Spanish Journal of Agricultural Research
18 (3), e0609, 8 pages (2020)
eISSN: 2171-9292
https://doi.org/10.5424/sjar/2020183-16247
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)

RESEARCH ARTICLE

OPEN ACCESS

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

Hassan S. Mohamed-Ali (Mohamed-Ali, HS), Khalid A. Abdoun (Abdoun, KA), Emad M. Samara (Samara, EM), Mohamed A. Al-Badwi (Al-Badwi, MA), Aly B. Okab (Okab, AB) and Ahmed A. Al-Haidary (Al-Haidary, AA)

King Saud University, College of Food and Agriculture Sciences, Dept. Animal Production. Riyadh 11451, Saudi Arabia

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_{sk} (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.

Citation: Mohamed-Ali, HS; Abdoun, KA; Samara, EM; Al-Badwi, MA; Okab, AB; Al-Haidary, AA (2020). Direct heat stress-induced effects on rumen fermentation characteristics and nutrients degradability in sheep pair-fed alfalfa hay. Spanish Journal of Agricultural Research, Volume 18, Issue 3, e0609. https://doi.org/10.5424/sjar/2020183-16247

Received: 18 Dec 2019. **Accepted:** 05 Aug 2020.

Funding agencies/institutions	Project / Grant
Deanship of Scientific Research at King Saud University	RGP-VPP-171

Competing interests: The authors have declared that no competing interests exist.

Correspondence should be addressed to Khalid A. Abdoun: kabdoun@ksu.edu.sa

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, which are the main confounding factors responsible for variation in rumen fermentation characteristics under hot environmental conditions. Such researches may improve our understanding of rumen fermentation characteristics under hot climatic conditions and may assist as well in the development of suitable strategies to counteract the potential negative effects of HS.

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 (24º48'20.9"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 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). 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 (~24ºC) to reach its maximum (~45ºC) at 12:00 h, and then maintained at this level until 14:00h. Thereafter, the temperature was slowly decreased to reach 29ºC 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$^{0.75}$) 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_a) 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_a and RH data

were used thereafter to calculate the temperature humidity index (THI) according to LPHSI (1990) using the following formula: THI = T_a – [(0.55 − 0.55 RH) × (T_a − 58)], where T_a is the ambient temperature in degrees Fahrenheit and 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, ºC) was measured using a calibrated digital thermometer (measures to the nearest 0.10 ºC) 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_{sk}) 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 osmomer, 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 Mehrez & 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, RH, 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_{sk} was ($p<0.05$) increased in rams exposed to HS compared to TN, while T_r 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

Table 1. Meteorological data (mean ± SE) inside the controlled climatic-chambers during both study periods.

Study period	Duration	T_a (ºC)	RH (%)	THI
TN (thermoneutral)	21 days	23.64 ± 0.14b	40.74 ± 0.96a	69.12 ± 0.13b
HS (heat stress)	21 days	44.26 ± 1.70a	19.18 ± 1.70b	86.97 ± 1.39a

TN, thermoneutral; HS, heat stress. T_a, ambient temperature; RH, relative humidity; THI, temperature humidity index. [a-b]Means within the same column bearing different superscripts are significantly different at $p<0.05$.

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).

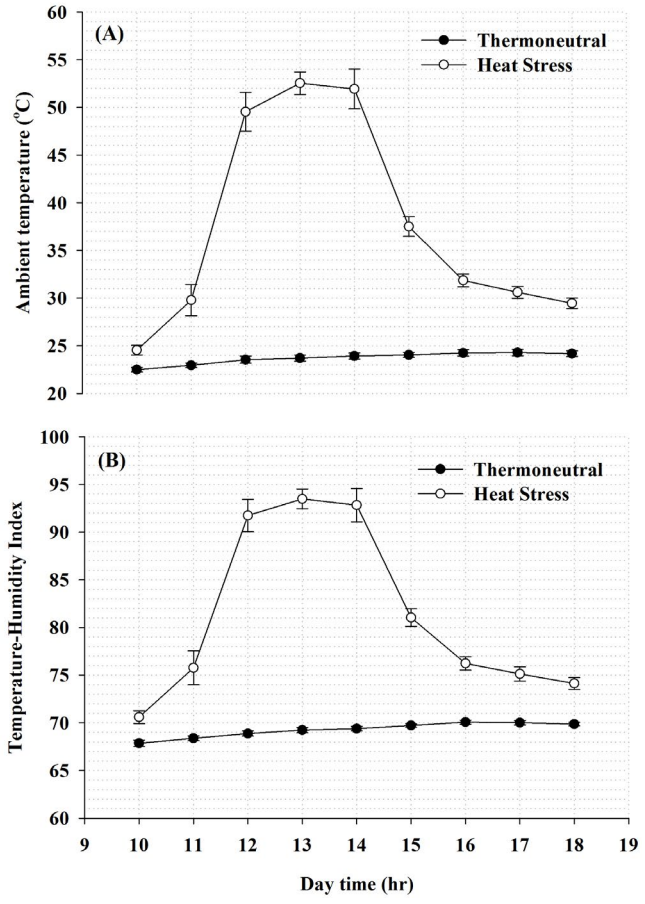

Figure 1. Variations in average ambient temperature (A) and temperature humidity index (B) during the day hours (10:00-18:00 h) of thermoneutral and heat stress periods.

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

Table 2. Physiological responses of desert rams during both study periods.

Measurements[1]	Study period[2]		SEM[3]
	TN	HS	
T_r (ºC)	38.84	38.92	0.17
T_{sk} (ºC)	32.00[b]	37.36[a]	0.19
RR (breaths/min)	21.23[b]	54.13[a]	6.15

[1]T_r, rectal temperature; T_{sk}, skin temperature; RR, respiration rate. [2]TN, thermoneutral; HS, heat stress. [3]SEM, standard error of mean. [a-b]Means within the same row bearing different superscripts are significantly different at $p<0.05$.

basis of THI under a particular weather condition (Bernabucci *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_{sk} 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_{sk} 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_r 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_a 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_r occurs when animal's body fails to maintain its heat balance (Abdel-Hafez, 2002), it has interestingly been reported that sheep can withstand T_a 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_{sk} 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 (Bernabucci *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.

Measurements[1]	Study period[2]		SEM[3]
	TN	HS	
Before feeding			
pH	7.48	7.38	0.86
NH_3-N (mmol/L)	4.96	7.02	0.67
Osmolality (mosmol/L)	163.20	216.60	17.49
VFAs			
Total (mmol/L)	22.58^b	32.75^a	2.28
Acetate (mol/100 mol)	40.20^b	47.60^a	0.80
Propionate (mol/100 mol)	18.10	19.60	0.62
Butyrate (mol/100 mol)	16.40	15.90	1.75
Acetate: Propionate	2.67	2.63	0.21
After feeding			
pH	6.80	6.74	0.10
NH_3-N (mmol/L)	6.68	6.06	0.94
Osmolality (mosmol/L)	174.20^b	233.20^a	17.08
VFAs			
Total (mmol/L)	42.16	47.66	0.28
Acetate (mol/100 mol)	48.00	50.40	1.10
Propionate (mol/100 mol)	21.60	22.80	0.72
Butyrate (mol/100 mol)	17.10	15.90	1.43
Acetate: Propionate	2.38	2.38	0.11

[1]VFAs: volatile fatty acids. [2]TN, thermoneutral; HS, heat stress. [3]SEM, standard error of mean. [a-b]Means 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.

Measurements	Study period[1]		SEM[2]
	TN	HS	
Dry matter	33.27	41.31	3.62
Organic matter	36.04	35.93	1.03
Crude fiber	12.67	11.10	1.54
Crude protein	43.65	56.52	5.37

[1]TN, thermoneutral; HS, heat stress. [2]SEM, standard error of mean.

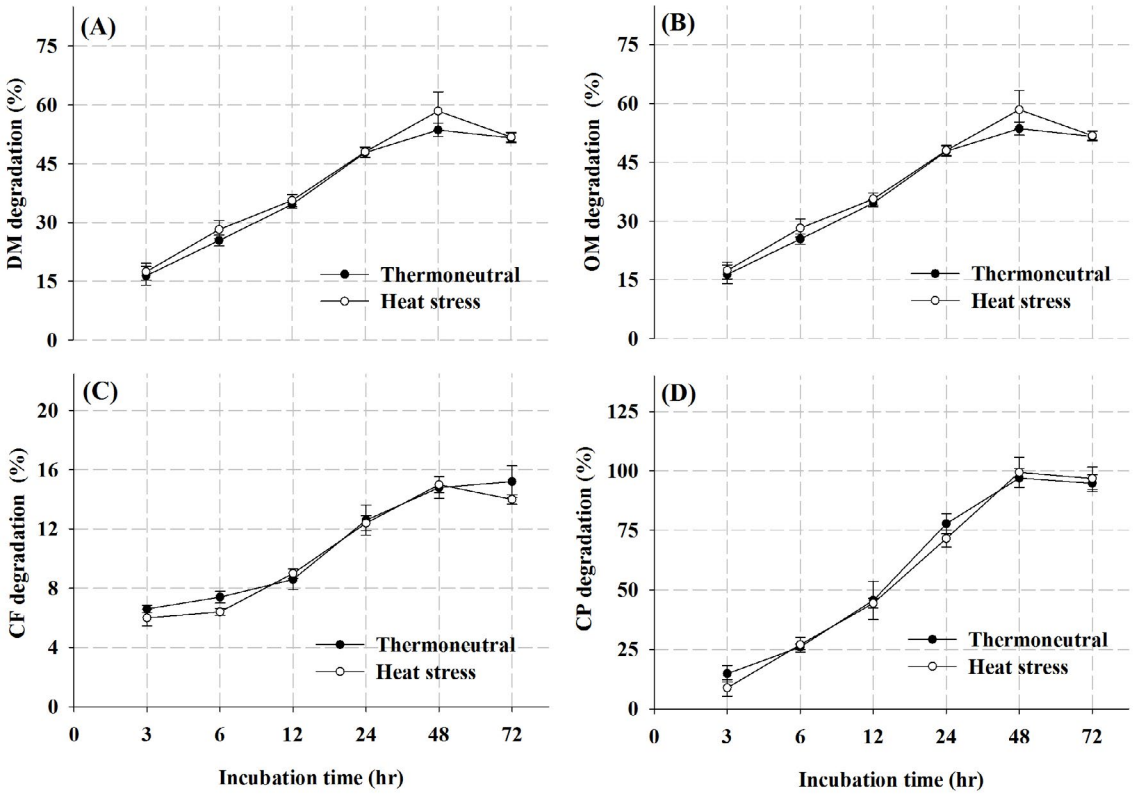

Figure 2. Ruminal degradation kinetics of dry matter (A), organic matter (B), crude fiber (C) and crude protein (D) in rams exposed to thermoneutral and heat stress periods.

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 T_a elevated their RR and T_{sk}; nevertheless, they did not show any noticeable changes in their T_r 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

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding of this research through the Research Group Project Nr. RGP-VPP-171. The authors thank the Deanship of Scientific Research and RSSU at King Saud University for their technical support.

References

Abdel-Hafez MAM, 2002. Studies on the reproductive performance in sheep. Doctoral thesis. Univ. Zagazig, Zagaziig, Egypt.

Adedeji TA, 2012. Effect of some qualitative traits and non-genetic factors on heat tolerance attributes of

extensively reared West African Dwarf (WAD) goats. Int J Appl Agric Res 8: 68-81.

Al-Haidary AA, Aljumaah RS, Alshaikh MA, Abdoun KA, Samara EM, Okab AB, Alfuraiji MM, 2012. Thermoregulatory and physiological responses of Najdi sheep exposed to environmental heat load prevailing in Saudi Arabia. Pak Vet J 32: 515-519.

Ames DR, Nellor JE, Adams T, 1971. Energy balance during heat stress in sheep. J Anim Sci 32: 748-788. https://doi.org/10.2527/jas1971.324784x

Anderson BE, 1989. Temperature regulation and environmental physiology, In: Dukes' physiology of domestic animals, 10th ed; Swenson MJ, (ed.). pp: 719-727. Cornell Univ Press, Ithaca, NY.

AOAC, 1997. Official methods of analysis. Association of Official Analytical Chemists, Washington, DC, USA.

Baumgard LH, Rhoads RP, 2013. Effects of heat stress on post-absorptive metabolism and energetics. Annu Rev Anim Biosci 1: 311-337. https://doi.org/10.1146/annurev-animal-031412-103644

Baumgard LH, Rhoads RP, Rhoads ML, Gabler NK, Ross JW, Keating AF, Boddicker RL, Lenka S, Sejian V, 2012. Impact of climate change on livestock production. In: Environmental stress and amelioration in livestock production; Sejian V *et al.* (eds.). pp: 413-468. Springer Verlag, Berlin. https://doi.org/10.1007/978-3-642-29205-7_15

Bergman EN, 1990. Energy contribution of volatile fatty acids from the gastrointestinal tract in various species. Physiol Revis 70: 567-590. https://doi.org/10.1152/physrev.1990.70.2.567

Bernabucci U, Lacetera N, Danieli PP, Bani P, Nardone A, Ronchi B, 2009. Influence of different periods of exposure to hot environment on rumen function and diet digestibility in sheep. Int J Biometeorol 53: 387-395. https://doi.org/10.1007/s00484-009-0223-6

Bernabucci U, Lacetera N, Baumgard LH, Rhoads RP, Ronchi B, Nardone A, 2010. Metabolic and hormonal acclimation to heat stress in domesticated ruminants. Animal 4: 1167-1183. https://doi.org/10.1017/S175173111000090X

Cain JW, Krausman PR, Rosenstock SS, Turner JC, 2006. Mechanisms of thermoregulation and water balance in desert ungulates. Wildlife Soc Bull 34: 570-581. https://doi.org/10.2193/0091-7648(2006)34[570:MOTAWB]2.0.CO;2

Chaidanya K, Soren NM, Sejian V, Bagath M, Manjunathareddy GB, Kurien E, Varma G, Bhatta R, 2017. Impact of heat stress, nutritional stress and combined (heat and nutritional) stresses on rumen associated fermentation characteristics, histopathology and HSP70 gene expression in goats. J Anim Behav Biometeorol 5: 36-48.

da Silva WE, Leite JHGM, de Sousa JER, Costa WP, da Silva WST, Guilhermino MM, Asensio LAB, Façanha DAE, 2017. Daily rhythmicity of the thermoregulatory responses of locally adapted Brazilian sheep in a semiarid environment. Int J Biometeorol 61: 1221-1231. https://doi.org/10.1007/s00484-016-1300-2

Das R, Sailo L, Verma N, Bharti P, Saikia J, Kumar R, 2016. Impacts of heat stress on health and performance of dairy animals- A review. Vet World 9: 260-268. performance of dairy animals- A review. Vet World 9: 260-268. https://doi.org/10.14202/vetworld.2016.260-268

Gauly M, Bollwen H, Breves G, Brugemann K, Dänicke S, Daş G, Demeler J, Hansen H, Isselstein J, König S, *et al.*, 2013. Future consequences and challenges for dairy cow production systems arising from climate change in Central Europe- A review. Anim Consort 7: 843-859. https://doi.org/10.1017/S1751731112002352

Ghassemi-Nejad J, Sung KI, 2017. Behavioral and physiological changes during heat stress in Corriedale ewes exposed to water deprivation. J Anim Sci Technol 59: 1-6. https://doi.org/10.1186/s40781-017-0140-x

Hall M, 2009. Heat stress alters ruminal fermentation and digesta characteristics, and behavior in lactating dairy cattle. Proc 11th Int Symp of Ruminant Physiology; Chilliard Y *et al.* (eds.). pp: 204-205. Wageningen, Netherlands.

INRA, 1978. Alimentation des ruminants. INRA Publ, Versailles.

Kadzere CT, Murphy MR, Silanikove N, Maltz E, 2002. Heat stress in lactating dairy cows: A review. Livest Prod Sci 77: 59-91. https://doi.org/10.1016/S0301-6226(01)00330-X

Kelly RO, Martz FA, Johsnon HD, 1967. Effects of environmental temperature on ruminal volatile fatty acids levels with controlled feed intake. J Dairy Sci 50: 531-533. https://doi.org/10.3168/jds.S0022-0302(67)87460-5

King CC, Dschaak CM, Eun JS, Fellner V, Young AJ, 2011. Quantitative analysis of microbial fermentation under normal or high ruminal temperature in continuous cultures. Profess Anim Sci 27: 319-327. https://doi.org/10.15232/S1080-7446(15)30495-2

LPHSI, 1990. Livestock and poultry heat stress indices agriculture engineering technology guide. Clemson Univ, SC, USA.

Marai IF, El-Darawany AA, Fadiel A, Abdel-Hafez MAM, 2007. Physiological traits as affected by heat stress in sheep a review. Small Rumin Res 71: 1-12. https://doi.org/10.1016/j.smallrumres.2006.10.003

Martz FA, Payna CP, Matches AG, Belyea RL, Warren WP, 1990. Forage intake ruminal dry matter disappearance and ruminal blood volatile fatty acids for steers in 18 and 32ºC temperatures. J Dairy Sci 73: 1255-1278. https://doi.org/10.3168/jds.S0022-0302(90)78793-0

McDonald I, 1981. A revised model for the estimation of protein degradability in the rumen. J Agric Sci 96: 251-252. https://doi.org/10.1017/S0021859600032081

McLeroy GB, 1961. The sheep of the Sudan: An extensive survey and system of classification. Sudan J Vet Anim Husb 2: 19-35.

Mehrez AZ, Ørskov ER, 1977. A study of artificial fiber bag technique for determining the digestibility of feeds in the rumen. J Agric Sci 88: 645-650. https://doi.org/10.1017/S0021859600037321

Nocek JE, Russell JB, 1988. Protein and energy as an integrated system: relationship of ruminal protein and carbohydrate availability to microbial protein synthesis and milk production. J Dairy Sci 71: 2070-2107. https://doi.org/10.3168/jds.S0022-0302(88)79782-9

Nonaka I, Takusari N, Tajima K, Suzuki T, Higuchi K, Kurihara M, 2008. Effects of high environmental temperatures on physiological and nutritional status of prepubertal Holstein heifers. Livestock Sci 113: 14-23. https://doi.org/10.1016/j.livsci.2007.02.010

O'Brien MD, Rhoads RP, Sanders SR, Duff GC, Baumgard LH, 2010. Metabolic adaptations to heat stress in growing cattle. Domest Anim Endocrinol 38: 86-94. https://doi.org/10.1016/j.domaniend.2009.08.005

Rathwa SD, Vasava AA, Pathan MM, Madhira SP, Patel YG, Pande AM, 2017. Effect of season on physiological, biochemical, hormonal and oxidative stress parameters of indigenous sheep. Vet World 10: 650-654. https://doi.org/10.14202/vetworld.2017.650-654

Rhoads ML, Rhoads RP, Van Baale MJ, Collier RJ, Sanders SR, Waber WJ, Crooker BA, Baumgard LH, 2009. Effects of heat stress and plane of nutrition on lactating Holstein cows: I. Production, metabolism and aspects of circulating somatotropin. J Dairy Sci 92: 1986-1997. https://doi.org/10.3168/jds.2008-1641

Robertshaw D, 1981. The environmental physiology of animal production. In: Environmental physiology aspects of housing for animal production; Clark JA (ed.). pp: 3-17. Butterworths, London. https://doi.org/10.1016/B978-0-408-10688-7.50007-2

Rout PK, Kaushik R, Ramachandran N, Jindal SK, 2017. Identification of heat stress-susceptible and -tolerant phenotypes in goats in semiarid tropics. Anim Prod Sci 58: 1349-1357. https://doi.org/10.1071/AN15818

Salles MSV, Zanetti MA, Salles FA, Titto EAL, Conti RMC, 2010. Changes in ruminal fermentation and mineral serum level in animals kept in high temperature environments. Revis Brasil Zootec 39: 883-890. https://doi.org/10.1590/S1516-35982010000400025

Saro C, Ranilla MJ, Tejido ML, Carro MD, 2014. Influence of forage type in the diet of sheep on rumen microbiota and fermentation characteristics. Livestock Sci 160: 52-59. https://doi.org/10.1016/j.livsci.2013.12.005

SAS, 2009. Statistical analysis system user's guide statistics. SAS Inst. Inc. Cary, NC, USA.

Silanikove N, 1992. Effects of water scarcity and hot environment on appetite and digestion in ruminants, a review. Livestock Prod Sci 30: 175-194. https://doi.org/10.1016/S0301-6226(06)80009-6

Silanikove N, 2000. Effects of heat stress on the welfare of extensively managed domestic ruminants. Livestock Prod Sci 67: 1-8. https://doi.org/10.1016/S0301-6226(00)00162-7

Smith FE, Murphy TA, 1993. Analysis of rumen ammonia & blood urea nitrogen. https://animalscience.unl.edu/Research/RumNut/RumNutLab/84-analysisofrumenammonia.pdf [4 Aug 2020].

Tajima K, Nonaka I, Higuchi K, Takusari N, Kurihara M, Takenaka A, Aminov RI, 2007. Influence of high temperature and humidity on rumen bacterial diversity in Holstein heifers. Anaerobe 13: 57-64. https://doi.org/10.1016/j.anaerobe.2006.12.001

Uyeno Y, Sekiguchi Y, Tajima K, Takenaka A, Kurlahara M, Kamagata Y, 2010. An RNA-based analysis for evaluating the effect of heat stress on the rumen microbial composition of Holstein heifers. Anaerobe 16: 27-33. https://doi.org/10.1016/j.anaerobe.2009.04.006

Wheelock JB, Rhoads RP, Vanbaale MJ, Sanders SR, Baumgard LH, 2010. Effects of heat stress on energetic metabolism in lactating Holstein cows. J Dairy Sci 93: 644-655. https://doi.org/10.3168/jds.2009-2295

Wojtas K, Cwynar P, Kołcz R, 2014. Effect of thermal stress on physiological and blood parameters in merino sheep. Bullet Vet Inst Pulawy 58: 283-288. https://doi.org/10.2478/bvip-2014-0043

Yadav B, Singh G, Wankar A, Dutta N, Verma AK, Chaturvedi VB, 2012. Effect of heat stress on digestibility in crossbred cattle. Proc 8th Biennial Conf of ANAC and Symp on Animal Nutrition Research Strategies for Food Security; Pattaniak AR (ed.). pp: 138. Bikaneer, Rajasthan.