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Diminishing Water-Intake may have Pragmatic Aspects in Lambs Fed on Pelleted Complete Diet and Reared under Thermo-Neutral Condition

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ABSTRACT

Some basic questions regarding sheep's ability to tolerate different levels of water intake (WI), especially when fed on a pelleted-complete diet (PCD) and reared under comfortable climatic conditions, are still open. To investigate the direct influence of different levels of WI in sheep fed PCD and reared under such conditions on the lambs' physiological (performance, thermal, blood and urine) status, 24 healthy male growing Najdi lambs were exposed under thermo-neutral conditions to three levels of WI (100%, 67%, and 33% of their *ad libitum* WI or water requirement) for 6 weeks. Meteorological, production performance, thermo-physiological, as well as blood and urine biochemical measurements, were all determined. The obtained findings clearly substantiate that reducing the level of water intake (p<0.05) produced tangible effects in both 67-WI and 33-WI lamb groups. However, 67-WI lambs showed resilience to limited water availability by inducing proportional physiological responses in their production performances, body temperatures, blood metabolites, and renal function but within the homeostatic ranges similar (p>0.05) to 100-WI lambs. Results collectively signify that diminishing WI up to 33% in lambs (fed on PCD and reared under comfortable conditions) would ensure that water is adequately conserved under prevailing water scarcity and can be implemented without compromising their homeostatic functions. Such applied approaches can consequently have a pragmatic aspect through improving water management approaches required for PCD-based sheep production under water scarcity conditions, which is crucial to economical animal production and responsible animal stewardship. Feasibility studies are thereby highly recommended.

***Keywords:** blood metabolites; homeostasis; renal function; sheep; thermophysiology*

INTRODUCTION

Water is considered one of the essential nutrients needed by animals to maintain homeostasis, digest feeds, excrete urine, produce milk, regulate body temperature, as well as intracellular and molecular transports (NRC, 2007). While the available drinking water for livestock production in arid and semi-arid regions is devastatingly declining, the estimated total water available for absolute consumption across the globe is deemed paltry (around 0.70%, Naqvi *et al.*, 2017). Moreover, climate change and global warming have exacerbated the dilemma of availability, quantity, and quality of freshwater resources for agriculture and livestock production (Sejian, 2013). As one of the major limiting factors of economic productivity, especially in arid and semi-arid regions (Napoli *et al.*, 2016), effective water management is essential for rationalizing water consumption but without adverse effects on livestock production and performance.

Unlike other animals, small ruminants (e.g., sheep and goats) are advantageously able to withstand water stress (in terms of water restriction or deprivation) mainly by evoking several changes in their basic physiological functions, including metabolic disruption of homeostatic balances, loss of appetite, as well as thermal, enzymatic, and hormonal reactions (Al-Ramamneh *et al.*, 2012; Akinmoladun *et al.*, 2019; De *et al.*, 2020). However, the increased usage of high-energy pelleted complete diet (PCD) in the livestock industry (with its low moisture characteristic and prevailing water scarcity ravaging the arid regions) deserves some attention. In a previous study (Adeniji et al., 2020), we evaluated the production performance of lambs subjected to different water restriction levels and fed on PCD. It was strongly evident that lambs could tolerate water restrictions of up to 33% of their daily requirement without compromising their production performance. However, there is an urgent need to identify the precise amount of water reduction that growing lambs can tolerate without compromising their well-being when fed on PCD and rear under thermo-neutral climatic conditions.

This experiment was therefore designed -as a continuum to our previous investigation- to bridge such a knowledge gap by determining the influence of three different water-intake levels on the physiological (performance, thermal, blood and urine) status of lambs, which could subsequently have pragmatic aspects through improving the management approaches required for PCD-based sheep production under water scarcity conditions.

MATERIALS AND METHODS

Location and Ethical Approval

This experiment was conducted at the experimental animal station affiliated with the Department of Animal Production, College of Food and Agriculture Sciences, King Saud University (24°48’20.8”N, 46°31’14.2”E). Animal use for scientific purposes as well as experimental procedures described herein followed the Animal Welfare Practices Act and was approved by the Research Ethics Committee, King Saud University (Process number: KSU-SE-20-18). Several indicators (i.e., skin turgor, clarity of the eyes, and shape and position of the eyes in orbit) were used every day to assess the hydration level of the experimental animals.

Animals and Experimental Design

A total of 24 healthy male growing Najdi lambs with an initial body weight of 35.10±0.24 kg and 4.50 months old were used in a 2-phases experiment. During the 3-week preparatory phase, the experimental animals were weighed, ear tagged, prophylactically treated for internal and external parasites, and individually housed in shaded pens (1.50 × 2.00 m) with concrete floor and cement block side walls. Additionally, the average daily WI of lambs was estimated in this phase to be 4.80 liters/day (calculated out of 6 liters/day offered as an excess of their daily requirement). Meanwhile, during the 6-week experimental phase, lambs were randomly allocated into one of three experimental groups (8 animals per group), namely 100-WI (*ad libitum* WI or 100% of their water requirement), 67-WI (67% of *ad libitum* WI), and 33-WI (33% of *ad libitum* WI). The initial WI was *ad libitum* (for 100-WI group), 3.20 liters (for 67-WI group), and 1.60 liters (for 33-WI group). Still, the offered amount of water was recalibrated weekly based on the lambs’ body weights to compensate for the increment of their water requirements concerning body weight.

On the other hand, lambs were fed throughout the experiment at 4% of their initial body weight on a commercial PCD diet containing 11·30 MJ/kg ME and 13·00% CP on DM basis (ALWAFI-ARASCO, KSA; feed ingredients included: barley, wheat, palm kernel meal, soybean hulls, wheat bran, alfalfa, salt, limestone, molasses, acid buffer, and commercial premix). It is noteworthy that both water and feed were given once at 08:00 hr daily, and lambs’ body weight was taken weekly. Feed intake (FI), average daily gain (ADG), and feed conversion ratio (FCR) were calculated as previously mentioned (Adeniji *et al.,* 2020). In brief, the weights of the provided and refused feed were taken daily to calculate the daily FI using a sensitive-scale measure to the nearest 10 g. Meanwhile, lambs were weighed prior to the morning feeding with an electronic-scale measure to the nearest 0.10 kg, at the beginning of the experiment and then weekly throughout the experimental phase to calculate ADG. After that, FCR was calculated by dividing the daily FI by ADG.

Experimental Measurements

Three high-precision data loggers (TW-USB-2-LCD+, ThermoWorks Inc., Lindon, Utah, USA) placed above the lambs at the height of approximately 2 m were used to continuously record the ambient temperature (T_a) and relative humidity (RH) at 30-minute intervals. A special software (Box-Car Pro 4, Onset Co, USA) was used to program and retrieve logger data. After that, the temperature humidity index (THI) was calculated to evaluate the severity of possible environmental stress using the method of Kelly & Bond (1971): [$THI=T_a-\{(0.55-0.55xRH)x(T_a-58)\}$], where T_a is the ambient temperature in degree Fahrenheit and RH is the relative humidity as a fraction of the unit. Figure 1 depicts the average weekly meteorological data that prevailed throughout the experimental period.

Rectal (T_r) and skin (T_{sk}) temperatures of experimental lambs were measured twice weekly (at 08:00 and 13:00 hr). A calibrated digital thermometer (Traceable Mini IRTM Thermometer, Friendswood, TX, USA) was used to estimate T_r, while an infrared thermometer (Traceable Mini IRTM Thermometer, Friendswood, TX, USA) was used to determine T_{sk} in two shaved areas (left shoulder and hip regions). Additionally, the formulas; [$T_{sk}-T_r$], [T_a-T_{sk}], and [T_a-T_r] were utilized according to da Silva & Maia (2013) to estimate the internal (BTG_{in}), external (BTG_{ex}), and total (BTG_{to}) body thermal gradients, respectively.

Furthermore, blood and urine samples were fortnightly collected from all lambs. Blood samples (~6 mL) were first collected before feeding via jugular venipuncture into both plain and EDTA vacutainer tubes, and then immediately transferred to the laboratory for further procedures. The EDTA tubes were used to analyze the packed cell volume (PCV) using the capillary tube method, while sera were separated by centrifuging the plain tubes at 1500 x g for 10 min at 4 °C and then stored in Eppendorf tubes at −20 °C until further analysis. On the other hand, urine samples were collected using a specially designed harness and then drawn into Eppendorf tubes and stored at −20 °C until further analysis. Serum concentrations of total protein (TP), albumin (ALB), glucose (GLU), non-esterified fatty acid (NEFA), blood creatinine (B-CRT) and urea nitrogen (B-UN), as well as urine concentrations of creatinine (U-CRT) and urea nitrogen (U-UN) were all analyzed using a semi-automated chemical analyzer (RX Monza, Randox Laboratories Ltd, Crumlin, UK) and commercial kits (Randox Laboratories, Antrim, United Kingdom) according to the manufacturer's procedures. Meanwhile, serum (B-OSMO) and urine osmolality (U-OSMO) were both analyzed using an Osmometer (VAPRO Pressure Osmometer, Model 5600, South Logan, USA).

Statistical Analyses

To evaluate the physiological response of lambs maintained under the thermo-neutral condition to the fixed effect of treatment (i.e., different levels of WI), all measured, estimated, and calculated data were analyzed as a completely randomized design using the PROC GLM procedure of SAS 9·4 (SAS Institute Inc., Cary, NC). The descriptive statistics for all parameters were calculated using the PROC MEANS method. Data were then subjected to repeated measure ANOVA, where means showing significant differences were tested using the PDIFF option. In fact, statistical means were compared using the least significant difference (LSD) test. However, unless otherwise indicated, the probability value that denotes statistical significance was set at $p<0.05$.

RESULTS

The descriptive analysis of all measured, estimated, and calculated parameters in the present experiment are available in the supplementary file (Table S1). Despite the high coefficient of variation in some parameters, results showed that all parameters were normally distributed.

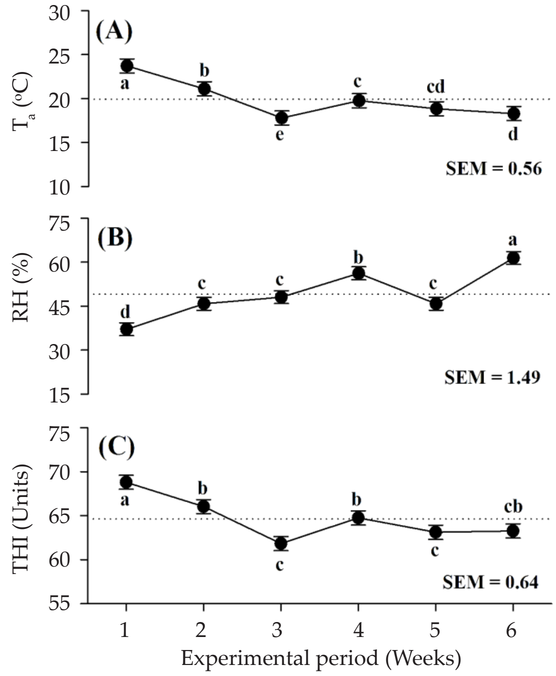

Figure 1. A meteorological chart showing weekly recorded ambient temperature (T_a, in A), relative humidity (RH, in B), and temperature-humidity index (THI, in C) throughout a 6 weeks experimental period. The dotted horizontal lines represent the average values as presented in Table 1. [a-c]Means bearing different superscripts are significantly different at $p<0.05$.

Table S1. Descriptive analysis of all measured, estimated, and calculated variables in this experiment

Variables	Descriptive analysis							
	Mean	Min	Max	SD	SE	CV	Skewness	Kurtosis
T_a (°C)	19.82	12.74	29.64	3.71	0.23	18.74	0.20	-0.76
RH (%)	49.78	25.62	75.19	11.63	0.73	23.36	0.12	-0.95
THI (Units)	64.58	55.49	74.15	4.26	0.27	6.60	-0.05	-0.85
T_r (°C)	39.25	38.55	40.95	0.39	0.04	0.98	2.64	8.85
T_{sk} (°C)	33.30	29.43	36.88	1.49	0.15	4.48	0.14	-0.79
BTG_{in} (°C)	-5.96	-9.63	-3.65	1.38	0.14	-23.09	-0.03	-0.83
BTG_{ex} (°C)	-13.47	-17.05	-9.60	1.49	0.15	-11.07	-0.14	-0.79
BTG_t (°C)	-19.43	-21.13	-18.73	0.39	0.04	-1.98	-2.64	8.85
PCV (%)	31.11	26.50	35.00	1.77	0.20	5.69	-0.26	-0.21
TP (g/dL)	6.52	5.14	9.97	1.33	0.14	20.46	1.24	0.43
ALB (g/dL)	3.55	2.67	4.86	0.47	0.05	13.33	0.82	0.23
GLU (mg/dL)	91.62	74.87	118.77	13.24	1.51	14.45	0.66	-087
NEFA (mmol/L)	0.57	0.32	0.88	0.12	0.01	21.39	1.13	0.63
B-CRT (mg/dL)	1.73	0.31	3.78	0.61	0.06	35.04	0.89	1.52
B-UN (mg/dL)	11.91	6.36	20.36	3.45	0.44	28.94	0.48	-0.70
B-OSMO (mosm/L)	305.88	277.00	349.00	19.36	2.02	6.33	0.21	-1.25
U-CRT (mg/dL)	103.13	58.53	216.56	30.77	4.27	29.83	1.41	3.06
U-UN (mg/dL)	2.01	0.43	4.02	0.92	0.13	46.16	0.35	-0.60
U-OSMO (mosm/L)	1612.84	1030.00	2550.00	488.17	50.62	30.27	0.51	-1.24

Note: Ta= ambient temperature; RH= relative humidity; THI= temperature-humidity index; T_r= rectal temperature; T_{sk}= skin temperature; BTG_{in}= internal body thermal gradient; BTG_{ex}= external body thermal gradient; BTG_{to}= total body thermal gradient; PCV= packed cell volume; TP= total protein; ALB= albumin; GLU= glucose; NEFA= non-esterified fatty acid; B-CRT= blood creatinine; B-UN= blood urea nitrogen; B-OSMO = serum osmolality; U-VOL= urine volume; U-CRT= urine creatinine; U-UN= urea nitrogen; and U-OSMO= urine osmolality.

Analysis of the meteorological indices (T_a, RH, and THI) recorded herein showed that all of them have (p<0.05) varied with the progress of the experiment. However, the overall averages of these parameters were 19.82±0.23 ˚C, 49.78±0.73%, and 64.58±0.27, respectively (Table S1). Besides, the dispersion of these datasets relative to their means (i.e., SD) were 3.71 ˚C, 11.63%, and 4.26, respectively (Table S1), and the maximum oscillation range between their overall weekly averages was 6.11 ˚C, 24.33%, and 7.97, respectively (Figure 1).

Under such conditions, implementing different levels of water restriction revealed no effects (p>0.05) on T_r and T_{sk} of growing lambs albeit the numerically lower values in 67-WI and 33-WI (Table 1, Figure 2). Similarly, no influence (p>0.05) was observed on all body thermal gradients (i.e., BTG_{in}, BTG_{ex}, and BTG_{to}), which remained relatively constant throughout the experiment (Table 1, Figure 2).

On the other hand, reducing the level of WI produced variable effects on animal performance as well as blood and urine constituents. As a matter of fact, FI was reduced (p<0.05) in 67-WI lambs and further reduced (p<0.05) in 33-WI lambs. Besides, reducing the level of water intake up to 33% of daily requirement resulted in a decline (p<0.05) in both ADG and FCR, but no effect was observed in 67-WI lambs (Table S1). Moreover, results revealed that no differences (p>0.05) were found between experimental groups in PCV level as well as B-CRT and U-UN concentrations (Table 1, Figure 2). However, it was clear that TP, ALB, GLU, NEFA, B-UN, B-OSMO, U-CRT, and U-OSMO concentrations had increased (p<0.05) in water-restricted lambs, whereas U-VOL exhibited the opposite trend (Table 1, Figure 2). In contrast to lambs receiving 67% of their *ad libitum* WI, it was notably evident that 33-WI lambs had particularly higher variation in the level of metabolites due to the increased level of water restriction, where TP, ALB, GLU, NEFA, B-OSMO, and U-OSMO had clearly (p<0.001) exhibited the most critical changes (Table 1).

DISCUSSION

Water scarcity is an inevitable scenario in an ever-changing climatic condition. Small ruminants are generally expected to drink up to 4 liters of water per day for optimal performance (NRC, 2007). Still, many producers provide more water to their livestock than needed to meet their demands, leading to higher water costs. Thus,

Table 1. Influence of different levels of water restriction on the physiological (performance, thermal, blood and urine) status of growing Najdi lambs (n= 8 animals per treatment) fed pelleted complete diet and reared under comfortable conditions (Ambient temperature= 19.82±0.23 °C, relative humidity= 49.78±0.73%, and temperature humidity index= 64.58±0.27; Mean±SE)

Variables[1]	Treatments[2]			SEM	P value
	100-WI	67-WI	33-WI		
Performance					
FI (kg)	1.28[a]	1.16[b]	0.91[c]	0.03	< 0.001
ADG (kg/day)	0.21[a]	0.17[a]	0.08[b]	0.02	0.003
FCR (FI/ADG)	6.10[a]	6.82[a]	11.38[b]	0.64	0.026
Thermal[3]					
Tr (°C)	39.31	39.3	39.15	0.06	0.148
Ts (°C)	33.44	33.19	33.26	0.13	0.337
BTG_{in} (°C)	-5.88	-6.12	-5.90	0.12	0.267
BTG_{ex} (°C)	-13.62	-13.37	-13.44	0.13	0.343
BTG_{to} (°C)	-19.49	-19.48	-19.33	0.06	0.148
Blood					
PCV (%)	30.87	31.04	31.37	0.38	0.606
TP (g/dL)	5.65[c]	6.60[b]	7.30[a]	0.06	< 0.001
ALB (g/dL)	3.39[b]	3.47[b]	3.80[a]	0.04	< 0.001
GLU (mg/dL)	85.74[c]	92.41[b]	96.15[a]	0.51	< 0.001
NEFA (mmol/L)	0.51[c]	0.57[b]	0.63[a]	0.02	< 0.001
B-CRT (mg/dL)	1.65	1.76	1.78	0.11	0.661
B-UN (mg/dL)	10.27[b]	12.41[a]	13.04[a]	0.67	0.013
B-OSMO (mosm/L)	290.86[c]	306.30[b]	317.75[a]	0.92	< 0.001
Urine					
U-VOL (mL/d)	1046.60[a]	338.60[b]	327.13[b]	70.21	< 0.001
U-CRT (mg/dL)	92.32[b]	101.04[ab]	115.89[a]	6.07	0.03
U-UN (mg/dL)	1.91	1.87	2.20	0.25	0.584
U-OSMO (mosm/L)	1197.80[c]	1659.50[b]	1939.25[a]	12.10	< 0.001

Note: [1]Details are shown in the text. [2]Treatments: 100-WI= *ad libitum* water intake; 67-WI= 67% of *ad libitum* water intake; 33-WI= 33% of *ad libitum* water intake.[3]Tr and Tsk were measured twice weekly at 08:00 and 13:00 hr, but average values were merely used herein. [a-c]Means within the same row bearing different superscripts are significantly different at p<0.05.
FI= feed intake; ADG= average daily gain; FCR= feed conversion rate; Tr= rectal temperature; Tsk= skin temperature; BTG_{in}= internal body thermal gradient; BTG_{ex}= external body thermal gradient; BTG_{to}= total body thermal gradient; PCV= packed cell volume; TP= total protein; ALB= albumin; GLU= glucose; NEFA= non-esterified fatty acid; B-CRT= blood creatinine; B-UN= blood urea nitrogen; B-OSMO = serum osmolality; U-VOL= urine volume; U-CRT= urine creatinine; U-UN= urea nitrogen; and U-OSMO= urine osmolality.

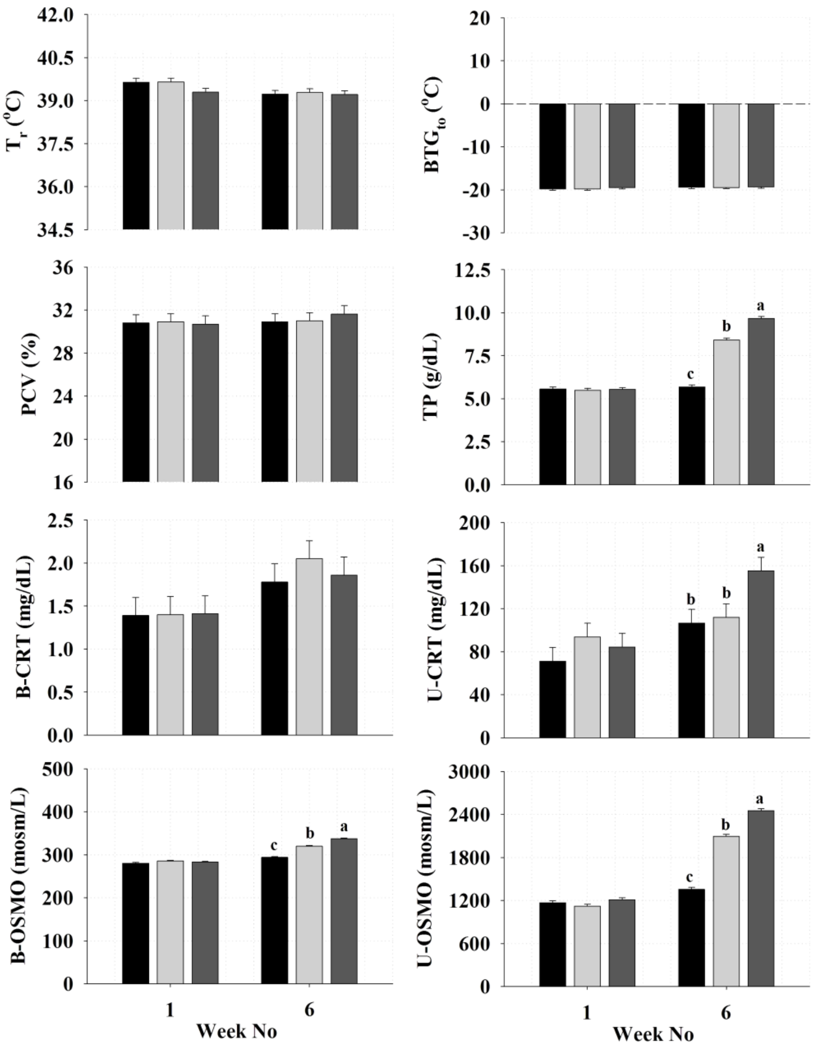

Figure 2. Time-dependent effect of different levels of water-restriction on some physiological variables of growing Najdi lambs (n= 8 animals per treatment) fed on pelleted complete diet and reared under comfortable conditions (Ambient temperature= 19.82±0.23 °C, relative humidity= 49.78±0.73%, temperature humidity index= 64.58±0.27; Mean±SE). 100-WI (■)= *ad libitum* water intake; 67-WI (■)= 67% of *ad libitum* water intake; 33-WI (■)= 33% of *ad libitum* water intake; T_r= Rectal temperature; BTG_{to}= total body thermal gradient; PCV= packed cell volume; TP= total protein; B-CRT= blood creatinine; U-CRT= urine creatinine; B-OSMO= serum osmolality; and U-OSMO= urine osmolality. [a-c]Means within the same experimental week bearing different superscripts are significantly different at p<0.05.

effective water management is undoubtedly vital for rationalizing water consumption without compromising livestock production and performance. Nevertheless, data on the precise amount of water restriction that growing lambs can tolerate without affecting their well-being when fed on PCD and reared under comfortable climatic conditions are still very scarce to nothing in the literature. The present experiment was conducted to address the physiological responses of growing Najdi lambs fed on PCD and reared under thermo-neutral condition when exposed to three different water-intake regimens (i.e., 100-WI, 67-WI, and 33-WI) for 6 weeks.

Based on the current findings (Figure 1), meteorological indices clearly indicated that lambs were within their thermo-neutral condition throughout the experiment (Rout *et al.,* 2017). Reducing the level of water intake under such conditions had no subsequent impacts on the thermal status of lambs fed on PCD based on the un-altered findings of their body temperatures and thermal gradients (Table 1, Figure 2). Exposure to hot weather conditions could trigger an increase in T_r, T_{sk}, heart rate, as well as other thermo-physiological indices (Abdoun *et al.,* 2012; Al-Haidary *et al.,* 2012; Samara *et al.,* 2016), however, this was not the case herein since lambs were reared under thermo-neutral condition. In fact, Alamer (2010) observed no change in T_r of water-deprived Awassi and Najdi sheep during the winter season. Few studies similarly have demonstrated that sheep are thermo-stable even under water restriction regimes (Saini *et al.,* 2013), but on the contrary, Al-Ramamneh *et al.* (2012) observed a decrease in T_r of water-restricted sheep and goats, which could be a consequence of a decrease in the endogenous metabolic heat production as a result of decreasing feed intake. Accordingly, the observed un-change in the thermo-physiological status of water-restricted lambs fed on PCD and reared under a thermo-neutral condition in the current experiment could primarily signify that diminishing water intake up to 33% of their WI can be implemented without compromising their well-being.

Notably, however, reducing the WI level had tangible effects on production performance and metabolic homeostasis (Table 1, Figure 2). Water restriction was expected to reduce lambs' blood and urine volumes, and results actually documented a decrease in U-VOL of lambs receiving 67-WI and 33-WI attributed mainly to the adaptive process of lowering the glomerular filtration rate of lambs' kidney. Surprisingly, however, lambs receiving 67-WI and 33-WI showed no alteration in PCV (albeit the numerically higher values) compared to their counterparts receiving 100-WI, which indicates that these lambs are still able to maintain their homeostatic balance of body fluids when subjected to low WI for 6 weeks. This was supported by a previous report, such as Jaber *et al.* (2004), who reported un-altered PCV in Awassi ewes watered every 3 days in a 3-week trial and every 4 days in a 6-week trial, respectively. Additionally, PCV of Yankasa ewes was as well not affected in a 5 days' water-deprivation study (Igbokwe, 1993). Furthermore, it was clearly evident that water restriction had increased TP, GLU, NEFA, B-UN, B-OSMO, and U-OSMO concentrations in both 67-WI and 33-WI lambs. The observed increase in TP, GLU, and NEFA levels could be attributed to the reduction in FI and ADG, which has been previously discussed by our team (Adeniji *et al.*, 2020). In fact, this was consistent with several reports (Igbokwe, 1993; Jaber *et al.*, 2004; Kaliber *et al.*, 2016; Kumar *et al.*, 2016), and in contrast with few (Silanikove & Tadmor, 1989; De *et al.*, 2015).

Regarding B-CRT, B-UN, U-CRT, and U-UN concentrations, water restriction might have activated the creatinine excretion and urea reabsorption processes in renal tubules to generate an osmotic gradient necessary for urine concentration and body water preservation; thereby, leading to the herein observed increase in B-UN and U-CRT concentrations as well as un-altered B-CRT and U-UN concentrations (Weiner *et al.*, 2015; Kaliber *et al.*, 2016). These findings, however, contradict a previous study on water-deprived small ruminants (Kaliber *et al.*, 2016), which could be related to the difference in climatic conditions, where water loss for evaporative cooling is a crucial factor affecting urine concentration and consequently the level of these metabolites.

On the other hand, the observed increase in B-OSMO of water-restricted lambs could be attributed to the increasing level of serum TP to maintain a relatively stable osmotic balance (Casamassima *et al.*, 2016). This outcome is in consistent with previous reports (Alamer, 2010; Al-Haidary *et al.*, 2012). As for the rise in U-OSMO level in both of 67-WI and 33-WI lamb groups, inevitably, U-OSMO level will accordingly rise as U-VOL level decreases in water-restricted lambs. It is worth mentioning that all metabolites assayed in this experiment were within their physiological reference ranges reported for sheep (Radostits *et al.*, 2000), except for the circulating concentration of GLU.

CONCLUSION

The present experiment shed some light on the amount of water restriction that growing lambs (fed on PCD and reared under thermo-neutral conditions) can tolerate without compromising their well-being. Results collectively signify that diminishing WI up to 33% in lambs (fed on PCD and reared under comfortable conditions) would not only ensure that water is adequately conserved under prevailing water scarcity but can also be implemented without compromising their homeostatic functions. This conclusion was demonstrated by the findings that 67-WI lambs were resilient to limited water availability by inducing proportional physiological responses in their production performance, body temperatures, blood metabolites, and renal function but within the homeostatic ranges similar to 100-WI lambs.

CONFLICT OF INTEREST

The authors certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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