Research Article

Oluwakamisi F. Akinmoladun*, Fabian N. Fon, Conference T. Mpendulo, Omobola Okoh

Intake, nutrient digestibility, nitrogen, and mineral balance of water-restricted Xhosa goats supplemented with vitamin C

https://doi.org/10.1515/opag-2020-0190
received February 19, 2020; accepted August 25, 2020

Abstract: The study objective was to evaluate the effect of single and/or extra doses of vitamin C (VC) on water-restricted (WR) Xhosa goats, by evaluating their intake, digestibility, nitrogen, and mineral balance during summer. Goats (42) were randomly divided into seven groups: GI (control, received ad libitum water daily), GII (WR-70% of ad lib.), GIII (WR-50% of ad lib.), GIV (WR-70% of ad lib. + VC [3 g/day orally]), GV (WR-50% of ad lib. + VC [3 g/day orally]), GV1 (WR-70% of ad lib. + VC [3 g/day orally + 5 g extras every eighth day]), and GVII (WR-50% of ad lib. + VC [3 g/day orally + 5 g extras on every eighth day]). The experiment was a complete randomized design. Data obtained were analysed using the general linear model (PROC GLM) of SAS procedure. The depression in nutrients intake was lessened with VC supplementation. Water-restriction effect was not significant on nutrient intake/metabolic weight. Retained nitrogen increased with water-restriction levels but not influenced by VC. NH₄-N significantly decreased as the level of water restriction increases, while the pH was similar across the WR groups. Supplementation of VC failed to significantly influence the depression in apparent digestibility and digestible nutrients induced by water restriction levels. The retained Ca, K, and Mg increased with levels of water restriction.

Keywords: water restriction, Xhosa goats, digestibility, nutrient intake, ascorbic acid

1 Introduction

The multiplicity role of livestock in supporting livelihood, especially to developing nations, ranges from household income generation, food security, and employment opportunities to many socioeconomic attribute (Moyo and Swanepoel 2010). These immense attributes and contributions require that their productions be increasingly sustained to meet the need of the ever growing human population. Unfortunately, a huge percentage of livestock population especially ruminants, widely distributed in water-limiting and dry zones of the world, are faced with the challenge of water scarcity and seasonal drought (Marino et al. 2016). This burden of water stress is further heightened by fluctuations in weather and rainfall patterns (global warming), consequently limiting the volume of water resources in most areas (IPCC 2007).

Drinking water is very important in the nutrition of livestock and suboptimal intake can critically impact on physiology and productivity. This is because of its role in the maintenance of body heat balance, efficient digestion, absorption of food, and as the main solvents for both intra- and extracellular fluids (Alamer 2010). In a review on the adaptation of livestock to water scarcity, small ruminants seem to be more resilient to limited water intake compared to other livestock species (Akinmoladun et al. 2019). However, the extent to which species can effectively use limited water differs with respect to breed and animal type. Desert adaptable breeds in arid regions have evolved survival mechanism in terms of efficient use of water and body reserves during the period of
water shortfalls (Alemneh and Akeberegn, 2019). Unlike monogastric where body water loss above 15% can be detrimental, water losses of 18, 20, and 25% of body weight can be tolerated by cattle, sheep and goat, and camel, respectively. Such resilience by ruminants to dehydration effects is because of the improvements in feed intake digestibility, lower energy deficiency induced by dry matter (DM) intake reduction, and the rumen capacity to conserve water up to 15% body weight for use during scarcity (Al-Ramamneh et al. 2012). Despite this adaptive mechanism, exposure of ruminants to limited water intake portends a stressful condition, negatively impacting on body weight, and production performances in animals (Mpendulo et al. 2020).

In ruminant nutrition, administration of vitamin C (VC) is a practice that is not common. This is because VC is usually biosynthesized in ruminants and may require no exogenous supplementation. However, stress and disease conditions can easily deplete plasma VC in livestock (Chambial et al. 2013). As an antioxidant, VC helps in the scavenging of free oxidative radicals induced by oxidative stress and also plays an active role in immunomodulation (Tan et al. 2018). Body weight loss, depression in feed intake as well as other affected physiological variables in water and transportation stressed sheep and goats were reduced following vitamin C supplementation (Ghanem et al. 2008; Kassab and Mohammed 2014; Akinmoladun et al. 2020).

Xhosa goat is an indigenous breed, adaptable to the Eastern Cape Province. The summer months in South Africa (December–March) are characterized by hot and dry temperature extremes and water scarcity. During this period, it is always difficult for ruminants to maintain their body weights because most of the water points are dried up and the goats are forced to trek long distance in search of water while grazing the sparsely distributed grasses and browse plants. Variations among species of ruminants to digestibility, water use abilities, and feed utilization have been documented (Jaber et al. 2013). It is hypothesized that different digestive mechanism may have been evolved by adaptable breeds to water scarcity and marginal feeding circumstances, and a possible improvement when VC is supplemented. This might likely result in differences in the efficiency of nutrient utilization. In addition, the limitation of VC bioavailability because of urinary excretion losses may reduce when boosted with extra VC doses. This research work was therefore undertaken to evaluate the intake, rumen products, digestibility, nitrogen, and mineral balance in water-restricted (WR) Xhosa goats supplemented with single and/or extra doses of VC.

2 Materials and methods

2.1 Location

The experiment was conducted at the Honeydale farm, University of Fort Hare, Alice campus, located in the False Thornveld of the Eastern Cape, South Africa (latitude, 32°46’ and longitude, 26°50’) and at an altitude of 535 m above sea level. It is a warm climate with a temperature range between 11.1 and 24.6°C, and an average annual rainfall of about 575 mm (Schulze et al. 2006).

2.2 Management of animals

A total of 42 female goats (Xhosa breed) with an average age of 12 months and body weight 15.92 ± 2.12 kg were used in a 75-day experiment. Before commencement of the experiment, de-worming using ivermectin and vaccination against foot-and-mouth disease was carried out on the goats. The indices of digestibility were taken during the last 10 days of the experiment. The goats were kept in individual metabolic cages (1.33 × 0.58 m), provided with feeder, water trough, and a separate system for collecting urine and faeces. The animals were offered feed as total mixed ration based on 4% of their body weights in the ratio of 70:30 of Lucerne hay and concentrated (maize gluten, 55.43%; sunflower husk, 42.42%; limestone, 0.70%; monocalcium diphosphate (MCDP), 0.75%; salt, 0.5%; mineral mix, 0.2%) on DM basis, respectively. The concentrated diet was formulated to meet the nutrient requirements for growing goats (NRC 1981). The diet composition is shown in Table 1. The physical condition of each animal was closely monitored daily and proper sanitary conditions were maintained during the trial period.

Ethical approval: The research related to animal use has been complied with all the relevant national regulations and institutional policies for the care and use of animals, and has been approved by the Animal Research Ethics Committee of the University of Fort Hare (Reference No: MUC011SAKI01).

2.3 Dietary treatments and design

The goats were allocated to the seven dietary treatment groups comprising of six animals per group: GI (control), WR-ad lib.; GII, WR-70%; GIII, WR-50%; GIV, WR-70% + 3 g VC; GV, WR-50% + 3 g VC; GVI, WR-70% + 3 g VC (+extra
was given every eighth day in the afternoon before feeding in the morning. Extra VC supplementation after it was dissolved in 50 mL drinking water shortly after feeding. Goats on VC treatment were given the respective amount of VC each day. The average temperature, humidity and the ambient temperature inside the pen. The degree of heat stress was then calculated based on the THI values (i.e., THI $\leq$ 22.2 absence of heat stress; 22.2 to $\leq$ 23.3 moderate heat stress; 23.3 to $\leq$ 25.6 severe heat stress; and $>25.6$ extreme severe heat stress) (Marai et al. 2007). Diurnal temperature and humidity pattern in the housing units are presented in Figure 1 as average values.

2.5 Digestibility trial

Feed offered and feed refused (if any) for each goat were collected and weighed every morning to obtain an estimate of feed intake. Total urine and faecal output from water at focal points in the pen, so that loss because of evaporation can be inputted when calculating for total water intake. WR-70% and WR-50% groups did receive drinking water daily at a level of 70 and 50% of the intake recorded in the W100 group, respectively. Before the commencement of the 7-day collection period, there was a 3-day period of adaptation of the animals to the metabolic crates. The feed and water intake were recorded daily all through the trial period.

### Table 1: Ingredient and chemical composition of experimental diet (expressed in g kg$^{-1}$ DM)

| Ingredient                  | Quantity |
|-----------------------------|----------|
| Lucerne                     | 700      |
| Maize gluten                | 166.3    |
| Sunflower husk              | 127.3    |
| Limestone                   | 2.1      |
| MCDP                        | 2.3      |
| Salt                        | 1.5      |
| Premix$^a$                  | 0.6      |

$^a$Ca = 220 g/kg; P = 55 g/kg; Mg = 35 g/kg; S = 22 g/kg; Cl = 105 g/kg; Na = 70 g/kg; Mn = 1,500 mg/kg; Fe = 500 mg/kg; Zn = 1,550 mg/kg; Cu = 440 mg/kg; Co = 50 mg/kg; I = 40 mg/kg; Se = 20 mg/kg.

5 g VC; GVIL WR-50% + 3 g VC (+extra 5 g VC). Experimental goats on VC treatment were given the respective amount after it was dissolved in 50 mL drinking water shortly before feeding in the morning. Extra VC supplementation was given every eighth day in the affected group. L-ascorbic acid (VC) used as a supplement was sourced from Minema Chemical Stores, Gauteng, South Africa. The 3 g/day VC dose was selected based on previous findings on its effectiveness and higher bioavailability at lower doses (Jaber et al. 2011). In addition, the bio-availability of VC could be increased with multiple dosing than single dosing (Hidiroglou et al. 1997). Experimental feed was offered twice daily, at 9:00 and 16:00 h in equal proportions. Water restriction percentages for experimental groups were calculated based on daily ad libitum intake of the control group. Water was supplied in containers of known volume and was topped-up once a day. In W100 group, the Xhosa goats receive water daily at two different times of the day, at 8:00 and 15:00 h to determine the quantity of water ingested. Total water intake was calculated as the difference between the amounts offered and left overs, rebating loss of water because of evaporation. Water loss because of evaporation was calculated by putting buckets filled with

Figure 1: Diurnal temperature and humidity pattern in the housing unit.
each animal were collected daily and weighed. Of the total faeces collected, 10% was taken and thereafter dried in an oven at 70°C for 48 h. Faecal DM and DM intake were calculated as appropriate. The dried samples of faeces and feed were screened through a 1 mm mesh size, sieved, and stored before analysis. Urine samples were collected in plastic containers that have been previously washed with 10% HCL for 4 h, rinsed with de-ionized water, and allowed to dry before use. Total urine output was collected daily into 10 L plastic container that contained 6N H2SO4 and de-ionized water to prevent the urine N from volatilization. Ten per cent of urine aliquot was daily collected in a 3 L plastic container during the 7-day collection period and frozen at −20°C until further analysis.

2.6 Rumen content sampling for pH and NH3–N

Rumen contents were sampled from goats for experimental meat studies following slaughtering at a registered abattoir (Adelaide abattoir, South Africa). Goats were electrically stunned for 5 s at 200 V, to render them unconscious prior exsanguinations and evisceration. Rumen samples were obtained from the reticulo-rumen of each goat and squeezed through two layers of cheesecloth. The ruminal pH was determined with the help of a pH meter (Dascor, Inc., Escondido, CA). It was inserted into the rumen fluid collected after calibration with buffer solutions, pH 4 and 7. After the pH was measured, a 5 mL bottle was used to collect rumen samples for NH3–N determination and followed by the addition of 1 mL of 1% H2SO4. Samples were kept frozen (−20°C) until NH3–N analysis.

2.7 Chemical analysis

Triplicates of feed, refusals, and faecal samples were used to determine DM, organic matter (OM), ether extract (EE), Ash, and crude protein (CP) according to the procedure of AOAC (2000). Approximately 1 g of each sample was measured in a glass beaker and dried for 48 h at 70°C. Samples were thereafter cooled in a desiccator for 2 h, and the weights of the dried samples were recorded. The dried samples were ashed in a muffle furnace (630°C for 6 h), cooled for 2 h in a desiccator, and weights recorded. OM intake (OM = 100-ash content) was calculated by multiplying the per cent OM of the feed sample with the amount consumed for each goat. A similar thing was done to calculate the faecal OM. Nitrogen (N) was determined using the Kjeldahl method (AOAC 2000) and CP calculated as N × 6.25. Nitrogen retained (NR) was calculated from the amounts of N (g/day) consumed and excreted in the faeces and urine as follows: NR (g/day) = Nconsumed − Nfaeces − Nunurine.

A dilution rate of 1:60 and 1:9 in 1% nitric acid for urine and faecal samples, respectively, was used in the analysis of Na, Ca, K, and Mg (Richter et al. 2012) with the aid of the optical emission spectrophotometry (ICP; Optima 7000 DV; PerkinElmer, Waltham, MA). Samples were analysed in triplicate and the mean values recorded. The mineral content of individual components of feed offered, refusals, total faeces, and urine was calculated by multiplying respective values of minerals determined by their total quantities. The daily mineral content of mineral intake, refusals, faecal output, and urine output was determined by dividing total mineral content of the respective components by the number of days of collection. Apparent mineral absorption was calculated by subtracting faecal mineral from consumed mineral and multiplying by 100.

The frozen rumen samples were thawed at 4°C. The NH3–N concentration in rumen fluid was analysed by the alkaline phenol-hypochlorite colorimetric method (Broderick and Kang 1980).

The coefficient of digestibility (%) (Givens et al. 2000), digestible nutrient, and total digestible nutrient (TDN) (Banerjee 1998) were calculated as follows:

\[
\text{Coefficient of digestibility} = \frac{\text{Nutrient consumed} - \text{Nutrient in faeces}}{\text{Nutrient consumed}} \times 100,
\]

\[
\text{Digestible nutrient} = \% \text{ Nutrient amount in feed} \times \% \text{ of nutrient digestibility} / 100
\]

\[
\text{TDN} = \% \text{DCP} + \% \text{DCF} + \% \text{DNFE} + (\% \text{DEE} \times 2.25),
\]

where DCP is the digestible crude protein; DCF is the digestible crude fibre (CF); DNFE is the digestible nitrogen-free extract (NFE); and DEE is the digestible ether extract (NRC 2001).

2.8 Statistical analysis

Data for intake, apparent digestibility of dietary constituents, digestible nutrients, nitrogen balance, mineral absorption, and rumen products were subjected to
PROC GLM procedure of SAS 8.0 (SAS 2001). Mean treatment differences were determined by Duncan multiple range test at a probability level of 0.05. The model used was

\[ Y_{ij} = \mu + T_i + \epsilon_{ij}, \]

where \( Y_{ij} \) = values of observation; \( \mu \) = general mean; \( T_i \) = effect of water treatments; and \( \epsilon_{ij} \) = residual error.

3 Results

The diurnal temperature (°C) and humidity (RH%) in the housing unit are shown in Figure 1. The average THI in the experimental room was 25.57 ± 0.15 throughout the experiment. DM intake (DMI; g/day), OM intake (OMI; g/day), and CP (g/day) intake were similar \((P > 0.05)\) in the entire WR groups but lower than \((P < 0.05)\) what was obtained in the control (GI) (Table 2). The EE and the nutrients’ intake per metabolic weight for both the WR groups and control were similar \((P > 0.05)\). The TDN was significantly higher \((P < 0.05)\) in the WR-treated groups (GII–GVII) than in GI (Figure 2). However, VC supplementation (single and/or extra) did not influence \((P > 0.05)\) the TDN values. The faecal and urine output decreased \((P < 0.05)\) with levels of water restriction. The total N intake decreased \((P < 0.05)\) with levels of water restriction and linearly increased in the VC-treated groups. Faecal N, faecal N/N%, urinary N, and urinary N/N% significantly decreased \((P < 0.05)\) with water restriction levels. The %N retained increases \((P < 0.05)\) with levels of water restriction, but was not affected \((P > 0.05)\) by VC supplementation. The pH was similar \((P > 0.05)\) in the WR groups, and values obtained were higher \((P < 0.05)\) than the control (GI). The NH₃–N decreased \((P < 0.05)\) as the water restriction levels increases and was not influenced \((P > 0.05)\) by VC supplementation at various doses.

The coefficient of digestibility and digestible nutrients percentages (CP, CF, NFE, EE, DM, and OM) among the differently watered groups (with or without VC treatment) is shown in Table 3. In the water-restriction groups (GII–GVII), the digestibility of DM, OM, CF, CP, and NFE increased \((P < 0.05)\) with water restriction levels and were higher than the control (GI). The digestibility of EE was similar \((P > 0.05)\) for treatments GII–GVII but lower \((P < 0.05)\) than in GI. Unlike the EE, the digestible nutrients (CP, CF, NFE, DM, and OM) in the WR groups were significantly higher \((P < 0.05)\) than the control (GI). The digestible nutrients (except EE) increase \((P < 0.05)\) with water restriction levels. Supplementation of VC at various doses to the WR groups did not improve \((P > 0.05)\) the digestible nutrients.

Macro-mineral intake, excretion, absorption, and retention are reported in Table 4. The number of macrominerals (Ca, P, Mg, Na, and P) intake significantly decreased \((P < 0.05)\) because of water restriction in GII–GVII compared to GI. However, a similar amount \((P > 0.05)\) of the macro minerals was consumed regardless of the levels of water restriction and VC dosage. Compared to other mineral elements, a greater amount of Ca \((g/day)\) and K \((g/day)\) \((P < 0.05)\) was excreted via urine than faecal. Supplementation of VC did not create a pattern of influence on the minerals excreted via faeces or urine. The apparent absorption of Ca was highest in GIII \((P < 0.05)\), while GVII had the highest absorption \((P < 0.05)\) of Mg and K. The retained Ca (%) was highest \((P < 0.05)\) in GIII and lowest in GI. The retained K (%) increased \((P < 0.05)\) with water restriction levels. A similar amount of P (%) and Na (%) were retained in GI, GIII, GV, and GVII. The retained Mg (%) increased \((P < 0.05)\) with levels of water restriction. The retentions of Ca, Mg, and K significantly increased with water restriction levels.

4 Discussion

The average THI recorded in the experimental house is above the threshold values (Marai et al. 2007), and this has resulted in severe stress on the goats. This implies a negative heat balance as more heat will be gained by the animal from the environment. Efficient digestion and absorption require a water medium for the physical softening of feed (Doreau et al. 2012). When water supply is adequate, the breakdown of feed is enhanced, thus facilitating fermentative and digestive processes. Giving the correlation between feed intake and water consumption (Thang et al. 2012), the reduced nutrients intake in the WR groups could be as a result of limited water intake. Usually, the amount of feed consumed by ruminants is affected by the osmolality of body fluids. This is because the secretion of gastric juices and saliva in response to feed intake usually results in hyperosmolality and hypovolemia, urging animals to drink while eating or stop when dehydrated (Langhans et al. 1991). Severe responses to feed intake under various water restriction regimen has been reported in Turkana goats, fat-tailed sheep, and Zebu (Maloiy et al. 2008). Other studies have also reported a drop in DM intake following water restriction (Casamassima et al. 2016).
Regardless of VC supplementation, the reduced urine and faecal output in the WR groups could be attributed to suboptimum water intake. During water scarcity, ruminants reduce water loss by reducing faecal water and urine volume, thereby resulting in higher urine osmolality (Naqvi et al. 2015). This drop in urine volume could be attributed to reduced filtration rate of the glomerulus and renal plasma flow in the WR groups (Schwartz and Furth 2007). This tendency of generating hyperosmotic urine is attributed to the length of the loops of Henle which is longer compared to non-adaptable breeds (McNab 2002). This greater urine concentration and reduced urine output are the adaptive mechanism that enhances their chances of survival. The decrease in nitrogen consumption could be attributed to reduced voluntary DM intake by the WR groups. In a similar vein, there was a decrease in the amount of nitrogen excreted because of limited water intake by the animal. When expressed as a percentage of N-intake, high losses of nitrogen were recorded through faeces compared to urine across the treatment groups. The high faecal nitrogen loss in the control group compared to the WR groups mirrors the high feed consumed and faecal output in the control group. The increased nitrogen retention obtained by the WR group in this study was facilitated by low nitrogen excretion. Contrarily, the control group had a negative nitrogen balance but the WR groups were able to keep hold of a significant amount of the consumed nitrogen. The higher digestible and retained N, and CP digestibility in the WR groups compared to the control is a reflection of the low nitrogen excretion in faeces and urine, resulting in positive N retention. This may be an indication of N recycling via the wall of the rumen and saliva for microbial synthesis (Nejad et al. 2014). Because of suboptimal water intake, degradation process in the rumen is slowed down and this helps to facilitate protein escape. The escaped protein may be digested in the abomasum and small intestine, leading to increased uptake of nitrogen from the small intestine. Thus, protein metabolism in the liver is increased as well as enhanced nitrogen recycling into the rumen from possible amino acid deamination for non-essential protein synthesis or energy utilization.

The decreased urinary N loss following water restriction in this study could be attributed to reduced filtration

### Table 2: Nutrient intake, output, nitrogen metabolism, and rumen product of WR Xhosa goats supplemented with VC

| Parameters                  | GI     | GII    | GIII   | GIV    | GV     | GVI    | GVII   | SEM  | P-value  |
|-----------------------------|--------|--------|--------|--------|--------|--------|--------|------|----------|
| **Nutrient intake**         |        |        |        |        |        |        |        |      |          |
| DMI (g/day)                 | 633.37 | 546.94 | 536.22 | 552.08 | 538.68 | 551.86 | 538.32 | 7.18 | 0.065    |
| DMI (g/BW<sub>0.75</sub>)  | 74.41  | 78.88  | 78.02  | 73.92  | 73.42  | 75.95  | 73.81  | 16.72| 0.996    |
| OMI (g/day)                 | 524.62 | 453.03 | 444.15 | 467.29 | 446.19 | 457.10 | 445.89 | 5.95 | 0.036    |
| OMI (g/BW<sub>0.75</sub>)  | 61.14  | 65.34  | 64.63  | 61.23  | 60.81  | 62.91  | 61.14  | 4.37 | 0.895    |
| CP (g/day)                  | 137.25 | 118.52 | 116.20 | 119.64 | 116.73 | 119.59 | 116.65 | 1.56 | 0.035    |
| CP (g/BW<sub>0.75</sub>)   | 16.12  | 17.09  | 16.91  | 16.01  | 15.91  | 16.46  | 15.99  | 0.35 | 0.766    |
| EE (g/day)                  | 11.08  | 9.57   | 9.38   | 9.66   | 9.42   | 9.66   | 9.42   | 0.13 | 0.045    |
| EE (g/BW<sub>0.75</sub>)   | 1.30   | 1.38   | 1.37   | 1.29   | 1.28   | 1.33   | 1.29   | 0.13 | 0.906    |
| **Output**                  |        |        |        |        |        |        |        |      |          |
| Faecal output (g/day)       | 199.59 | 144.39 | 124.23 | 143.98 | 126.07 | 144.10 | 123.56 | 5.28 | <0.001   |
| Urinary output (mL/day)     | 383.33 | 233.95 | 156.67 | 223.33 | 157.38 | 226.43 | 153.81 | 14.94| <0.001   |
| **Nitrogen metabolism**     |        |        |        |        |        |        |        |      |          |
| N intake (g/day)            | 21.96  | 18.96  | 18.59  | 19.14  | 18.68  | 19.13  | 18.66  | 0.12 | 0.033    |
| N faeces (g/day)            | 4.77   | 2.25   | 2.02<sup>c</sup>d | 2.42b | 2.17c | 2.19f | 1.90g | 0.09 | 0.003    |
| Digest. N (g/day)           | 19.30  | 16.95  | 16.43  | 16.44  | 15.91  | 16.48  | 16.77  | 0.54 | 0.041    |
| N urine (g/day)             | 1.55   | 1.00b  | 0.31d  | 0.80c  | 0.34d  | 0.92b  | 0.37d  | 0.05 | 0.004    |
| N retention (g/day)         | 15.63c | 15.71c | 16.26<sup>a</sup>b | 15.92c | 16.18  | 16.02<sup>ab</sup> | 16.43<sup>a</sup> | 0.15 | 0.021    |
| Faecal N/N%                 | 21.70c | 21.15c | 10.87<sup>d</sup> | 12.62<sup>b</sup> | 12.5<sup>d</sup> | 11.3<sup>d</sup> | 11.6<sup>d</sup> | 10.17e | 0.23 | 0.003    |
| Urine N/N%                  | 7.04<sup>a</sup> | 5.28b | 1.68<sup>d</sup> | 4.17<sup>c</sup> | 1.83d | 4.86<sup>c</sup> | 1.98<sup>d</sup> | 0.49 | 0.022    |
| Retained N/N%               | 71.16<sup>c</sup> | 82.86<sup>c</sup> | 87.45a | 83.18<sup>a</sup>b | 86.60a | 83.75b | 88.04a | 1.05 | 0.012    |
| **Rumen parameters**        |        |        |        |        |        |        |        |      |          |
| pH                          | 6.78   | 7.09a  | 7.02a  | 7.03a  | 7.05a  | 7.10a  | 7.08a  | 0.08 | 0.001    |
| NH<sub>3</sub>−N            | 27.06<sup>a</sup> | 25.02b | 22.44<sup>c</sup> | 24.90<sup>b</sup> | 21.42<sup>d</sup> | 27.16a | 21.68<sup>d</sup> | 0.18 | <0.001    |

<sup>a,b,c,d</sup>Means with different superscripts across the row are significantly different (P<0.05); GI: W100%; GII: W70%; GIII: W50%; GIV: W70% + 3 g VC; GV: W50% + 3 g VC; GVI: W70% + 3 g VC + extra 5 g VC; GVII: W50% + 3 g VC + extra 5 g VC; DMI: dry matter intake; OMI: organic matter intake.
Ammonia-N decreased with decreased water intake in this study. According to Mathews et al. (2019), when intake is below optimum, rumen ammonia production drops because of low rate of degradation. The increased rumen pH in the WR groups could be because of the combined effect of heat and water stress. Lactating dairy cattle undergoing heat stress had their ruminal pH increased from 5.82 to 6.03 (Hall 2009) and was slightly different from the 6.78 to 7.10 recorded in this study. In a similar study on growing heat-stressed Korean cattle steers fed rumen-protected fat, the rumen pH was found to slightly increase from 6.88 to 7.02 and 6.74 to 7.10 in July and August, respectively (Kang et al. 2019).

Water restriction of any form would generally enhance the digestibility of nutrients by enhancing feed intake reduction and allowing sufficient time for digesta. Increased retention of digesta implies that rumen microbes will have enough time for degradation and synthesis (Casamassima et al. 2016). In this study, digestibility of nutrients significantly increases as water intake decreases. This is similar to the increased total tract digestibility of DM and OM in WR St. Croix sheep (Hussein et al. 2018). However, the findings of Nejad et al. (2014) showed no differences in nutrient digestibility between 2 and 3 h water restrictions after feeding in Corriedale ewes. This non-significant effect maybe as a result of the limited time of exposure to reduced water intake, compared to the 75 days of prolonged exposure in this study. Single and/or multiple dosing with VC did not improve nutrient digestibility, as values obtained between treated and untreated WR groups were similar.
The amount of minerals in dietary constituents and what is required by animals compared to protein, carbohydrates, and/or fat are often low. This does not undermine their importance, as an absence or imbalance may elicit deficiencies, and consequently impair performance (Suttle 2010). In addition, the mineral nutrition of goats is yet to be fully worked out, as recommended mineral consumption values are still based on extrapolations from intermediate values between sheep and cattle (Meschy 2000). The low mineral intake in the WR groups may be as a result of reduced feed intake, given the fact that nutrients ingested by livestock are positively correlated with feed intake. As an adaptive mechanism coupled with the role of water for proper digestion, a reduction in feed intake usually accompanies limited water intake in small ruminants (Akinmoladun et al. 2019), consequently impairing the amount of nutrients ingested. However, a marginal improvement in DMI intake following VC supplementation in the WR groups did not significantly translate to higher mineral intake. In this study, GI ingested higher minerals but excreted more of these minerals, especially Ca, Mg, P, and K, in faeces and urine compared to the water restriction groups. The affirmation of Boswald et al. (2017) on higher excretion of P in herbivores through faeces agrees with the report of this study. However, the excretion of Ca in this study is higher in urine but decreased with water restriction levels. When dehydrated, animals adapt by reducing faecal water and urine output. This might explain the higher retention of Ca, K, P, and Mg among the WR groups compared to the control. However, a minimum threshold of 45% in apparent Ca absorption is recommended for ruminants.

| Parameters | GI | GII | GIII | GIV | GV | GVI | GVII | SEM | P-value |
|------------|----|-----|------|-----|----|-----|------|-----|---------|
| Dose (n)   | 4  | 4   | 4    | 4   | 4  | 4   | 4    |     |         |
| **Mineral intake (g/day)** |    |     |      |     |    |     |      |     |         |
| Ca         | 9.94 a | 8.58 b | 8.42 b | 8.67 b | 8.66 b | 8.66 b | 8.45 b | 0.32 | 0.033   |
| P          | 2.41 a | 2.08 b | 2.04 b | 2.10 b | 2.06 b | 2.09 b | 2.04 b | 0.08 | 0.047   |
| Mg         | 3.80 a | 3.28 b | 3.22 b | 3.31 b | 3.23 b | 3.30 b | 3.22 b | 0.12 | 0.048   |
| Na         | 1.43 a | 1.24 b | 1.21 b | 1.25 b | 1.22 b | 1.24 b | 1.21 b | 0.05 | 0.021   |
| K          | 5.26 a | 4.54 b | 4.45 b | 4.58 b | 4.67 b | 4.58 b | 4.46 b | 0.17 | 0.017   |
| **Faecal excretion (g/day)** |    |     |      |     |    |     |      |     |         |
| Ca         | 2.11 a | 1.83 b | 1.36 d | 2.47 a | 1.08 d | 1.82 b | 1.50 c | 0.28 | 0.051   |
| P          | 1.36 a | 0.63 a | 1.00 c | 1.14 b | 1.05 c | 1.89 b | 0.57 a | 0.08 | <0.001  |
| Mg         | 0.94 a | 0.72 b | 0.57 d | 0.52 a | 0.68 b | 0.60 b | 0.32 c | 0.06 | <0.001  |
| Na         | 0.41 a | 0.37 ab | 0.30 bc | 0.35 ab | 0.33 b | 0.16 d | 0.20 c | 0.05 | 0.048   |
| K          | 1.33 ab | 0.90 d | 1.11 bc | 1.68 a | 1.06 c | 0.76 d | 0.69 d | 0.11 | 0.002   |
| **Urinary excretion (g/day)** |    |     |      |     |    |     |      |     |         |
| Ca         | 7.29 a | 3.77 bc | 2.77 c | 4.00 b | 3.98 bc | 3.41 b | 3.68 bc | 0.59 | 0.003   |
| P          | 0.49 a | 0.28 b | 0.22 bc | 0.50 a | 0.27 bc | 0.12 d | 0.07 c | 0.05 | <0.001  |
| Mg         | 0.78 a | 0.53 b | 0.24 d | 0.42 a | 0.30 d | 0.35 d | 0.39 b | 0.07 | 0.003   |
| Na         | 0.69 ab | 0.53 bc | 0.72 ab | 0.30 d | 0.36 d | 0.76 a | 0.81 b | 0.11 | 0.044   |
| K          | 3.71 a | 2.25 b | 1.35 c | 2.85 b | 2.08 b | 2.25 b | 2.17 b | 0.39 | 0.023   |
| **Apparent absorption (%)** |    |     |      |     |    |     |      |     |         |
| Ca         | 78.82 b | 78.50 b | 83.88 b | 70.75 b | 87.30 a | 78.97 b | 82.26 b | 3.70 | 0.135   |
| P          | 43.18 b | 68.67 a | 50.98 b | 45.42 b | 48.49 b | 43.35 b | 61.82 a | 4.23 | 0.004   |
| Mg         | 75.32 a | 78.06 a | 82.24 a | 84.39 a | 78.98 a | 81.74 b | 89.00 a | 1.52 | 0.001   |
| Na         | 72.41 a | 70.52 b | 75.02 b | 71.84 b | 72.82 a | 87.08 a | 83.84 a | 3.36 | 0.019   |
| K          | 75.03 a | 80.14 a | 75.10 b | 67.33 a | 76.22 a | 83.32 a | 84.66 a | 2.24 | 0.001   |
| **Mineral retention (%)** |    |     |      |     |    |     |      |     |         |
| Ca         | 5.60 d | 33.87 bc | 50.65 a | 24.79 a | 40.13 ab | 39.60 abc | 38.73 abc | 7.72 | 0.025   |
| P          | 23.09 c | 54.52 a | 40.13 b | 21.35 a | 38.66 b | 37.56 b | 58.31 a | 6.25 | 0.006   |
| Mg         | 55.33 d | 61.87 a | 74.64 ab | 71.70 b | 69.78 b | 71.20 b | 77.09 a | 2.59 | 0.001   |
| Na         | 24.25 bc | 28.61 abc | 14.77 c | 47.64 a | 43.45 b | 26.50 abc | 17.60 c | 11.28 | 0.036   |
| K          | 5.35 b | 29.29 a | 44.73 a | 5.70 b | 29.61 b | 34.25 b | 36.04 b | 7.68 | 0.017   |

Means with different superscript across the row are significantly different (P < 0.05); GI: W100%; GII: W70%; GIII: W50%; GIV: W70% + 3 g VC; GV: W50% + 3 g VC; GVI: W70% + 3 g VC + extra 5 g VC; GVII: W50% + 3 g VC + extra 5 g VC.
(McDowell 1992). In the current study, apparent absorption of Ca was higher than the threshold recommended, regardless of water restriction levels. The increase in apparent absorption of Na and K in the WR groups following multiple VC supplementations could probably facilitate Na flux in the fibroblast cells (Morla et al. 2016) because of the increased bioavailability of this vitamin (Hidirogloou et al. 1997). The more significant result of Na and K retention compared to absorption is similar to what was reported by Salles et al. (2008). The reason is that urine is the main excretion path for these minerals, and it is usually factored in the calculation for retention. Improvement in the amount of K and Na retained following water stress is very important in the mineral balance. This is because of their role in transporting substrates into and out of the cells and also in osmotic pressure regulation. A similar improvement in K retention was reported in heat stressed steers fed diets supplemented with monensin (Salles et al. 2008). Water restriction levels and multiple VC supplementations significantly improve the absorption and retention of Mg. This may be linked to decreased excretion through the urine.

## 5 Conclusions

Exposure of small ruminants to limited water affects nutrients intake and digestibility and can be more pronounced under high thermal load. Supplementation of VC at various doses failed to significantly improve the depression in digestibility and nutrients intake induced by water restriction. The mineral balance was affected by levels of water restriction, with higher losses of Ca and K observed in the urine than faeces, compared to other minerals.

### Acknowledgments

The authors express their gratitude to the late Prof. Voster Muchenje for his immense contributions towards the success of this project and also to Govan Mbeki Research and Development Centre, University of Fort Hare, South Africa for providing the financial assistance to publish this article.

### Funding information

This research was funded by NRF-TWAS Africa Renaissance Doctoral Award. Grant number 110851.

### Author contribution

OFA: conceptualization, funding acquisition, investigation, methodology, formal analysis, and writing—original draft; FNF and OO: writing – review and editing, and supervision; CTM: supervision.

### Conflict of interest

The authors state no conflict of interest.

### Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

### References

[1] Abdelatif AM, Elsayed SA, Hassan YM. Effect of state of hydration on body weight, blood constituents and urine excretion in Nubian Goats (*Capra hircus*). World J Agric Sci. 2010;6(2):178–88.

[2] Akinmoladun OF, Fon FN, Mpendulo CT, Okoh O. Performance, heat tolerance and blood metabolites of water restricted Xhosa goat supplemented with vitamin C. Transl Anim Sci. 2020;4(2):1–15.

[3] Akinmoladun OF, Muchenje V, Fon FN, Mpendulo CT. Small ruminants: farmers’ hope in a world threatened by water scarcity. Animals. 2019;9(7):456.

[4] Alamer M. Effect of water restriction on thermoregulation and some biochemical constituents in lactating Aardi goats during hot weather conditions. J Basic Appl Sci Res. 2010;11:189–205.

[5] Alemneh T, Akeberegn D. Adaptation strategies of farm animals to water shortage in desert areas. Am J Biomed Sci Res. 2019;2(6):245–50.

[6] Al-Ramamneh D, Riek A, Gerken M. Effect of water restriction on drinking behaviour and water intake of German black-head mutton sheep and Boer goats. Animal. 2012;6:173–8.

[7] Mpendulo CT, Akinmoladun OF, Ikusika OO, Chimonyo M. Effect of hydric stress on intake, growth performance and nutritional status of Nguni goats. Ital J Anim. 2020;19(1):1071–8.

[8] AOAC. Official methods of analysis of the official analytical chemists. In: Horwitz W (ed.). 17th edn. Washington DC USA: Association of Official Analytical Chemists; 2000.

[9] Banerjee GC. Feeds and principles of animal nutrition. Revised edition of animal nutrition. New Delhi: Oxford and IBH Publishing Co. Pvt. Ltd; 1998.

[10] Boswald IF, Dobenecker B, Clauss M, Kienzle E. A comparative meta-analysis on the relationship of faecal calcium and phosphorus excretion in mammals. J Anim Physiol Anim Nutr. 2017;2017(102):370–9.

[11] Broderick GA, Kang JH. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. J Dairy Sci. 1980;63:64–74.

[12] Casassamassima D, Vizzarri F, Nardola M, Palazzo M. Effect of water restriction on various physiological variables on intensively reared Lacauna ewes. Vet Med. 2016;6:623–34.

[13] Chambial S, Dwivedi S, Shukla KK, John PJ, Sharma P. Vitamin C in disease prevention and cure: an overview. Indian J Clin Biochem. 2013;28(4):314–28.
[14] Doreau M, Corson MS, Wiedemann SG. Water use by livestock: a global perspective for a regional issue? Anim Front. 2012;2(2):9–16.

[15] Ghanem AM, Barbour EK, Hamadeh SK, Jaber LS, Abi Said M. Physiological and chemical responses in water-deprived Awassi ewes treated with vitamin C. J Arid Environ. 2008;72:341–9.

[16] Givens DI, Oxford RFE, Omed HM. Forage evaluation in East African ruminants. Wallingford, Oxon (UK): CAB International; 2000.

[17] Hall MB. Heat stress alters ruminal fermentation and digesta characteristics, and behavior in lactating dairy cattle. In: Chilliard Y, Glasser F, Faulconnier Y, Bocquier F, Veissier I, Doreau M (eds.). Proceeding of 11th international symposium on ruminant physiology. Wageningen, The Netherlands: Wageningen Academic Publ; 2009. p. 204.

[18] Hidiroglou M, Batra TR, Zhao X. Comparison of vitamin C bioavailability after multiple or single oral dosing of different formulations in sheep. Reprod Nutr Dev. 1997;37:443–8.

[19] Hussein A, Puchala R, Gipson T, Tadesse D, Wilson B, Husseini A, Puchala R, Gipson T, Tadesse D, Wilson B, Doreau M, Corson MS, Wiedemann SG. Water use by livestock: a global perspective for a regional issue? Anim Front. 2013. p. 115

[20] Intergovernmental Panel on Climate Change (IPCC). Synthesis report. In: Pachauri RK, Reisinger A (eds.). Contribution of working groups I, II and III to the fourth assessment report of the Intergovernmental Panel on Climate Change. Geneva, Switzerland: IPCC; 2007. p. 104.

[21] Jaber LS, Chedid M, Hamadeh S. Water stress in small ruminants. Jaber LS, Hanna N, Barbour N, Abi Said EK, Rawda M, Chedid N, Mathews C, Crispie F, Lewis E, Reid M, Omid HM. Forage evaluation in East African ruminants. J Arid Environ. 2011;75:625–33.

[22] Marai IFM, El Kanga HJ, Piao MY, Park SJ, Na SW, Kim HJ, Baik M. Effects of heat stress and rumen-protected fat supplementation on growth performance, rumen characteristics, and blood parameters in growing Korean cattle steers. Asian Austral J Anim. 2019;32(6):826–33.

[23] Jaber LS, Hanna N, Barbour N, Abi Said EK, Rawda M, Chedid N, et al. mobilization in water restricted Awassi ewe supplemented with vitamin C. J Arid Environ. 2011;75:625–6.

[24] Kang HJ, Piao MY, Park SJ, Na SW, Kim HJ, Baik M. Effects of heat stress and rumen-protected fat supplementation on growth performance, rumen characteristics, and blood parameters in growing Korean cattle steers. Asian Austral J Anim. 2019;32(6):826–33.

[25] Kassab AY, Mohammed AA. Ascorbic acid administration as anti-stress before transportation of sheep. Egyptian J Anim Prod. 2014;51(1):19–25.

[26] Langhans W, Scharre E, Meyer AH. Changes in feeding behaviour and plasma vasopressin concentration during water deprivation in goats. J Vet Med. 1991;38:11–20.

[27] Malooy GMO, Kanui TI, Towett PK, Wambugu SN, Miron JO, Wanyolke MM. Effects of dehydration and heat stress on food intake and dry matter digestibility in East African ruminants. Comp Biochem Physiol. 2008;151:185–90.

[28] Maral IFM, El Darawawy AA, Fadiel A, Abdel-Hafez MAM. Physiological traits as affected by heat stress in sheep – a review. Small Rumin Res. 2007;71:1–12.

[29] Marino R, Alzori AS, D’Andrea M, Iovane G, Trabalza-Marinucci M, Rinaldi L. Climate change: production performance, health issues, greenhouse gas emissions and mitigation strategies in sheep and goat farming. Small Rumin Res. 2016;135:50–9.

[30] Mathews C, Crispie F, Lewis E, Reid M, O’toole PW, Cotter PD. The rumen microbiome: a crucial consideration when optimizing milk and meat production and nitrogen utilization efficiency. Asian Austral J Anim. 2019;10(2):115–32.

[31] McDowell LR. Minerals in animal and human nutrition. San Diego: Academic Press; 1992.

[32] Meschy F. Recent progress in the assessment of minerals requirements of goats. Livest Prod Sci. 2000;64:9–14.

[33] Morla L, Edwards A, Crambert G. New insights into sodium regulation in the distal nephron: Role of G-protein coupled receptors. World J Bio Chem. 2016;7(1):44–63.

[34] Moyo S, Swanepoel FJC. Multifunctionality of livestock in developing communities. In: Swanepoel F, Stroebel A and Moyo S (eds.). The role of livestock in developing communities: enhancing multifunctionality. South Africa: The Technological Centre for Agricultural and Rural Cooperation (CTA) and University of the Free State; 2010.

[35] Naqqi SMK, Kumar D, De K, Seijan V. Climate change and water availability for livestock: impact on both quality and quantity. In: Seijan V, Gaughan J, Baumgard L, Prasad C, eds., Climate change impact on livestock: adaptation and mitigation. New Delhi: Springer; 2015.

[36] National Research Council (NRC). Nutrient requirements of dairy cattle. 7th rev. edn. Washington, DC: National Academy Press; 2001.

[37] National Research Council (NRC). Nutrient requirements of goats: angora, dairy, and meat goats in temperate and tropical countries. Washington, DC: National Academy of Sciences. National Academic Press; 1981.

[38] Nejad GJ, Lohakare JD, West JW, Sung KI. Effects of water restriction after feeding during heat stress on nutrient digestibility, nitrogen balance, blood profile and characteristics in Corriedale ewes. Anim Feed Sci Tech. 2014;193:1–8.

[39] Richter EL, Drewoski ME, Hansen SL. The effect of dietary sulfur on performance, mineral status, rumen hydrogen sulfide, and rumen microbial populations in yearling beef steers. J Anim Sci. 2012;90:3945–53.

[40] Salles MSV, Zanetti MN, Salles FA. Effect of monensin on mineral balance in growing ruminants reared under different environmental temperatures. Anim Feed Sci Tech. 2008;141:233–45.

[41] SAS Institute. SAS/STAT user’s guide, statistics, version 8. Cary, NC: SAS Institute; 2001.

[42] Schulze RE, Lynch SD, Maharaj M. Annual precipitation. In: Schulze RE, ed. South African atlas of climatology and agrohydrology. Water research commission report 1489/1/06, section 6.2. Pretoria: Water Research Commission; 2006.

[43] Schwartz GJ, Furth SL. Glomerular filtration rate measurement and estimation in chronic kidney disease. Pediatr Nephrol. 2007;22:1839–48.

[44] Suttle NF. Mineral nutrition of livestock. 4th ed. London, UK: Cabi; 2010.

[45] Tan BL, Norhaizan ME, Liew WP, Sulaiman RH. Antioxidant and oxidative stress: a mutual interplay in age-related diseases. Front Pharmacol. 2018;9:1162.

[46] Thang TV, Sunagawa K, Nagamine I, Kishi T, Ogura GA. Physiological stimulating factor of water intake during and after forage feeding in large-type goats. Asian Austral J Anim. 2012;25:502–14.