Direct heat stress-induced effects on rumen fermentation characteristics and nutrients degradability in sheep pair-fed alfalfa hay

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Abstract

**Aim of study:** To investigate the direct effect of heat stress on rumen fermentation characteristics and nutrients degradability of pair-fed rams to subsequently eliminate the confounding effects of dissimilar feed intake induced by heat stress exposure.

**Area of study:** Saudi Arabia.

**Material and methods:** Five rumen-cannulated desert rams (45 ± 1.63 kg body weight; 2–3 years of age) were placed individually in controlled climatic-chambers to be exposed to two successive periods. The 1st period was a control thermoneutral period (TN; 23.64 ± 0.14 ºC; extended for 21 days) followed by a 2nd period of heat stress (HS; 44.26 ± 1.70 ºC, for another 21 days). Each period was consisted of a temperature acclimation phase (7 days) and a data collection phase (14 days). Alfalfa hay was offered twice daily during both periods in a pair-fed manner (800 g DM per head and day).

**Main results:** Exposing pair-fed desert rams to elevated ambient temperature had ($p<0.05$) elevated their respiration rate and skin temperature, without noticeable ($p>0.05$) changes in their rectal temperature. Most of the rumen fermentation characteristics and nutrients degradability were not affected by HS ($p>0.05$). However, exposure to HS increased ($p<0.05$) pre-feeding rumen total volatile fatty acids concentrations, pre-feeding molar proportion of acetate, and post-feeding rumen osmolality.

**Research highlights:** HS had no direct effect on post-feeding rumen fermentation characteristics and nutrients degradability in desert sheep. This implies that strategic approaches to mitigate the adverse effects of HS have to be directed towards promoting feed intake and nutrients utilization under such conditions.

**Additional key words:** body temperature; feed intake; hot climate; rumen ecosystem.

**Abbreviations used:** CF (crude fiber); CP (crude protein); DM (dry matter); HS (heat stress); OM (organic matter); RH (relative humidity); RR (respiration rate); $T_a$ (ambient temperature); THI (temperature humidity index); TN (thermoneutral); $T_r$ (rectal temperature); $T_s$ (skin temperature); VFAs (volatile fatty acids).

**Authors’ contributions:** Conceived and designed the experiment: HSMA, KAA and AAAH. Performed the experiments: HSMA, EMS, ABO and MAAB. Analyzed the data: HMA, KAA and EMS. Wrote the paper: KAA, HSMA, EMS and AAAH.

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**Funding agencies/institutions**

<table>
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<th>Project / Grant</th>
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<td>Deanship of Scientific Research at King Saud University</td>
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**Introduction**

Sheep are forced to induce several physiological changes when exposed to environmental challenges, which include decrease in feed intake and disturbance of nutrients metabolism (Rhoads et al., 2009; Wheelock et al., 2010; Adedeji, 2012; Wojtas et al., 2014), as well as reduction in blood flow to the rumen epithelium, thereby lowering ruminal motility, reducing saliva production and decreasing rumen pH (Kadzere et al., 2002; Yadav et al., 2012; Das et al., 2016). These changes collectively create a less favorable environment for rumen microbes to properly function. Consequently, ruminal microbial fermentation products, which are the primary energy
and protein source for the host (Nocek & Russel, 1988; Bergman, 1990) are likely to be adversely affected, and thus hampering the productivity and performance of the animal.

Despite the fact that the effect of adverse environmental temperatures on rumen fermentation have been extensively characterized in recent years, results of these studies were inconsistent. Reduced, elevated or unaltered volatile fatty acids (VFAs) production as a result of elevated environmental temperatures have all been reported in several studies (Martz et al., 1990; Kadzere et al., 2002; Tajima et al., 2007; Nonaka et al., 2008; Bernabucci et al., 2010; Salles et al., 2010; Chaidanya et al., 2017). These discrepancies can be attributed to the difference in feeding procedures and the types of ration provided (Gauly et al., 2013), as well as the variation in feed intake induced by heat stress (HS). Available data associated with the effect of HS on rumen fermentation are mostly sourced from studies that have used concentrate-based diets (Chaidanya et al., 2017). Even though high-forage diets are extensively used in the feeding of small ruminants in large parts of the world (Saro et al., 2014), less attention has been paid to the effect of HS on forage fermentation. Some reports showed that increasing rumen ammonia N concentrations could be induced under hot environmental conditions (Hall, 2009; Salles et al., 2010; King et al., 2011). These studies have relied on the estimation of rumen ammonia N as an indicator of higher ruminal protein degradation. In the present study, both ruminal fluid ammonia N content and in situ ruminal degradation of nutrients were measured. Besides, most conventional HS experiments have not been conducted under controlled feed intake conditions (O’Brien et al., 2010), which complicates differentiating between the direct and indirect (mediated by reduced feed intake) effects of HS. Therefore, animals herein were subjected to pair-feeding in order to eliminate confounding results of dissimilar feed intake (FI) induced by the exposure of HS. Accordingly, rams were offered similar amount of alfalfa hay [800 g dry matter (DM) per head and day]. Such amount of offered feed was estimated according to the recommended maintenance energy requirements for sheep (95 kcal/kg maintenance energy requirements for sheep (95 kcal/kg) by INRA (1978) as well as a pilot study performed before conducting this study. On the other hand, rams have a continuous access to fresh water and vitamins/minerals blocks. The present study was carried out in accordance with the current laws on animal welfare and research in Saudi Arabia as well as the standards established by the Faculty Research Ethics Committee at King Saud University (Ethics Reference No: KSU-20-66).

Study measurements

During the data collection phase (14 days), several measurements were recorded, measured, and/or estimated. Ambient temperature (T<sub>a</sub>) and relative humidity (RH%) inside the chamber were recorded continuously throughout the study at 30 min intervals by two data loggers (HOBO Pro-Series data logger, Model H08-032-08, Onset Comp, USA) fixed approx. 2 m above the animals. A special data logging software (Box-Car Pro 4, Onset Comp, USA) was used for programming the loggers as well as for data retrieval. The collected T<sub>a</sub> and RH data

Material and methods

Animals, management, and study design

Five rumen-cannulated desert rams were housed individually at the Animal Research Station affiliated to the Department of Animal Production, King Saud University, Riyadh, Saudi Arabia (24°48′20.9″N 46°31′14.2″E). Rams were 2–3 years old, with a mean body mass of 45 ± 1.63 kg, and had flabby ears, thick necks with a dewlap, long-legs, fat tails, and a brown hairy coat.

The present study was extended for 42 days and preceded by 14 days of acclimation to the environment, feed, and human handling. Controlled climatic-chambers were used to expose the rams to 2 successive periods. The 1<sup>st</sup> period was a control thermoneutral period (TN; 23.64 ± 0.14 °C; extended for 21 days) followed by a 2<sup>nd</sup> period of heat stress (HS; 44.26 ± 1.70 °C; for another 21 days). Each period was consisted of a temperature acclimation phase (7 days) and a data collection phase (14 days). However, the HS period was designed to simulate the natural conditions during summer, where the ambient temperature inside the controlled climatic-chamber was set to rise daily at 10:00 h from its basal TN level (~24°C) to reach its maximum (~45°C) at 12:00 h, and then maintained at this level until 14:00h. Thereafter, the temperature was slowly decreased to reach 29°C at 18:00 h, before it gradually returned back to its basal TN level at 20:00 h.

Regarding the feed, alfalfa hay was offered twice daily (8:00 and 14:00 h) during both periods. The hay contained on DM basis; 17.60 % crude protein, 1.60 % ether extract, 29.40 % crude fiber, 9.60 % ash, and 2.10 Mcal/kg metabolizable energy. It is worthwhile to mention that feed was offered in a pair-fed manner to eliminate the confounding effects of dissimilar feed intake (FI) induced by the exposure of HS. Accordingly, rams were offered similar amount of alfalfa hay [800 g dry matter (DM) per head and day]. Such amount of offered feed was estimated according to the recommended maintenance energy requirements for sheep (95 kcal/kg<sup>0.75</sup>) by INRA (1978) as well as a pilot study performed before conducting this study. On the other hand, rams have a continuous access to fresh water and vitamins/minerals blocks. The present study was carried out in accordance with the current laws on animal welfare and research in Saudi Arabia as well as the standards established by the Faculty Research Ethics Committee at King Saud University (Ethics Reference No: KSU-20-66).
were used thereafter to calculate the temperature humidity index (THI) according to LPHSI (1990) using the following formula: \[ \text{THI} = T_a - [(0.55 - 0.55 \times \text{RH}) \times (T_a - 58)] \], where \( T_a \) is the ambient temperature in degrees Fahrenheit and \( \text{RH} \) is the relative humidity as a fraction of unit.

Meanwhile, several physiological responses were measured in duplicate from each ram at 14:00 h during both study periods at two consecutive days (i.e. day 8 and 9 of the data collection phase). Respiration rate (RR, breaths/min) was measured by counting the number of flank movements during 60 sec. Then, rectal temperature (\( T_r \), °C) was measured using a calibrated digital thermometer (measures to the nearest 0.10 °C) by gently inserting it 10 cm inside the rectum and attaching it to the interior wall until a fixed reading was obtained. Meanwhile, a calibrated infrared thermometer (Traceable Mini IR™ Thermometer, Friendswood, TX, USA) was used to measure skin temperature (\( T_s \)) in shaved areas at right shoulder and hip regions.

On days 10 of both periods, on the other hand, rumen contents were collected from each animal before feeding (07:30 h) and 4 hours after feeding (12:00 h). Samples of the rumen contents were taken through the fistula using a tube attached to a vacuum pump. The collected rumen contents were then strained through 4 layers of cheesecloth. The pH of the rumen fluid was immediately measured using an electronic pH electrode, and rumen fluid osmolality was determined in duplicate samples using an Osmometer (VAPRO pressure osmometer, model 5600, South Logan, USA). Thereafter, 8 mL of filtered rumen fluid was transferred to acid-resistant plastic test tubes containing 2 mL of 1 N sulfuric acid and stored at -20 °C until measurement of VFAs and ammonia N contents. The rumen fluid samples were analyzed for their total and molar proportions of individual VFAs (acetic, propionic and butyric) using gas chromatography (Nukol, SupelcoTM WSAF-2 Mix from SUPELCO Co., Bellefonte, PA, USA), while rumen fluid ammonia N concentration was determined according to the procedure described by Smith & Murphy (1993).

Furthermore, these rams were used to assess ruminal nutrients degradability during both periods. In situ procedure was performed as described by Mehrrez & Ørskov (1977) along three consecutive days (i.e. day 12 to 14 of the data collection phase). Nylon bags 15 × 10 cm in size with a pore size of 45 ± 10 µm were used. Accurately weighed (5 g) feed samples (alfalfa hay) were transferred into each nylon bag, which were then carefully sealed and placed into the ventral sac of the rumen of each individual rumen-cannulated ram. Feed samples in the nylon bags were incubated for periods of 3, 6, 12, 24, 48, and 72 hours. After removal of the nylon bags at the end of each incubation period, the nylon bags were washed with cold tap water until no further color appeared. Feed sample residues in the nylon bags were then dried at 65°C for 48 hours, and weighed to determine their dry matter (DM) content. Duplicates of individually weighed feed sample residues were then pooled by incubation period to analyze organic matter (OM), crude fiber (CF) and crude protein (CP) contents, and potential degradability (PD) of DM, OM, CF and CP was then determined according to the exponential model adopted by McDonald (1981). Alfalfa hay and feed samples residues were chemically analyzed for DM, OM, CF, CP, ether extract and total ash content according to AOAC (1997).

### Statistical analyses

Recorded, measured, and estimated data during the study were all analyzed as a completely randomized design using the PROC GLM procedure of statistical analysis system (SAS, 2009) to determine the differences in all the variables as a function of the fixed effect of the treatment and the random effect of the animal. The PROC MEANS procedure was also used to obtain the descriptive statistics of all parameters. Thereafter, data were subjected to ANOVA using \( \alpha = 0.05 \), and mean differences were elaborated using the PDIFF option. The probability value, which denotes statistical significance, was set at \( p<0.05 \).

### Results

The overall means of all meteorological measurements (\( T_a, \text{RH}, \text{and THI} \)) in both TN and HS periods are presented in Table 1, while the average daily variations in \( T_a \) and THI during both periods are presented in Fig. 1. The obtained results indicate the overall means of \( T_a \) and THI were (\( p<0.05 \)) higher, while the overall mean of RH was (\( p<0.05 \)) lower during HS period compared to TN period. The average calculated THI for HS period was 86.97 ± 0.98, suggesting that desert rams were exposed to severe heat stress during this period.

The overall means of measured RR and \( T_a \) was (\( p<0.05 \)) increased in rams exposed to HS compared to TN, while \( T_a \) did not differ (\( p>0.05 \)) in these rams (Table 2). Additionally, chromatographic analysis of rumen fluid samples revealed that HS did not (\( p>0.05 \)) change the concentrations of total VFAs, the molar proportions of acetate, propionate and butyrate, and the acetate to propionate ratio as determined 4 hours after feeding compared to TN. However, total VFAs concentration and molar proportions of acetate measured before feeding were higher (\( p<0.05 \)) during HS compared to TN period (Table 3). Similarly, rumen fluid ammonia N concentration tended to be higher (\( p=0.061 \)) before feeding during HS
study period, but 4 hours after feeding they were almost similar during both periods (Table 3). However, rumen osmolality was increased ($p<0.05$) during HS period after feeding compared to TN period (Table 3). Meanwhile, HS treatment did not induce any change ($p>0.05$) in rumen pH before or after feeding compared to TN (Table 3). Likewise, *in situ* ruminal degradability rates and the calculated potential degradability of DM, OM, CF and CP were not affected ($p>0.05$) during the HS period, where the degradation rates during both study periods were almost similar (Table 4, Fig. 2).

### Discussion

Exposure of sheep to elevated ambient temperature induces an increase of dissipation of excess body heat via panting and sweating, in order to negate the excessive heat load (Silanikove, 2000; Bernabucci *et al.* , 2010). However, when environmental temperature increases above 36 °C, the physiological mechanisms of the animal fail to negate the excessive heat load, resulting in an increase of their body temperature and reduce feed intake, which consequently could evoke a series of drastic changes in rumen functions and subsequently could negatively affect the rumen ecosystem and fermentation end products. Therefore, it is the intention of the current study to investigate the direct effect of heat stress on rumen fermentation characteristics and nutrients degradability of pair-fed rams in order to subsequently eliminate the confounding effects of dissimilar feed intakes induced by the exposure to heat stress.

According to the measured data, rams herein were exposed to high $T_a$ and THI. LPHSI (1990) categorized THI levels for sheep and goats as follows: values<82 = absence of HS; 82 to<84 = moderate HS; 84 to<86 = severe HS and>86 = extreme severe HS. Therefore, it can be assumed that rams were exposed to severe heat stress during HS period. However, because the ability of ruminants to regulate their body temperature is species and breed dependent, it is difficult to define the severity of HS on the...
basis of THI under a particular weather condition (Bernaubucci et al., 2010; Rout et al., 2017). Further, animals interact with their environment in a much more complex manner than is represented by THI alone (Silanikove, 2000), and thus physiological responses are a much better reflection of the degree of HS experienced by animals.

Evaporation (mainly panting) is the most important method of heat dissipation in sheep (Marai et al., 2007; da Silva et al., 2017). The normal resting RR for sheep has been reported to be 20–38 breaths/min, and it can reach above 40 breaths/min when T_a start to reach 26°C (Ames et al., 1971; Silanikove, 2000). The observed increase in RR is likely an attempt to increase heat loss by evaporative cooling, while the increase in T_s may be a result of skin capillary bed vasodilation and the consequent increase in blood flow to the body surface areas to facilitate heat dissipation (Wojtas et al., 2014). Similar changes in RR and T_a to those observed herein have been commonly found by other studies on sheep (Al-Haidary et al., 2012; Ghassemi-Nejad & Sung, 2017; Rathwa et al., 2017). The lack of HS-induced change in T_s herein could be explained by the higher heat tolerance and adaptability to hot environments of desert sheep as previously reported by McLeroy (1961). Moreover, the relatively long time (~14 h) at low T_s during the HS period as well as the observed low RH throughout the study could have largely facilitated the heat dissipation via evaporative cooling. Despite the fact that high T_s occurs when animal’s body fails to maintain its heat balance (Abdel-Hafez, 2002), it has interestingly been reported that sheep can withstand T_s as high as 43°C, relying mainly on panting as thermoregulatory mechanisms if RH is below 65% (Anderson, 1989). Therefore, it seems that rams studied herein have managed to maintain their heat balance and body temperature by increasing their RR and T_a to dissipate excess body heat.

Moreover, the direct effect of heat stress on rumen fermentation indices of pair-fed rams was carried out both before feeding and 4 hours after feeding. HS induced increases in ammonia N, total VFAs concentration, and molar proportions of acetate prior to feeding. This might return to the reduction of rumen motility and the increase of the mean retention time, which might be triggered by the exposure to a hot environment (Robertshaw, 1981). In fact, this subsequently could result in an increase in structural carbohydrates digestibility and protein degradability (Silanikove, 1992; Nonaka et al., 2008). In fact, sheep exposed to HS in the current study tended to exhibit an increased potential degradability of protein (Table 4). Interestingly, HS induced reductions in feed intake have been repeatedly reported (Bernaubucci et al., 2009; Salles et al., 2010; Adedeji, 2012; Baumgard et al., 2012; Baumgard & Rhoads, 2013; Chaidanya et al., 2017), and changes in microbial populations and fermentation patterns have been attributed to variation in feed intake (Kelly et al., 1967; Uyeno et al., 2010). Therefore, the un-altered

### Table 3. Ruminal fermentation indices measured shortly before feeding and 4 hours post-feeding in pair-fed desert rams exposed to different ambient temperature condition.

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Study period</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TN</td>
<td>HS</td>
</tr>
<tr>
<td><strong>Before feeding</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.48</td>
<td>7.38</td>
</tr>
<tr>
<td>NH₃-N (mmol/L)</td>
<td>4.96</td>
<td>7.02</td>
</tr>
<tr>
<td>Osmolality (mosmol/L)</td>
<td>163.20</td>
<td>216.60</td>
</tr>
<tr>
<td><strong>VFAs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (mmol/L)</td>
<td>22.58⁺</td>
<td>32.75⁺</td>
</tr>
<tr>
<td>Acetate (mol/100 mol)</td>
<td>40.20⁺</td>
<td>47.60⁺</td>
</tr>
<tr>
<td>Propionate (mol/100 mol)</td>
<td>18.10</td>
<td>19.60</td>
</tr>
<tr>
<td>Butyrate (mol/100 mol)</td>
<td>16.40</td>
<td>15.90</td>
</tr>
<tr>
<td>Acetate: Propionate</td>
<td>2.67</td>
<td>2.63</td>
</tr>
<tr>
<td><strong>After feeding</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>6.80</td>
<td>6.74</td>
</tr>
<tr>
<td>NH₃-N (mmol/L)</td>
<td>6.68</td>
<td>6.06</td>
</tr>
<tr>
<td>Osmolality (mosmol/L)</td>
<td>174.20⁺</td>
<td>233.20⁺</td>
</tr>
<tr>
<td><strong>VFAs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (mmol/L)</td>
<td>42.16</td>
<td>47.66</td>
</tr>
<tr>
<td>Acetate (mol/100 mol)</td>
<td>48.00</td>
<td>50.40</td>
</tr>
<tr>
<td>Propionate (mol/100 mol)</td>
<td>21.60</td>
<td>22.80</td>
</tr>
<tr>
<td>Butyrate (mol/100 mol)</td>
<td>17.10</td>
<td>15.90</td>
</tr>
<tr>
<td>Acetate: Propionate</td>
<td>2.38</td>
<td>2.38</td>
</tr>
</tbody>
</table>

1VFAs: volatile fatty acids. 2TN, thermoneutral; HS, heat stress. 3SEM, standard error of mean. a,bMeans within the same row bearing different superscripts are significantly different at p<0.05.

### Table 4. Estimated ruminal nutrients potential degradability (%) in pair-fed desert rams during both study periods.

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Study period</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TN</td>
<td>HS</td>
</tr>
<tr>
<td>Dry matter</td>
<td>33.27</td>
<td>41.31</td>
</tr>
<tr>
<td>Organic matter</td>
<td>36.04</td>
<td>35.93</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>12.67</td>
<td>11.10</td>
</tr>
<tr>
<td>Crude protein</td>
<td>43.65</td>
<td>56.52</td>
</tr>
</tbody>
</table>

1TN, thermoneutral; HS, heat stress. 2SEM, standard error of mean.
ruminal fermentation indices observed post-feeding could be attributed to the pair-feeding procedure utilized herein. On the other hand, HS increased ruminal osmolality after feeding with an inclination to be increased before feeding. This could be attributed to the fact that animals lose water through evaporative means when thermoregulatory mechanisms are employed (Marai et al., 2007), which subsequently causes disturbances in electrolyte and fluid balances and forces animals to mobilize water from the rumen to replenish fluid homeostasis (Cain et al., 2006).

In conclusion, exposing pair-fed desert rams to elevated Ta elevated their RR and Tsk; nevertheless, they did not show any noticeable changes in their Tr and most of the tested rumen fermentation parameters and ruminal degradation of feed nutrients. This clearly indicates that there is no direct effect of HS on post-feeding ruminal fermentation indices, and confirms as well that the inconsistency of previous reports might be due to the difference in feeding procedures and could be secondary to the HS-induced reduction in feed intake. Studying the interaction between environmental temperature and rumen function is very complicated; there are many interlinked factors that affect rumen function individually or in combination, hence contributing to this complexity. Therefore, identification of these factors and their potential combinations is paramount. This necessitates a comprehensive detailed study encompassing all aspects of rumen function and HS interaction. The present study actually implies that strategic approaches to mitigate the adverse effects of HS on ruminal microbial fermentation and nutrients degradation have to be directed towards promoting feed intake under such conditions. Nevertheless, there is limited knowledge pertaining HS influence on ruminal microbial degradation of feed nutrients. Therefore, future studies should focus on the changes of ruminal microbial population in response to HS.

Acknowledgement

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