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Hierarchical cluster analysis: A statistical tool to study sheep behavior under heat stress

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Abstract

Sheep farming, and the income generated from this endeavor, contributes significantly to the global economy and rural livelihoods. Therefore, it is vital to maintain the productivity of this industry in the face of changing climate patterns. In the context of sheep farming, animals are exposed to a higher heat load as global temperatures are increasing, leading to heat stress. This heat stress conditions can adversely affect animal productivity and welfare resulting in reduced feed intake, physiological (panting, higher respiration, higher heart rate, etc.) and behavioral changes (lying down, standing, seeking shade, etc.) to compensate for increased heat load ultimately leading to lowered productivity. Considering this, it is important to monitor sheep behavior in order to implement improved management practices to compensate for changes in climate. The current short study investigated sheep behavior at different times throughout the day. The data generated was analyzed using the hierarchical cluster analysis method in order to integrate all variables into a single representative dendrogram that could summarize sheep behavior. While hierarchical cluster analysis has been applied in diverse scientific fields, as far as we know, the statistical application reported here is novel in the context of sheep behavior in response to climate change. We studied sheep behavior throughout the day (9:00 – 9:50; 10:00 – 10:50; 11:00 – 11:50; 14:00 – 14:50; 15:00 – 15:50; 16:00 – 16:50). Shade was provided in the form of trees. The following indicators were recorded over a period of twelve months at two week intervals: number of animals eating grass, ruminating and resting. The statistical evaluations undertaken resulted in the generation of a dendrogram which integrated all evaluated variables to categorize the behaviors undertaken at different times during the day. The dendrogram indicated three groupings of sheep behavior that were distinctly different from each other. The analysis shown here indicates that the use of hierarchical cluster analysis culminating in the construction of a dendrogram can effectively synthesize large datasets to outline similar relationships (in this study, this was in the context of observed behaviors). This statistical method applied to sheep physiological studies may help interpret experimental data in the context of climatic change.

Keywords: Animal physiological stress; Biostatistics; Climate change; Heat stress; Ovis aries

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1. Introduction

Agriculture and livestock farming are facing significant challenges due to global changes in climatic conditions. The adverse effects of global warming are being experienced across the world resulting in not only increased ambient temperatures but also changing patterns in rainfall, drought, etc. These adverse effects are being accelerated by anthropogenic activities with one of the causal agents being increased emissions in greenhouse gases. In order to ensure that productivity remains stable despite changing climate, scenarios need to be investigated to determine the effect of adverse effects, for example, increased temperatures, on livestock and plant production. In the context of livestock, the demand for meat and associated products has shown an increasing trend. In the context of sheep farming, there has been a 7% increase in production between 2006 and 2016 [1], thereby highlighting the importance of the industry globally. Farming with sheep contributes not only to the Gross Domestic Product of countries but also supports rural livelihoods.

In the commercial sector, farming is undertaken with domesticated sheep with breeds being developed over time with improved, desirable traits. In Africa and Asia, indigenous sheep breeds are also found which display adaptations to survive under harsh environmental conditions, marginal lands, etc. Both indigenous and domesticated sheep represent valuable Animal Genetic Resources (AGR) and the Food and Agricultural Organization (FAO) has highlighted concerns surrounding the loss of this germplasm. Hence, recommendations have been made to conserve the genetic resources of sheep and other farm animals not only in developed countries but also in developing regions by incorporating phenotypic and molecular characterization [2].

As mentioned above, conservation of sheep genetic resources is critical to ensure that a diverse gene pool is available to have access to existing breeds, or develop new ones, that can tolerate emerging pests, diseases and altered environmental conditions [2, 3, 4, 5, 6]. This is particularly important when the effects of climate change (increasing temperature) are considered in the context of sheep farming. Whether in the commercial, smallholder or rural context, generally sheep are grazing animals that feed in pastures. While grazing, sheep are exposed to the elements ranging from the harsh, cold European conditions to the hot arid conditions in the deserts of Africa. Climate change is considered to be one of the most significant challenges facing livestock farmers. Increased ambient temperature results greater exposure to solar radiation and possibly increased relative humidity) making it difficult for animals to regulate energy, thermal, water, hormonal and mineral balances [7]. When ruminants are exposed to heat stress, these animals will attempt to dissipate the heat load in order to maintain euthermia [8]. However, this can be an energy intensive process. Some breeds are better adapted to prevailing environmental conditions, for example the Najdi breed of domestic sheep in the deserts of Saudi Arabia [8]. It has been reported that the thermal comfort range for sheep is from 15 – 30 °C with an upper critical limit of 35 °C [9]. Sheep respond to stresses such as high temperatures through behavioral or physiological mechanisms (body temperature, respiration rate, blood serum changes, etc.) or a combination of both [8, 10]. Observation of sheep behavior provides an early indication of animal welfare. Behavioral attributes that are commonly recorded include standing time, feeding behavior (e.g. frequency), time spent lying down, shade seeking behavior, etc. [10]. Hence, the current study investigated the behavioral response of sheep at intervals throughout the day.

The data generated from many scientific experiments are usually statistically analyzed using parametric or non-parametric tests to determine if there are statistically significant differences between means. Depending on the experimental design, the statistical analysis will be either uni-variate or multi-variate with post-hoc tests included to specify where the differences lie [11, 12, 13, 14, 15]. However, the abovementioned tests present some weaknesses when large datasets are considered with numerous measurements over time. Therefore, we investigated the application of hierarchical cluster analysis for data collected from trials investigating sheep behavior in response to heat stress. Cluster analysis is a useful technique that allows for the synthesis of large datasets into manageable groupings. Essentially, cluster analysis enables data reduction that results in summarizing data into manageable subgroups [16]. When data is analyzed through cluster analysis, dependent and independent variables are not distinguished from each other, instead all relationships are examined collectively. In hierarchical cluster analysis, many clusters are combined in a sequential manner with a reduction in clusters at each step until one cluster remains. Clustering occurs based on dissimilarities (or distances) between samples. The Euclidean distance is one of such cluster analysis method [16]. Hierarchical cluster analysis has been applied in a wide range of fields, however, to our knowledge; the statistical application reported here has not been frequently utilized in studies of sheep behavior in the context of climate change.

The present study involved recording of behavioral indicators of sheep exposed to full solar radiation or some shade in the form of trees. The following behaviors were monitored: number of animals eating grass, ruminating and resting. Shaded conditions were provided by trees so sheep had the option to seek shade. The behaviors were monitored every 10 min from 9:00 to 11:50 and 14:00 to 16:50 on specific days, i.e. on two days each month over a period of 12 months (i.e. on a total of 24 days) The collected data were statistically analyzed with integrated variables to evaluate the most significant effects of heat stress on sheep behavior. This data was subsequently used to generate a dendrogram for analysis of behavioral patterns.

2. Material and methods

For the current study, female sheep of the Pelibuey race were used [17]. This breed is common in the Caribbean, Mexico and South America. It is raised primarily for meat since it is a breed of hair sheep that does not usually grow wool. Healthy sheep that were two to four years old (at reproductive growth stage) were used. Each ach animal weighed approximately 35 to 40 kg. Observations of twelve sheep were made over a period of 12 months (from July 2018 to June 2019). Sheep had access to shade from trees provided by 10 adult trees of Casuarina esquisetifolia and five of Samanea saman. A series of behavioral indicators was recorded, viz. the number of animals eating, ruminating and resting. Sheep were monitored at 10 minute intervals from 9:00 – 11:50 and 14:00 – 16:50 on two days each month over the course of one year. Observations were made visually as described by Czacko [18]. When the trial was not in progress (17:00 – 08:50; 12:00 – 13:50), all animals remained in pens. While animals were confined, a source of water and feed was provided (mixture of Pennisetum purpureum (50%), Saccharum officinarum (50%) and sodium chloride).

The trial was conducted at the "La Orlinda" farm in Ciego de Avila, Cuba (21 52´48.6´´ N, 78 41´32.6´´ W; 53 meters above sea level). The soil was a typical, lixiviated, yellow and quarzitic ferralytic type. Average min-max ambient temperature conditions ranged from 19.7oC to 33.4oC with relative humidity between 72 and 97% and 600-800 mm of rainfall per annum [19]. During the trial, sheep grazed on 2 ha of land. The main grasses available were Paspalum notatum (45% coverage), Bothriochloa pertusa and Dichanthium caricosum (with a combined coverage of 40%). The remaining 15% was comprised of Sporobolus indicus and Sida rhombifolia.

All data generated were statistically analyzed using SPSS (Version 8.0 for Windows, SPSS Inc., New York, NY) to perform Analysis of Variance (ANOVA) and Tukey tests ($p<0.05$). Moreover, to identify the most significant integrated effects of heat stress on sheep behavior, hierarchical cluster analysis was performed.

Data were standardized with 0 to 1 by the min-max normalization method [20]. Following identification of the minimum and maximum values for each variable, a formula was applied to standardize the data: (Value to be normalized – Min value observed in the experiment) / (Max value observed in the experiment – Min value observed in the experiment). The dendrogram was generated using average linkage (between groups). Intergroup bonding was used for the clustering method with the square Euclidean distance being calculated.

3. Results and discussion

The current work evaluated the behavioral attributes of Pelibuey sheep over a twelve month period. The primary activities monitored were actively eating, ruminating or resting. These activities were observed at intervals throughout the day (from 9:00 to 16:50) on two days each month. A summary of the results are presented in Fig. 1 A-C which indicate the number of animals eating, ruminating or resting at different times during the day. Sheep were able to seek cover from direct solar radiation by accessing the shaded conditions (and consequent reduced temperatures) during the day. Overall, the results showed that the preferred activity of animals during the day was eating since sheep spent most of the day engaging in this activity (Fig. 1 A) rather than ruminating (Fig. 1B) or resting (Fig. 1C). In terms of eating behavior, the results showed that the times when the greatest proportion of sheep was eating was from 9:00 to 10:50 and again from 16:00 to 16:50 (Fig. 1A). During the warmest part of the day, i.e. from midday to early afternoon, a fewer number of sheep were engaged in eating. This observation might be related to thermoregulatory considerations. It has been reported that livestock typically respond to increased temperature by reducing food intake as an adaptive response to heat stress [21]. This is because ruminal fermentation results in the production of heat and since feeding ultimately generates heat load in ruminants. Reducing feeding will contribute towards reducing the heat load during warm conditions [10, 21, 22, 23]. As expected, the highest proportion of sheep was resting during the hottest part of the day (Fig. 1C). Other researchers have also investigated sheep behavior for a range of reasons, for example, thermoregulatory and physiological responses of sheep to increased temperature [8], sheep behavior in a semi-arid tropical environment [24], cyclic heat stress on physiological and blood characteristics [25]. The current study contributes to this growing body of knowledge and investigates a breed not evaluated in the abovementioned studies.

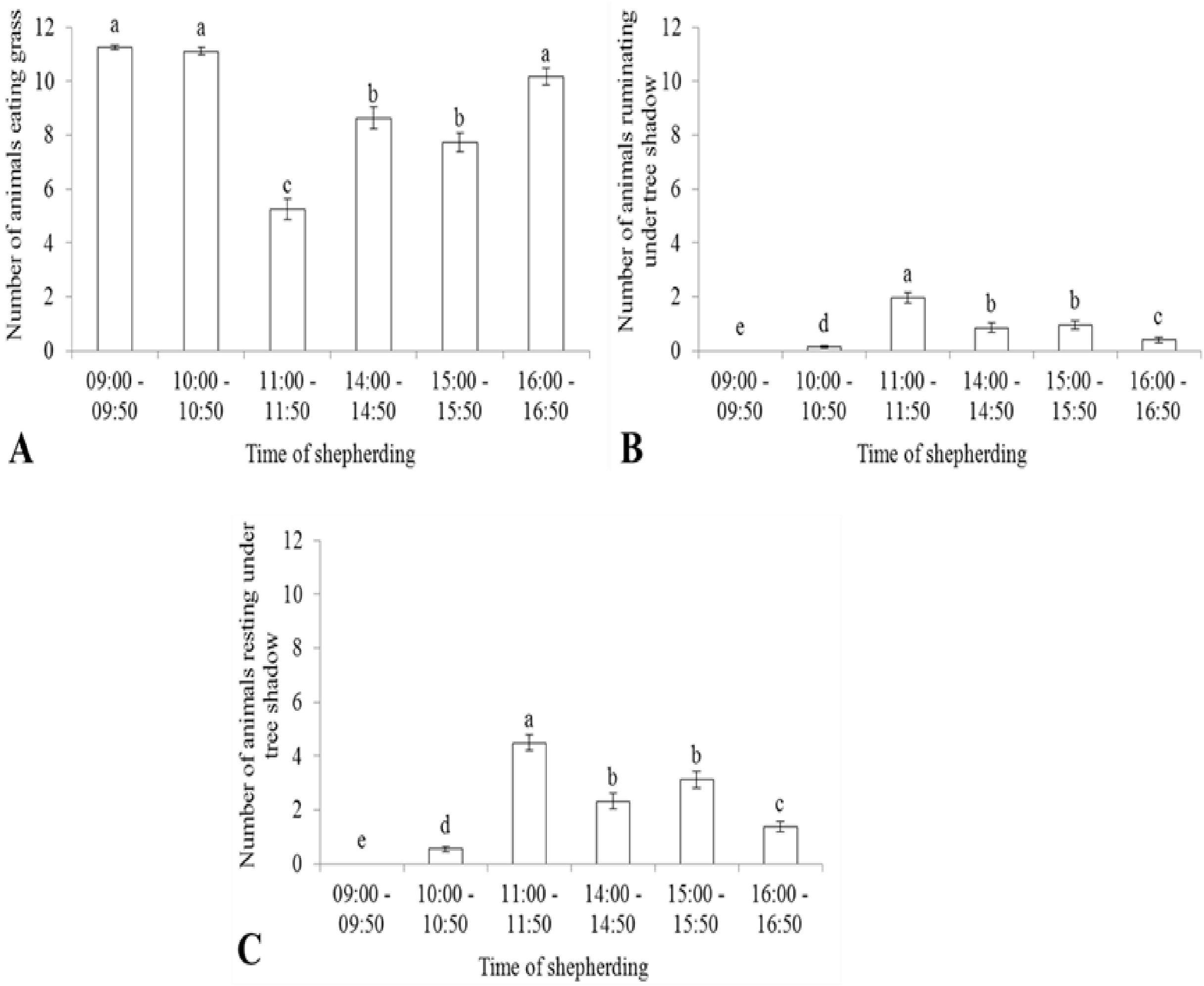

Figure 1 Effect of the time of shepherding on the numbers of sheep eating grass (A), ruminating under tree shadow (B) and resting under tree shadow (C). Results with the same *letter* are not statistically different (One-Way ANOVA, $p>0.05$). Vertical bars represent SE. Sheep rarely ruminated or rested under sun radiation (data not shown).

In this study, it was observed that sheep preferred shadow to ruminate and rest. This shade seeking behavior is not unexpected as animals attempt to regulate body temperatures during the day. As mentioned above, these two activities have less importance to animals compared with eating, at least during daylight hours (Fig. 1). For the initial statistical analysis, each dependent variable was analyzed separately, giving insight into the behavioral responses of sheep induced by environmental temperature at different times throughout the day. However, this method of analysis has weaknesses. For example, the contribution of experimental noise and the sensitivity of individual measurements can influence the analysis which will ultimately lead to challenges in using such methods to rank the treatments in terms of severity. Considering this, a method is needed that can overcome such weaknesses by using a more holistic analysis technique. In order to achieve this, we combined all data collected and generated a dendrogram to integrate results (Fig. 2).

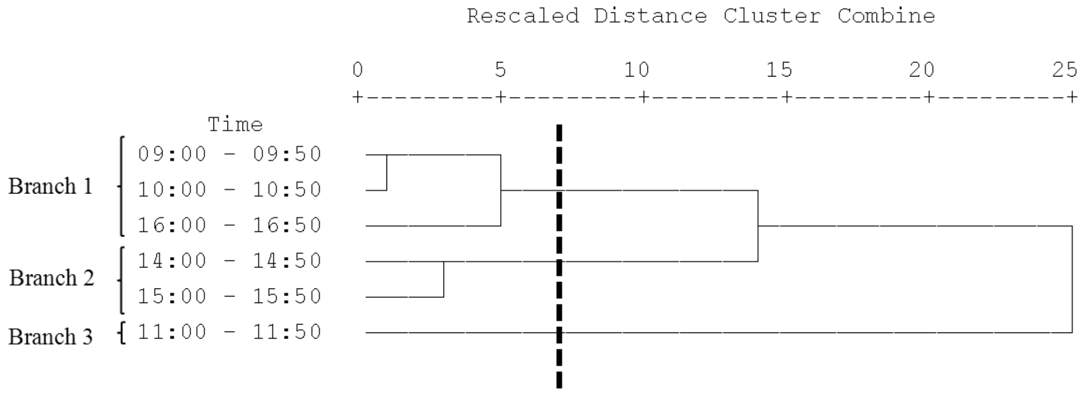

Figure 2 Hierarchical cluster analysis using measurements shown in Fig. 1. The dendrogram was built using average linkage (between groups). Variables were standardized to vary from 0 to 1 according to Kantardzic (2003). Clustering method: intergroup bonding. Measurement: square Euclidean distance. This dendrogram indicates that from 11:00 to 11:50 (branch 3), sheep behaviour is remarkably different from the other periods of shepherding. Branch 1 groups the shepherding times between 9:00 and 10:50, and between 16:00 and 16:50 which are fresher. Sheep behaviour is intermediate between 14:00 and 15:50 (branch 2). Heavy dotted line indicates separation of the three branches of the dendrogram.

Before the hierarchical cluster analysis can be performed the variables first need to be standardized to ensure that distance determinations are not influenced by large numerical values [20, 26]. Once this is done, the hierarchical cluster analysis can be performed and a dendrogram can be produced. A dendrogram provides a graphical illustration of hierarchical relationships between samples. Dendrograms are a useful output to indicate how objects cluster together following hierarchical analysis [27, 28]. It summarizes large datasets into meaningful groupings showing relationships between the measured parameters.

In the current study, hierarchical cluster analysis was performed using the data generated in the study. The dendrogram was constructed using average linkage between groups (where the distance between two groups was defined as the average distance between the objects in the first and second groups). At each successive step during clustering, the two clusters with the smallest linkage distance were linked together. The method used for clustering was intergroup bonding and the square Euclidean distance was applied. The horizontal axis of the dendrogram (Fig. 2) represents the distance or dissimilarity between clusters [29]. Evaluation of the dendrogram indicates three groupings of behavior observed throughout the day (indicated by branch 1 – 3). The dendrogram shows that from 11:00 to 11:50, sheep behavior is remarkably different from the other periods (Fig. 2, branch 3). During this time, the fewest number of sheep are engaged in actively eating while the highest numbers are ruminating and resting (Fig. 1). This coincides with the warmest part of the day where sheep might be using energy to regulate body temperature (through physiological and behavioral) means to tolerate elevated temperatures. Sheep engage in a number of behaviors to lower body temperature. For example, one of the primary ways sheep dissipate heat load during warm temperatures is by panting leading to the evaporation of moisture from the respiratory tract [21]. Sheep behavior between 9:00 and 10:50, and 16:00 and 16:50 (branch 1) are similar (early morning and later in the afternoon). At these times the highest numbers of sheep are eating and the fewest number are ruminating or resting. Hence, during this period sheep are expending energy to promote feed intake during cooler times in the day. Sheep behavior is intermediate between 14:00 and 15:50 (branch 2) where an intermediate number of sheep were eating, ruminating and resting. Hence, the hierarchical cluster analysis allowed for the separation of behaviors into three discrete categories indicated by branches 1, 2 and 3 which was not apparent from the analysis performed in Figure 1.

As discussed earlier, dendrograms have been widely used in other scientific fields, particularly in investigations of molecular characterization. In this regard, the genetic diversity of sheep has been studied and the relationships presented in dendrograms [30, 31, 32, 33]. However, to our knowledge this method has not been used to integrate sheep response parameters to heat stress. The analysis shown here (Fig. 2) indicates that the use of the hierarchical cluster analysis could contribute to establishing a more *integrated* evaluation of behavior. It can also be used as a non-dimensional indicator when comparing different environmental conditions. Ultimately, this type of analysis using behavioral and other data can be used to improve management practices (such as provision of shade, feed supplementation, etc.) which are especially relevant in attempts to promote sustainable livestock production in the face of changing climate patterns.

4. Conclusion

This statistical method applied to sheep physiological studies may help interpret experimental data in the context of climatic change.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

Authors do not have any competing interests.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study. Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

Author's contributions

JOS, AV, FDMM, JMM, CM, AB, EH, NFF and JCL designed the research; JOS, AV, FDMM and JMM conducted the experiment; JOS, JMM, CM, AB, EH, NFF and JCL analyzed the data and wrote the paper; and JOS and JCL had primary responsibility for the final content. All authors have read and approved the final manuscript.

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