.org/10.1016/j.livsci.2014.06.001