Impacts of short-term water restriction on Pelibuey sheep: physiological and blood parameters

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Abstract
One of the projected effects of climate change is a reduction in rainfall in certain regions of the world. Hence, the agricultural and livestock sectors will have to cope with increasing incidences of water shortage while still maintaining productivity levels to feed an ever increasing global population. This short communication reports on the effect of a 2-week water stress on Pelibuey sheep in Cuba. Three treatments were compared, viz. supply of water ad libitum, water supplied once every 3 or 6 days. Following exposure to the water stress, the results showed no changes in sheep body weight or rectal temperature. However, respiration frequency was affected with water stress causing a reduction from 23.3 to 13.3 respirations per min in control and water-deprived animals, respectively. Furthermore, there was evidence for hemoconcentration in response to water stress (levels of hemoglobin increased from 9.2 to 13.1 g L\(^{-1}\) and hematocrits from 27.6 to 39.3% in the control group and animals restricted to water once every 6 days. The imposed water stress was also evident in the reduction of lymphocytes (from ±63 to 43%), and in increase of neutrophils (from approximately 38 to 54%) and leukocytes (from 3133 to 4933 per mm\(^3\)). The results indicated a decline in the levels of antioxidants, i.e., SOD from approximately 13 to 10 U mg\(^{-1}\) protein and CAT activity from 23 to 9 U mg\(^{-1}\) protein. To the best of our knowledge, this is the first report on the response of Pelibuey sheep to short-term water shortage stress under Cuban environmental conditions.

Keywords Animal physiological stress · Climate change · Ovis aries · Water stress

Introduction
Livestock farming contributes towards global food production to feed an ever increasing population. On the other hand, climate change is driven primarily by the release of greenhouse gases (GHG) into the environment which leads to global warming (IPCC 2013). The impacts of changing climate patterns on the livestock sector will have a dual effect on both animal nutrition and well-being. In the context of the former, the availability and quality of feed, forage, and water will be influenced by increasing temperatures, the predicted rise in carbon dioxide (CO\(_2\)) levels, and alterations in rainfall patterns while animal reproduction and health (including disease transmission) will also be affected by similar factors (Rojas-Downing et al. 2017; Escarcha et al. 2018). As a result, a self-perpetuating cycle is created where livestock farming accelerates climate change which subsequently has adverse effects on animal production. Hence, it is imperative that appropriate management strategies are implemented to ensure efficient food production while preserving the environment and natural resources (Wright et al. 2012; de Sousa Oliveira et al. 2020; Ocak Yetisgin and Şen 2020).

Water is essential for life, and animal welfare is strongly dependent on a stable supply of this resource (Serrano et al.
In the context of livestock farming, it is known that sheep and goats display greater resilience to unfavorable environmental conditions than cattle (Louw 1984; Silanikove and Koluman 2015). Sheep, being ruminants like goats, can tolerate the loss of more than 20% of body water (Jaber et al. 2004); however, the imposition of this stress has implications on animal performance and the maintenance of homeostasis (Abdelatif et al. 1994; Alamer and Al-Hozab 2004). Furthermore, as much of sheep farming is undertaken in arid and semi-arid regions of the world, indigenous species have the ability to out-perform conventional breeds under these adverse conditions (Silanikove 1992). In the present study, Pelibuey sheep were used; this species has become domesticated in Cuba and is also reared in other regions of the Caribbean and Mexico (Gutiérrez et al. 2005; Porter et al. 2016).

Livestock farming contributes towards food production but also requires a significant input of water to achieve this (Serrano et al. 2021a). In the current scenario of constrained water resources with predictions of further declines in the future, it is imperative to consider the impacts of a reduction in water supply on high input sectors. Therefore, it is critical to undertake research to identify vulnerabilities in current production systems as well as opportunities that would enable farmers to develop and implement strategies that would allow for an appropriate response to adverse effects to ensure sustainability of the livestock sector (Leite et al. 2019).

The present contribution reports on the effect of a short-term (2 weeks) water stress on Pelibuey sheep. The physiological responses of animals were recorded as well as evaluation of blood chemistry. To the best of our knowledge, this is the first report on the response of Pelibuey sheep to short-term water shortage stress under Cuban conditions.

Materials and methods

The trial was undertaken at the Field Experimental Station of the Bioplant Centre, University of Ciego de Ávila, Cuba (21°52′48.6″ N, 78°41′32.6″ W; 53 m above sea level) on Ferralitic-red soil. The animals used in the trial were young male Pelibuey sheep in good health that were 5 to 6 months old with a mass of between 12 and 17 kg. For exposure to the water stress, three levels of supply were compared: (1) water supplied ad libitum; (2) water supplied for 4 h (h); and water supplied for 4 h (8:00–12:00) every 3 days (days 1, 4, 7, 10, and 13); and water supplied for 4 h (8:00–12:00) every 6 days (days 1, 7, and 13). There were three replicates of three animals for each tested treatment (9 animals per treatment) and evaluations were recorded on the last day of the trial (day 14).

For the duration of the trial, animals were confined in pens with shade provided and with a living space of 2 m² allocated per animal. Sheep were provided with ground forage comprised of Pennisetum purpureum and Saccharum officinarum (1:1, v:v) with 2.5 kg of fresh mass supplied to each animal daily. In order to ensure that all animals were fed at the same time, linear feeders were used (at a distance of 50 cm from the front of the feeder per animal). For the provision of water, animals in each treatment had access to individual drinkers. Ambient temperatures were between 19.7 and 33.4 °C, with a relative humidity of 72 to 97% (during May 2021).

A range of physiological indicators were measured to assess the response of sheep to the reduced water supply. This included body weight (Alfa Laval Agri, 5 kg ± 1 g), body weight increase per day [calculated as (final weight – initial weight)/14 days]; rectal temperature recorded at 14:00 h (Sharma et al. 2013; Cerqueira et al. 2016); respiration frequency (Casamassima et al. 2016); and biochemical markers, i.e., levels of hemoglobin, hematocrits, lymphocytes, neutrophils, leukocytes, soluble proteins, and specific activities of superoxide dismutase (SOD) and catalase (CAT) in blood samples. For the measurement of blood parameters, samples were taken by venipuncture (between 8:30 and 9:00) in a tube with an anticoagulant [(20 mmol L⁻¹ ethylenediaminetetraacetic acid (EDTA)] to assess the hematology of the blood (Huerta Aragonés and Cela de Julián 2018).

To measure levels of soluble proteins, SOD- and CAT-specific activities, an aliquot (1.5 mL) of the blood sample supplemented with anti-coagulant was centrifuged at 20 000 ×g for 5 min at 4°C (Heat Force®, Neofuge 15R). The supernatant was discarded and the precipitate was washed three times with isotonic saline serum [0.9% NaCl (w:v)]. The precipitated red blood cells were diluted (1:4, v:v) with distilled water. The diluted samples were frozen at −6 °C for 30 min and then re-warmed to obtain hemolyzed samples which were stored at 2 °C until further analysis. To measure levels of soluble proteins and activities of enzymes, hemolyzed samples were supplemented with 50 mM KH₂PO₄ - KOH [pH 7.1; 1:500 (v:v)].

Levels of soluble proteins were determined according to the Bradford method (1976). A 20-μL aliquot of sample was supplemented with 1000 μL of Bradford reagent and the absorbance was measured at 595 nm following a 20-min incubation period. Bovine serum albumin (fraction V) was used as a standard.

The CAT (EC 1.11.1.6)-specific activity was measured according to Aebi (1984). One milliliter of red blood cells and 0.5 mL of H₂O₂ (15 mM) were combined in a quartz cuvette and the decomposition of H₂O₂ was evaluated at 240 nm and at 25 °C for 30 s. The CAT activity was determined using the molar extinction coefficient of H₂O₂ (ε = 0.0436 μM⁻¹ cm⁻¹). A unit of enzymatic activity was defined as the amount of catalase that degraded 1 μM of H₂O₂ per min.

The SOD (EC 1.15.1.1)-specific activity was determined by the inhibition of the auto-oxidation of pyrogallol...
(Marklund and Marklund 1974). In this method, 400 μL of red blood cells was added to a glass cuvette; thereafter, 530 μL of TRIS-HCl (50 mM, pH 8.2) and 70 μL of bovine liver catalase (0.3 U/mL, Sigma-Aldrich) were added. The reaction was initiated by the addition of 0.13 mM pyrogallol (Sigma-Aldrich). The reaction was permitted to proceed at 25 °C for 90 s and thereafter the decomposition kinetics of pyrogallol at 420 nm was measured for 60 s. A unit of superoxide dismutase was defined as the amount of enzyme capable of reducing the auto-oxidation of pyrogallol by 50%.

All data collected were statistically analyzed using SPSS (Version 8.0 for Windows, SPSS Inc., New York). Kolmogorov-Smirnov and Levene tests were performed to check normal distribution of data and homogeneity of variances, respectively. Analysis of variance (ANOVA) and Tukey tests (p < 0.05) were performed to identify significant differences between treatments.

**Results**

The present contribution reports on the response of Pelibuey sheep to the imposition of a 2-week water stress. The results showed that there was no discernible change in the body weight of animals in response to water restriction (Fig. 1a). However, water restriction reduced body weight increase per day from 0.052 to 0.012 kg day⁻¹ (Fig. 1b). The temperature of animals remained consistent irrespective of the amount of water consumed (Fig. 1c). However, the respiratory rate was found to be the significantly highest in animals that had constant access to water, intermediate in those supplied with water every 3 days, and the significantly lowest in those having access to the least amount of water (i.e., once every 6 days) (Fig. 1d).

When the blood parameters were measured, it was found that the levels of hemoglobin and hematocrit were significantly highest in those animals supplied with water every 6 days (Fig. 2a, b) while the levels of these were similar in the other two treatments. Overall, the amount of hemoglobin in blood increased from 9.2 g L⁻¹ in sheep supplied with regular access to water and those watered every 3 days up to 13.1 g L⁻¹ in animals that had water every 6 days. Similarly, hematocrit levels rose from 27.6% (in sheep exposed to no water limitation and those receiving water every 3 days) to 39.3% in those supplied with the least amount of water. In contrast, lymphocytes (Fig. 2c) were highest in sheep without water restrictions (63%) and lowest in sheep that were exposed to limited water supply (approximately 43%). The trend observed in neutrophils (Fig. 2d) and leukocytes (Fig. 2e) were similar as all animals that faced water restrictions displayed significantly higher levels for both measurements (±54% for neutrophils and 4933 leukocytes per mm³) than the control treatment (38% for neutrophils and 3133 leukocytes per mm³).

The level of proteins in the blood was significantly highest in sheep supplied with water every 3 days (Fig. 3a; 237 mg mL⁻¹) while there was no difference in the remaining two treatments. When antioxidants in blood were considered, the ad libitum treatment resulted in the highest levels of SOD (Fig. 3b) and CAT (Fig. 3c) specific activity (13 and 23 U mg⁻¹ protein, respectively) compared with animals with restricted water which displayed similarly low levels.
Discussion

The restriction of water in the diet of animals could translate into reduce blood circulation, impede the exchange of nutrients, and restrict basal metabolism, with a consequent reduction in energy production. These results are supported by Al-Ramamneh et al. (2012), Ghassemi and Sung (2017), and Vosooghi-Postindoz et al. (2018) in studies on water restriction, salinity, and ruminal digestibility in sheep. In the current trial, it was observed that the respiratory rate of sheep declined with the imposition of increasing levels of water stress (Fig. 1d from 23.3 to 13.3 respirations per min in control and water stressed animals,
respectively). This could suggest a thermoregulatory adaptation to cope with water stress, as discussed above.

The blood chemistry of animals can also be influenced by environmental variables and therefore is routinely measured to follow the progression of the physiological responses of animals to stresses (Okoruw a 2014; Al-Tamimi et al. 2019). It is noted that the average hemoglobin levels of sheep exposed to limited water supply and the control group, from our study were within the normal reference ranges of 9 to 16 g L−1 (Wang et al. 2015; Ahmadi-hamedani et al. 2016). Sejian et al. (2017) highlighted that determinations of hematocrit (packed cell volume) and hemoglobin levels provide good indications of thermo tolerance in sheep as these factors correlate with the body water balance. In the current study, hemoglobin and hematocrit levels displayed a similar trend — they were stable in the control and 3-day water-restricted treatments but were elevated with further water stress (Fig. 2a and b). Hemoconcentration (i.e., increased hemoglobin and packed cell volume) has been identified as a consequence of water stress in sheep (Abdelatif et al. 1994; Ghanem et al. 2008). However, others have reported conflicting results, e.g., Igboke (1993) and Jaber et al. (2004). It has been suggested that the observation of increased hemoglobin levels might be as a result of reduced plasma volume as a direct consequence of water loss (Kaliber et al. 2016; Kumar et al. 2016; Ay and Ulutas 2020). The maintenance of plasma volume levels might be indicative of an adaptive response by animals to tolerate stress (Sneddon 1993) and the inability to maintain levels could suggest some degree of stress. Interestingly, the phenomena of hemoconcentration and increase in hemoglobin and hematocrit levels have also been noted under conditions of heat stress. It has been suggested that the increase in blood hemoglobin levels may be a consequence of the higher oxygen demand (Okoruw a 2014; Al-Dawood 2017; Ribeiro et al. 2018; Vicente-Pérez et al. 2018). One hypothesis is that the mobilization of fluids into the circulatory system causes hemoconcentration due to the loss of water through evaporative means (Kumar et al. 2016), similar to the hemoconcentration that occurs when there is restriction of drinking water.

Leukocytes are commonly measured as an indicator of stress in vertebrates (Davis et al. 2008). Leukocytosis (describes an increase in the overall number of white blood cells) has been used to infer a stress response (Ots et al. 1998). However, this must be interpreted with caution as while this parameter can indicate whether an animal has been exposed to a comparatively lesser or greater degree of stress, it is not necessarily indicative of the ability of the animal to mount an immune response (Davis et al. 2008). In terms of an immune response, a reduction in leukocyte numbers is typical (Dhabhar 2002). In the current trial, an increase in leukocytes was observed in response to water stress (Fig 2e). It has been reported that changes associated with stress conditions include an increase in the prevalence of neutrophils (neutrophilia) and a decrease in the lymphocytes (lymphopenia or lymphocytopenia) (Davis et al. 2008). This was apparent in the current work where lymphocyte levels in water-deprived animals were reduced relative to the control. The opposite trend was observed for neutrophils. This suggests that the imposition of water stress was apparent in the blood chemistry of sheep. The observation of leukocytosis might also be linked the concurrent decrease in SOD activity as this enzyme has been reported to play an important role in defense against the toxicity caused by leukocytopenia that appears in infections associated with the generation of free radicals (Flores and Medina 2013). Donia et al. (2014) also observed lower serum activity of SOD in pneumonic sheep.

Some researchers have reported an increase in protein levels in response to water stress in sheep, e.g., Jaber et al. (2004), Nejad et al. (2014), Casamassima et al. (2016), and Vosooghi-Postindoz et al. (2018). This was attributed to hemoconcentration due to water loss (Casamassima et al. 2016). The observed results were inconsistent in the present study since blood protein levels increased in sheep supplied with water every 3 days but declined to levels similar to the control in those supplied with water every 6 days (Fig. 3a).

It has been well established that reactive oxygen species (ROS) are produced in response to stress conditions. Animals possess a suite of antioxidant systems which allow for the quenching of ROS (Halliwell and Gutteridge 2007). When ROS are produced in excess (or are not adequately quenched), oxidative damage results leading to membrane deterioration, protein, DNA and RNA denaturation, etc. (Mujahid et al. 2007). Superoxide dismutase (SOD) and catalase (CAT) are two antioxidants. Superoxide dismutase catalyzes the breakdown of the oxygen radical leading to the generation of $\text{H}_2\text{O}_2$, which is subsequently decomposed by CAT (Ghosh and Deb 2014). The current study investigated the effect of short-term water stress on the antioxidant capacity of sheep. The results indicated a decline in the levels of both antioxidants (SOD and CAT) when animals were exposed to water limitation. The effect was more pronounced in the case of catalase (Fig 3e). There has been limited work on the effect of water stress on antioxidant systems in animals as more effort has been focused on heat stress. For example, it has been shown that thermal stress reduced the activity of SOD and CAT in broiler chickens (Zhang et al. 2015). Furthermore, Shi et al. (2020) demonstrated that heat stress resulted in reduced serum levels of CAT in lambs following 28 days of stress. However, it must be highlighted that the short duration of the water stress imposed in the present study could have been insufficient to trigger protection from antioxidant mechanisms (Rathwa et al. 2017). Furthermore, sheep have other adaptive mechanisms that largely mitigate against the negative effects of limiting factors such as reduced plasma volume due to water stress. In sheep, there is a restriction of drinking water.
as lack of water. In addition, reduced SOD activity (Fig. 3b) could also be related to a deficiency of minerals that act as cofactors for the SOD enzyme (Flores et al. 2011). Even in animals under grazing conditions, serum SOD activity can be used as an indicator for the nutritional metabolic balance of certain elements such as copper, manganese, and iron (Flores et al. 2011). However, the increase in body weight per day was affected (Fig. 1b), which is related to an impairment of consumption and consequently a decrease in weight gain. This was similar to that described by Akinmoladun et al. (2019) and Ay and Ulutas (2020), which refer to the influence of the type of food and the association of heat stress.

This study reports on the imposition of short-term water stress in Pelibuey sheep. This type of research is critical in order to develop meaningful strategies to protect livestock production in the face of changing climate patterns. The short- and long-term consequences of water scarcity on sheep well-being and productivity during different life cycle stages will have ultimately have impacts on the longevity of this important sector in years to come.

Author contribution JOS, AVG, NCH, AGM, LPB, LH, GL, EH, NFF, JMM, and JCL designed the research; JOS, AVG, NCH, AGM, LPB, LH, and GL conducted the experiment; JOS, EH, NFF, JMM, and JCL analyzed the data and wrote the paper; and JOS, JMM, EH, and JCL had primary responsibility for the final content.

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Availability of data and material Not applicable.

Declarations

Ethics approval and consent to participate 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.

Ethics approval The Ethical Committee that provides licenses for experiments on animals approved the animal experimentations.

Consent for publication All authors have read and approved the final manuscript.

Competing interests The authors declare no competing interests.

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