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Abstract:	Water available to livestock in the tropical lowlands region is generally high in acidity. Therefore, the effects of acid water were investigated in this study. Nine Kacang goats were stratified based on body weight (BW) and then assigned to three treatment groups: 6.9, 5.2, and 3.8 which were offered drinking water varying pH levels: 6.9, 5.2, and 3.8, respectively. All goats were offered ad libitum Asystasia gangetica hay and dried cassava chips at 1% of BW (dry matter (DM) basis) followed a crossover design with three treatments tested in three periods. Total DM intake (%BW) was lowered ($P < 0.05$) as lower drinking water intake (DWI) ($P = 0.09$) at the water pH of 5.2. Ruminal pH also declined (6.98, 6.94, and 6.58 at the pH levels of 6.9, 5.2, and 3.8, respectively) ($P < 0.01$). Metabolizable energy and daily gain tended to be higher at 6.9 and 3.8 pH levels compared to those at pH 5.2 level ($P = 0.08$). There were no significant adverse effects of acid water on nutrient intake, utilization, and growth. Moreover, elevated ambient temperature was followed by the increased DWI ($P < 0.01$) at 6.9 pH level, but no such significant relationship was found at other pH levels that indicate a better capability of thermoregulation response under a high-temperature exposure.
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- A trial with 6.9, 5.2, and 3.8 pH levels of drinking water was conducted
- Ruminal pH was declined by acid drinking water
- No adverse effects of the acid water on nutrient intake, utilization, and growth
- Water intake correlated with maximum ambient temperature at 6.9 pH level

Conflict of Interest Statement

The authors declare that they have no competing interests.

Effects of acid drinking water on nutrient utilization, water balance, and growth of goats under hot humid tropical environment

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Abstract

Water available to livestock in the tropical lowlands region is generally high in acidity. Therefore, the effects of acid water were investigated in this study. Nine Kacang goats were stratified based on body weight (BW) and then assigned to three treatment groups: 6.9, 5.2, and 3.8 which were offered drinking water varying pH levels: 6.9, 5.2, and 3.8, respectively. All goats were offered *ad libitum Asystasia gangetica* hay and dried cassava chips at 1% of BW (dry matter (DM) basis) followed a crossover design with three treatments tested in three periods. Total DM intake (%BW) was lowered ($P < 0.05$) as lower drinking water intake (DWI) ($P = 0.09$) at the water pH of 5.2. Ruminal pH also declined (6.98, 6.94, and 6.58 at the pH levels of 6.9, 5.2, and 3.8, respectively) ($P < 0.01$). Metabolizable energy and daily gain tended to be higher at 6.9 and 3.8 pH levels compared to those at pH 5.2 level ($P = 0.08$). There were no significant adverse effects of acid water on nutrient intake, utilization, and growth. Moreover, elevated ambient temperature was followed by the

increased DWI ($P < 0.01$) at 6.9 pH level, but no such significant relationship was found at other pH levels that indicate a better capability of thermoregulation response under a high-temperature exposure.

Keyword acid drinking water, ruminal pH, livestock, high ambient temperature

1. Introduction

Water is one of the most important nutrients in the animal body since it plays important physiological roles related to nutrient transport, maintenance of proper fluid and ion balance, biochemical reactions, and body thermoregulation. A sufficient supply of good quality water is often considered as a limiting factor for all animals to maintain their health and optimal productivity (NRC, 2001). However, the supply of clean water resources is decreasing trend globally, driven by population and economic growth. In the next decades, there would be additional pressure on water resources to meet the elevated demand of agriculture, household use, and industry. Moreover, the adequate supply of clean water is challenged by extreme weather events due to climate change (Boretti and Rosa, 2019).

In humid tropical lowlands, most of the water is characterized by high acidity due to natural oxidation processes of pyrite and ferric ion. The pH of the surface water could drop to 3 and the most potential contaminants are sulfate (SO_4), iron (Fe), manganese (Mn), and aluminum (Al) (Manders et al., 2002; Sahrawat, 2004). Another water source in the lowland region is groundwater where the water has less acidity and contaminants (Winkel et al., 2008). Whilst recommended minimum pH levels for livestock is 5.5 (Bagley et al., 1997) or 6.0 (Olkowski, 2009), the effects of the acid water on ruminant animals have not been clearly documented. Therefore, it is necessary to identify the effects of the water on the animal's performance. The present study will have significant implications for water quality standards and for intervention options for the animal particularly reared in the lowland region. Thus, the

hypothesis was planned to elucidate the impact of acid water on water consumption, nutrient intake, and utilization under hot tropical climates.

2. Materials and Methods

2.1. Study site

Approval of the experiment was granted from the Faculty of Agriculture, Universitas Sriwijaya, Indonesia. The site is situated at an altitude of ±6 m above sea level and 3°11'38.4"S, 104°39'30.5"E. The animals were cared for according to the Animal Welfare Guidelines of the Indonesian Institute of Sciences. The environmental variables in the site (indoor temperature and relative humidity, RH), rainfall, sunshine, and wind speed are shown in Table 1.

2.2. Experimental animal, treatments, and feeding management

Nine Kacang goats, based on body weight (BW), were stratified and then assigned to three treatment groups. Animals in different treatment groups of 6.9, 5.2, and 3.8 were offered drinking water with varying levels of pH i.e: 6.9, 5.2, and 3.8, respectively. The animals were treated orally with Oxfendazole (25 mg/5 kg BW) and housed in individual pens (1.5 m × 0.75 m) in an open-sided type of house. Each pen was equipped with two identical feed troughs and an individual water bucket (diameter 23 cm, 5 L capacity). Goats were acclimatized to feeding and environmental conditions for 15 d and then subjected to the respective water treatments. All animals were weighed at the beginning of the study and then on every Sunday and Thursday to know changes in the BW on an electronic weighing balance before offering feed and water.

The experimental design was a crossover design that consisted of three levels of pH in three periods. Each experimental period lasted for four weeks with three weeks of adaptation followed by one week of sample collection where feed intake along with feces and urine

excretion were measured. Each measurement period was followed by one week of recovery where all animals received only pH 6.9 drinking water.

Diet consisted of *Asystasia gangetica* hay and dried cassava chips (Table 2). The hay was harvested at the pre blooming stage and chaffed to ±5 cm particle length and then sun-dried for 4 d. Cassava tubers were chapped to ±2 cm particle size and then sun-dried for 5 d. Feeding and drinking started at 9:00 after refusals from the previous day had been removed and weighed. The hay was offered ad libitum, according to 15% of the previous intake, while the amount of cassava chips was referred to 1% of individual BW and was adjusted after each BW measurement. Animals always had *ad libitum* access to salt-mineral lick and drinking water.

2.3. Preparation of different pH levels of water

Naturally available high acidity surface water was collected from non-tidal swamp area (3°10'29.7"S, 104°41'34.5"E) while the underground water (pH= 5.2) was collected from a well in the experimental site. The swamp water was manually collected using a 20-L bucket while the well water was pumped from the well. The swamp water had an acidulous taste and a 3.8 pH level. The level of pH was checked using a portable pH meter (Hanna HI 98130). The pH level 6.9 water was prepared from the well water by aeration for 4 d in a 50-L bucket using an aerator (Amara BS-410). Before the offering, each of the water was stored in separate 50-L buckets.

2.4. Sample collection, preparation, and analysis

The indoor temperature and RH were recorded by a climate data logger (Benetech G1365) at a 10-minutes interval. In addition, Rainfall, sunshine, and wind speed were taken at a meteorological station.

After weighing, refusals were homogenized and a subsample (~100 g) was taken and stored in paper bags at room temperature. Samples of the offered feeds were taken every

week and stored as for the refusals. The offered diet and refusals were homogenized and subsamples retained for processing and analysis at the end of each period. Total fecal and urinary excretion was determined by daily collection over 7 d. Total feces excreted by each animal was thoroughly mixed by hand, weighed, and a subsample of ~100 g fresh matter was taken and then dried at 45°C for three consecutive days. Dried feed and fecal samples were ground to pass a 1-mm mesh. At the end of each period, the feed and fecal samples were pooled per animal proportionally to the daily amount of each animal during the sampling week. The dried samples were stored in zipper plastic bags prior to laboratory analyses.

Each animal's total daily urine was homogenized and urine volume was measured then recorded after homogenizing and filtering with a surgical gaze. A sample of urine (~100 mL) was taken daily and stored at -20 °C for N analysis. The water sample was collected every week and stored in a 250-mL bottle at 5 °C. At the end of each period, the samples were pooled proportionally and then analyzed.

To measure rumen fluid pH, the animals were not supplied with drinking water for two h prior to the fluid collection. The fluid was collected using a stomach tube (diameter 6 mm) at one h after the goats consumed the water.

The dried feces, feed, and refusals were analyzed as follows: DM, ash (AOAC, 1990; Method 924.05), N (AOAC, 1990; Method 988.05), ether extract (EE; Method 920.39), NDF, and acid detergent fiber (ADF) with alpha-amylase and including residual ash (Van Soest et al., 1991). The DM content of urine was determined by drying a 3 mL urine sample at 60 °C for 12 h. Total N in urine samples was determined by the micro Kjeldahl method (AOAC, 1990; Method 988.05). Neutral detergent-insoluble N (NDIN) and Neutral detergent-insoluble ash (NDIash) were estimated according to Licitra et al. (1996). Water samples were analyzed for TDS (conductivity method, Orion Star A212, Thermo Scientific), Fe, Mn, Al (spectrometric techniques, inductively coupled plasma atomic emission spectroscopy Varian

715-ES, Agilent), nitrate (NO_3), nitrite (NO_2), ammonia (NH_3), sulfate (SO_4), hydrogen sulfide (H_2S) (spectrometric techniques, Spectrophotometer UV-VIS Lambda 45, Perkin Elmer), organic substances (permanganometric titration method).

2.5. Data calculation and statistical analysis

Organic matter (OM) concentrations were calculated by subtracting the ash concentration from 100, while the CP content was calculated as N×6.25. Neutral detergent fiber corrected for ash and crude protein (NDF_{acp}) was calculated by subtracting the NDIN and NDIash. Non fibrous carbohydrates (NFC) was calculated by subtracting the concentration of NDF_{acp}, CP, EE, and ash from 100 (Mertens, 1997).

Daily feed intake was calculated as the difference between the amount of feed offered and the amount of feed refusals for each animal across the sampling week. Individual drinking water intake (DWI) was calculated as the difference between the amount of water offered and left in the bucket. Three buckets with water were placed in the barn to estimate daily evaporative water loss, and then the daily DWI was corrected by the evaporative loss. The amount of water in the consumed feed (FWI) was calculated by the difference between the amount of water in the feed offered and refusals. Metabolic water was estimated using the factors 0.62, 0.42, and 1.10 for digestible carbohydrates, protein, and fat, respectively (Taylor, 1970). Apparent total water intake (TWI) was determined as the sum of DWI, FWI, and metabolic water. Fecal water was obtained from the amount of fecal excretion and the content of water. The amount of urinary water was the amount of urine corrected by the DM content of urine. Water retention was calculated by subtracting the amount of water in fecal and urinary excretion by the amount of TWI.

Metabolizable energy (ME, MJ/kg) content was calculated based on (AFRC, 1993) using digestible organic matter content in intake (g/kg DM). Total tract apparent digestibility of DM, OM, NDF, and ADF were obtained from the difference between the number of

nutrient ingested and the quantity of nutrients excreted in feces over the 7 d of sampling week. Nitrogen absorption was calculated by subtracting fecal N excretion by the amount of N intake (feed and DWI), whilst N retention was calculated by subtracting the amount of urinary N loss by the amount of absorbed N.

The data generated from 3 treatments, 3 periods, and 9 animals were analyzed using SAS 9.1 and presented as mean ± standard error. Data were analyzed by the mixed model procedure using the following model:

$$Y_{ijk}=\mu + T_i + P_j + TP_{ij} + a_k + e_{ijk};$$

Where Y_{ijk} is observed response at a particular *ijk* case; μ is overall mean; *Ti* is the fixed effect of treatment *i*; *Pj* is the fixed effect of period *j*; *TPij* is the fixed effect of the interaction between treatment *i* and period *j*; a_k is the random effect of animal *k*; and e_{ijk} is experimental error.

Differences between means were determined using the Tukey test. The Significance level was declared at $p < 0.05$, where p-values of 0.05 to 0.10 were considered as a trend. The relationship between the daily maximum (T_{max}) and average (T_{av}) temperature and DWI and DM intake during the collection weeks was tested by Pearson correlation analysis.

3. Results

The composition of drinking water offered to animals in different treatment groups showed increases in Fe, Mn, Al, NH_3, SO_4, and organic substances with the decrease in pH level. Nitrate was the lowest at 5.2 pH level, whereas for NO_3 and NO_2, the highest concentrations were found at 3.8 pH level (Table 3). Table 4 presents feed intake, nutrients digestibility, rumen pH, and daily gain of the goats. Total DM intake in the 5.2 group was lower ($P < 0.05$) than those subjected to the other treatments that comparable to the lower ($P < 0.05$) DM intake of hay (%BW) in the group. Metabolizable energy intake (MJ/kg $BW^{0.75}$) and daily gain were only influenced by trends ($P = 0.06$). Rumen pH was lowered ($P < 0.01$)

as the decrease of pH level where the pH in the 3.8 group was lower than those in the 6.9 and 5.2 group. Apparent DM, OM, NDF, and ADF digestibility were not significantly different (*P* > 0.05).

Drinking water intake and FWI (%BW) were tended to be lowered at the 5.2 group (P = 0.09) but metabolic water and TWI were not influenced (P > 0.05). Fecal water excretion (%BW) was lowered (P < 0.05) at the 5.2 group, which was not different from those on the 6.9 group (P > 0.05) but higher than those at the 3.8 group. Urinary water excretion and apparent water retention were not significantly affected by the pH level (P > 0.05) (Table 5). Intake of N (%BW) and fecal N excretion (g/d) were also lowered at 5.2 level. However, N absorption, urinary N excretion, and N retention did not vary among the different groups (P > 0.05) (Table 6).

During the collection weeks, maximum ambient temperature (Tmax) had a positive correlation with DWI of the 6.9 group but not of the 5.2 and 3.8 groups. . Dry matter intake did not significantly correlate with T_{max} among all the groups (P > 0.05). Ratio DWI/DMI had a positive correlation with T_{max} in the 6.9 group (P < 0.01), while in 3.8 group, the ratio tended to be correlated (P = 0.09). Positive correlations were also found in group 6.9 for T_{av} with DWI and ratio DWI/MWI, while in the group 3.8 a negative correlation was significant for T_{av} with DMI (%BW) (Table 7).

4. Discussion

The decreased DM intake has likely resulted from the lower DWI at 5.2 pH level. Water contaminant concentrations were different among the different pH levels of drinking water. However, the tendency of lower DWI in the 5.2 pH group could not be associated with the contaminant concentrations in the water where the higher concentrations were found in the 3.8 pH group compared to the 5.2 pH group. Referred to the maximum limits of contaminants concentrations in the drinking water, concentrations of total dissolved solids

(TDS), Fe, NO_3, NO_2, SO_4 were much lower (Table 3). Besides contaminants concentrations, the intake level of DWI might be more related to the palatability of the water. A Similar decrease in DWI at a lower level of contaminant was also reported (Sharma et al., 2017) for buffalo calves on five TDS levels in drinking water where the DWI was lower at 557 levels than those at 2571 mg/L level.

The rumen pH was declined by the acid drinking water in the present study, but was still in the normal range. Acid drinking water may cause rumen acidosis (Olkowski, 2009) when the rumen pH less than 5.5 (Morgante et al., 2007; O'Grady et al., 2008). However, the rumen pH values at the pH levels of 5.2 and 3.8 in this experiment increased to the normal range at 1 h post-drinking (Table 4). The animals' normal eating and ruminating behavior during the experiment and the sufficiency of the minerals-salt supplement might indicate a normal secretion of saliva to maintain the range of rumen pH when the animal continuously consumed the acid drinking water. As a result, the nutrients' digestibility did not affect. A similar OM and NDF digestibility was also reported when the ruminal pH was decreased from 7.0 to 6.2 (Shriver et al., 1986).

The daily gain was only affected by a trend ($P = 0.06$), although the gain of goats at the 5.2 level was 48 and 29% lower than those at the 6.9 and 3.8 levels, respectively. Similarly, a higher N retention of the goats at the 6.9 level did not significantly differ from those on the 5.2 and 3.8 levels that likely due to a higher standard deviation (Table 6). Thus, the positive gain and N retention along with feed intake and nutrients digestibility indicate that the acid water did not have detrimental effects on the goat performances.

The positive correlation for T_{max} - DWI, T_{max} - DWI/DMI, and T_{av} - DWI (Table 7) might be due to an increased demand for water by the goats under a higher ambient temperature in response to a higher loss of water through evaporation and sweating although this only applied for the 6.9 group. In e group 3.8, this was only shown by a trend for T_{max} -

DWI/DMI. A positive correlation for T_{max} - DWI was also reported for buffalo calves on five levels of TDS in drinking water (Sharma et al., 2017), while positive correlations of T_{max} – DWI and T_{av} – DWI (%BW) were also reported for lactating (Olsson and Dahlborn, 1989) and goat kids (Al-Tamimi, 2007).

When ambient temperature increased from 20 to 32^{o}C, DWI increased by 63% in cattle (Olkowski, 2009), while Gengler et al. (1970) reported an 80% increase of DWI when the temperature increased 18 to 35 ^{0}C. By plotting the DWI intake again T_{max} at 28 and 36 ^{o}C, the increases of DWI were 69% in the 6.9 group. The drinking water was offered at ad libitum level in the present study. Therefore, the animals could freely fulfill the additional requirement of water for the thermoregulation proceses. The stronger correlations in the 6.9 group reflect an important aspect of clean and good palatability water for maximum intake when the animals under high ambient temperature.

5. Conclusions

In conclusions, the effect of lowering pH level in drinking water always relates to the concentration of contaminants in the water. In the present study, the lowering pH level from 6.9 to 3.8 level did not result in adverse effects on the nutrient intake, balance, and growth due to the minimum levels of the contaminants in the water and the animal's ability to maintain the water range of normal rumen pH. However, the better ability of the animal in the 6.9 group to the high temperature was evidenced by the positive correlation between drinking water intake and ambient temperature. A further study with a more extended period of acid drinking water offering with thermoregulation and drinking behavior responses of the animals on the different pH levels is needed.

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Author contribution

Original intellectual concept and study design: A. I. M. Ali; Methodology: A. I. M. Ali, S. Sandi; Data curation, formal analysis, and investigation: E. Sahara, A. I. M. Ali; Writing - original draft preparation: A. I. M. Ali; Writing - review and editing: M. N. Rofiq, Dahlanuddin; Funding acquisition: A. I. M. Ali. All authors read and approved the final manuscript.

Data availability

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

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Tables

Table 1

Environmental variables observed during the trial.

Variable	Mean ± standard error	Range
Maximum temperature (T_{max}) (°C)	32.9 ± 0.20	28.4 - 36.8
Minimum temperature (T_{min}) (°C)	24.7 ± 0.10	23.0 - 26.4
Maximum relative humidity (%)	91.6 ± 0.26	86.5 - 94.7
Minimum relative humidity (%)	65.0 ± 0.86	54.6 - 87.5
Rainfall (mm/d)	3.4 ± 1.21	0.0 - 36.2
Sunshine (h)	5.4 ± 0.34	0.0 - 9.7
Wind speed (m/s)	1.8 ± 0.09	1.0 - 3.0

Table 2

Chemical composition (mean ± standard error) of Chinese violet (*Asystasia gangetica*) hay and cassava chips offered during the trial

	Chinese vioet hay	Cassava chips
Dry matter	88.4 ± 0.70	88.3 ± 1.06
Organic matter	89.8 ± 0.11	97.9 ± 0.13
Crude protein	14.3 ± 0.36	4.2 ± 0.25
Ether extract	1.7 ± 0.04	0.3 ± 0.02
Ash	10.2 ± 0.50	2.1 ± 0.13
Non fibrous carbohydrates[a]	27.6 ± 0.98	72.9 ± 1.50
Neutral detergent fiber	48.1 ± 0.75	20.8 ± 0.07
Neutral detergent fiber $_{acp}$[b]	46.2 ± 0.71	21.9 ± 0.08
Acid detergent fiber	30.5 ± 0.24	4.0 ± 0.18
Acid detergent lignin	14.9 ± 0.12	1.5 ± 0.07

[a]100-CP (%)-EE (%)-NDF (%)-NDICP (%)-TA (%).

[b]Neutral detergent fiber corrected for ash and crude protein.

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Table 3

Concentrations of contaminant substances (mg/L, mean ± standard error) in drinking water offered to treatment groups and their permissible limits

Element	Treatment groups			Permissible limits
	6.9	5.2	3.8	
Total dissolved solids	51.0 ± 2.31	48.3 ± 2.96	87.7± 8.67	4000[a], 3000[b]
Iron	0.008 ± 0.002	0.010 ± 0.000	0.223± 0.074	2
Manganese	0.001 ± 0.001	0.004 ± 0.003	0.027± 0.003	0.3[b]
Aluminum	0.014 ± 0.003	0.036 ± 0.001	2.870± 0.067	NA
Nitrate	14.1 ± 3.52	12.8 ± 0.51	24.8± 1.03	100[a], 77[b]
Nitrite	0.01 ± 0.011	0.02 ± 0.022	0.02± 0.02	33[a], 10[b]
Ammonia	0.27 ± 0.033	0.30 ± 0.058	0.47± 0.033	NA
Sulfate	3.3 ± 1.67	5.4 ± 2.11	25.6± 5.66	500[a], 1000[b]
Hydrogen sulfide	ND	ND	ND	NA
Organic substances	1.9 ± 0.07	1.7 ± 0.16	2.6± 0.28	NA
pH	6.9 ± 0.03	5.2 ± 0.06	3.8± 0.02	5.5[a], 6.0[b]

Minimum limit for pH and maximum limits for other elements based on United States Environmental Protection Agency (Bagley et al., 1997)[a] and Canadian Council of Ministers of the Environment (Olkowski, 2009)[b] for Livestock;

ND: not detected;

NA: not available

320

Table 4

Dry matter (DM) intake, metabolizable energy (ME) intake, digestibility of DM, organic matter (OM), neutral detergent fiber (NDF), and acid detergent fiber (ADF), rumen pH, and daily gain (mean ± standard error) of Kacang goats offered water having different pH levels

Parameter	pH level			*P*-value
	6.9	5.2	3.8	
Chinese violet hay				
g DM/d	389 ± 36.6	332 ± 32.5	390 ± 48.3	0.154
%BW	2.1 ± 0.15^{b}	1.8 ± 0.13^{a}	2.1 ± 0.17^{b}	0.035
Cassava chips				
g DM/d	159 ± 15.2	166 ± 15.6	158 ± 11.3	0.715
%BW	0.9 ± 0.06	0.9 ± 0.05	0.9 ± 0.05	0.683
Total DM intake				
g/d	548 ± 41.8^{B}	498 ± 39.9^{A}	549 ± 49.6^{B}	0.078
%BW	3.0 ± 0.13^{b}	2.7 ± 0.11^{a}	2.9 ± 0.13^{b}	0.026
ME intake				
(MJ/d)	5.8 ± 0.44	5.3 ± 0.40	5.8 ± 0.43	0.137
MJ/kg BW$^{0.75}$	0.65 ± 0.03^{B}	0.59 ± 0.02^{A}	0.64± 0.02^{B}	0.078
Digestibility (%)				
DM	68.1 ± 0.94	68.5 ± 0.99	67.7 ± 1.21	0.379
OM	67.9 ± 1.04	68.5 ± 1.04	67.5 ± 1.28	0.339
NDF	41.6 ± 1.61	41.9 ± 2.06	40.3 ± 2.46	0.448
ADF	23.4 ± 2.55	19.8 ± 3.91	23.6 ± 2.95	0.866

Rumen pH	6.98 ± 0.06^{b}	6.94 ± 0.05^{b}	6.58 ± 0.08^{a}	0.002
Daily gain (g/d)	73.4 ± 8.74^{B}	49.7 ± 8.42^{A}	64.2 ± 6.16^{AB}	0.062

Means within the same row with different superscripts are significantly different ($P < 0.05$); Means within the same row with different uppercase superscripts tended to differ at $0.05 \leq P < 0.10$;

BW: body weight

Table 5

Water balance (mean ± standard error) of Kacang goats offered water having different pH levels

Parameter	pH level			*P*-value
	6.9	5.2	3.8	
Drinking water intake				
ml/d	1456 ± 173	1218 ± 118	1460 ± 173	0.243
%BW	7.8 ± 0.59^{B}	6.6 ± 0.58^{A}	7.7 ± 0.55^{B}	0.091
Feed water intake				
ml/d	83.9 ± 6.64^{B}	73.6 ± 5.54^{A}	82.4 ± 7.07^{B}	0.091
%BW	0.45 ± 0.02	0.40 ± 0.02	0.44 ± 0.02	0.056
Metabolic water				
ml/d	209.2 ± 15.8	191.6 ± 14.4	206.2 ± 14.6	0.330
%BW	1.13 ± 0.05	1.02 ± 0.04	1.11 ± 0.03	0.186
Total water intake				
ml/d	1750 ± 192	1484 ± 133	1749 ± 192	0.231
%BW	9.4 ± 0.63	8.0 ± 0.63	9.3 ± 0.58	0.187
Faecal water excretion				
ml/d	261 ± 32.4^{AB}	202 ± 21.9^{A}	277 ± 45.5^{B}	0.055
%BW	1.4 ± 0.15^{ab}	1.1 ± 0.08^{a}	1.4 ± 0.17^{b}	0.034
Urinary water excretion				
ml/d	418 ± 56.2	321 ± 37.6	385 ± 66.4	0.392
%BW	2.3 ± 0.24	1.8 ± 0.21	2.0 ± 0.23	0.397
Apparent water retention				

ml/d	1070 ± 132.1	960 ± 97.9	1087 ± 88.4	0.421
%BW	5.7 ± 0.45	5.2 ± 0.49	5.8 ± 0.27	0.406

Means within the same row with different superscripts are significantly different ($P < 0.05$); Means within the same row with different uppercase superscripts tended to differ at $0.05 \leq P < 0.10$;

BW: body weight

Table 6

Nitrogen (N) balance (mean±standard error) of Kacang goats offered water having different pH levels

Parameter	pH level			*P*-value
	6.9	5.2	3.8	
N intake				
g/day	10.4 ± 0.907^{B}	9.0 ± 0.812^{A}	10.3 ± 1.112^{AB}	0.074
%BW	0.056 ± 0.003^{b}	0.048 ± 0.003^{a}	0.055 ± 0.004^{ab}	0.036
Fecal N				
g/d	4.41 ± 0.403^{b}	3.84 ± 0.357^{a}	4.43 ± 0.527^{b}	0.037
%BW	0.024 ± 0.002^{B}	0.020 ± 0.001^{A}	0.024 ± 0.002^{AB}	0.062
N absorb				
g/day	5.98 ± 0.526	5.18 ± 0.487	5.87 ± 0.620	0.313
%BW	0.03 ± 0.002	0.03 ± 0.002	0.03 ± 0.002	0.240
Urinary N				
g/day	3.32 ± 0.615	2.80 ± 0.413	3.10 ± 0.698	0.531
%BW	0.02 ± 0.003	0.02 ± 0.002	0.02 ± 0.003	0.469
N retention				
g/day	2.66 ± 0.542	2.38 ± 0.465	2.78 ± 0.439	0.789
%BW	0.01 ± 0.003	0.01 ± 0.002	0.02 ± 0.002	0.728

Means within the same row with different superscripts are significantly different ($P < 0.05$);

Means within the same row with different uppercase superscripts tended to differ at $0.05 \leq P < 0.10$;

BW: body weight

Table 7

Pearson correlation coefficients and significance levels[1] of the relationship between daily maximum (T_{max}) and average (T_{av}) temperature and drinking water intake (DWI) and dry matter intake (DMI) in Kacang goats offered water having different pH levels

Parameter	pH level		
	6.9	5.2	3.8
T_{max} - DWI			
ml/d	0.61 **	0.28 n.s.	0.18 n.s.
%BW	0.52 *	0.02 n.s.	0.18 n.s.
T_{max} - DMI			
g/d	0.04 n.s.	0.20 n.s.	-0.28 n.s.
%BW	-0.18 n.s.	-0.30 n.s.	-0.29 n.s.
T_{max} - DWI/DMI	0.59 **	0.13 n.s.	0.38 (*)
T_{av} - DWI			
ml/d	0.60 **	0.32 n.s.	-0.14 n.s.
%BW	0.52 *	-0.01 n.s.	-0.13 n.s.
T_{av} - DMI			
g/d	0.17 n.s.	0.29 n.s.	-0.45 (*)
%BW	-0.10 n.s.	-0.29 n.s.	-0.46 (*)
T_{av} - DWI/DMI	0.55 **	0.11 n.s.	0.08 n.s.

[1] Significance levels: n.s., not significant, (*) $p \leq 0.10$, *$p \leq 0.05$, **$p \leq 0.01$;

BW: bodyweight

Asep Indra Munawar Ali fp <asep_ali@fp.unsri.ac.id>

Rumin-D-21-539 Revision Requested

RUMIN <em@editorialmanager.com> 25 Oktober 2021 pukul 15.13
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Ms. No. Rumin-D-21-539
Effects of acid drinking water on nutrient utilization, water balance, and growth of goats under hot humid tropical environment
Small Ruminant Research

Dear Dr. Ali,

I can now inform you that the Editorial Board has evaluated your manuscript. The Editor has advised that the manuscript will be reconsidered for publication after major revision.
Besides the comments of the reviewers, you are kindly asked to pay a great attention to revise the final version with a native english speaker. I have also some minor comments:
How was water acidified?
What means RH at l. 95?
In Table 2, what is the unit: DM or as fed?
In Table 3, Statistics are unclear

The comments listed below should be taken into account when revising the manuscript. Along with your revision, you will need to supply a response letter ('Revision Note'), which is a thorough, detailed response to the referees' comments, specifically noting each comment made by the referees and/or Editor, and describing all changes. Should you disagree with any comment(s), please explain why. In case the Associate Editor or a reviewer has supplied a detailed list of small changes please use red type in the text to signal the changes you have made.

Please submit your revision online by logging onto the Editorial Manager for Small Ruminant Research using the following combination:
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We are looking forward to receiving the revised submission.

With kind regards,

Small Ruminant Research

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Reviewer #1: The manuscript is considering a little investigated question: How does water quality (pH) influence water intake of ruminants (like goats). I found almost no literature considering this topic directly. In your approach, it may be doubted that the effect of pH and other, potentially not analysed constituents of water, can be distinguished. Not to be misunderstood: I do not think that this distinction can be done in a more appropriate easily, but you should be aware of this noteworthy, but inevitable shortcoming of your approach (and maybe point this out to the reader). You are looking at the overall palatability of water (as correlated to pH, but maybe pH is not the most direct influence?).
Overall, it is appreciated that you measured variables in some breadth, while all appear justified.
The overall readibility of the manuscript is ok, but it will still benefit from some language editing (although I am no native speaker by myself, this is encouraged; please see also some of the comments and suggestions below; sorry, this is not meant to be unpolite, but it will help to give your data the deserved frame).

General comments:
L14: „…is generally high in acidity." Why is that?
L16/17; L63/64: Avoid repeating the numbers
L20/21: Rephrase the sentence.
L50-52: Please rephrase (No real hypothesis has been formulated; I would not consider a hypothesis necessary in your approach, but you simply investigate the influence of the water on several variables)
L61: Please add information on body weights of animals.
L66: Comment (plus maybe short description; half a sentence) necessary that the housing allowed fecal and urine sampling; have animals been kept for 3 weeks under these conditions?
L76: Important: Which origin/type of water was used in the recovery phases?
L83: Give more details on salt-mineral lick (composition; ideal would be the amount used in the trials)
L86: More details possible on the swamp water? Humic acids present?
L98: Start with the sampling of the offered feeds, then mention the refusals.
L113-115: The explanations of sampling rumen fluid are contradictory (2 h of water deprivation bevor sampling, but sampling one hour after drinking?)
L116: You mention that refusals were analysed, but have they been included in the calculations? (are nutrient intakes as used for digestibility calculations corrected for refusals?)
L118: Amylase is only used for NDF
L129-153: All this information should be explained in the respective sections before (point 2.5 just statistical analysis, all other calculations directly when the method is outlined)
L148: Maybe add the equation used for estimating ME from digestible nutrients.
L151: „Nitrogen net absorption was calculated…" (be aware that fecal N represents considerable amounts of endogenous N, secretions into the gut, cell debris etc.)
L313 (Table 1): Since you correlate average temperature with DMI or DWI/DMI (in table 7), please add values for Tav to table 1.
L314 (Table 2): Table is missing units(!)
L314 (Table 2): NDF is lower than NDFacp (does not make sense, must be lower)
L314 (Tab 2): „a 100-CP (%)-EE (%)-[NDF (%)-NDICP (%)]-TA (%)"; if CP is already subtracted, NDICP should be subtracted from NDF (add square brackets)
L314 (Tab 2): what does TA mean (ash is used in Tab 2)
L314 (Tab 2): best say: „bNeutral detergent fiber corrected for residual ash and crude protein" (to distinguish between „ND-residual ash" and „ash" as part of proximate/Weende analysis
Tab 4- Tab 6: You may consider skipping „…within the same row…" (different variables in each row)
Specific comments:
L19: following
L20/21: Change sentence
L37: …a decreasing trend…
L38: … there will be additional…
L46: level (singular)
L79: chopped?
L96: rainfall
L108/109: „…was measured then recorded after…" Please rephrase.
L123: TDS - abbreviation has not been explained before.

L147: …on AFRC (1993).
L178: …(% BW) tended to be lowered…
L202: A similar…
L207: …rumen pH becomes less than…
L212: …digestibility was not affected.
L215: „…was affected by a trend…" Please rephrase. (e.g. There was a trend for an effect on daily gain…)
L218: „…that likely due to a higher standard deviation…" Please rephrase
L224: „In group 3.8,…"
L227: „…reported for lactating goats (Olsson…"
L231: against
L234: processes
L236: …animals experience high…
L242/243: …ability of the animal in the 6.9 group to cope with the high temperature…
L244-246: Please rephrase the last sentence.
L314 (Table 2): violet
L317: Limits for pH (minimum) and other elements (maxima) for livestock drinking water based on Badgley et al. (1997)a or Olkowski (2009)
L317: Delete Hydrogen sulfite? (although interesting, but not measured and no limits included)
L328 (last line): body weight

Reviewer #2: The article could be of interest, however it needs a more thorough interpretation of the results. The discussion is too brief and does not fully explain all the major observations, particularly as related to the fact that the 5.2 treatment seems to stand out in its effects although the 3.6 is more acidic.
In addition, it is recommended to review the manuscript for language errors and structure.
It is recommended to provide the average weather data for each of the three periods, not just the average and range for the whole experiment. If possible to also calculate the THI as it gives a better reflection of the experienced heat stress, if any, by the animals during the three different periods.
It is also recommended to refer to newer publications, preferably on small ruminants, as applicable.
I also recommend to remove the upper superscripts in the tables (0.05<p<10).

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Asep Indra Munawar Ali fp <asep_ali@fp.unsri.ac.id>

Revision Confirmation for Rumin-D-21-539R1

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Ms. No. Rumin-D-21-539R1
Effects of acid drinking water on nutrient utilization, water balance, and growth of goats under hot-humid tropical environment

Dear Dr. Ali,

Thank you for the revised version of your submission to the journal Small Ruminant Research.

You will be able to check on the progress of your paper by logging onto the Editorial Managers as an Author using the following information:

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Kind regards,

Small Ruminant Research

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Small Ruminant Research

Effects of acid drinking water on nutrient utilization, water balance, and growth of goats under hot-humid tropical environment

--Manuscript Draft--

Manuscript Number:	Rumin-D-21-539R1
Article Type:	Research Paper
Section/Category:	Nutrition and Feeding Systems
Keywords:	acid drinking water; ruminal pH; livestock; heat stress
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	Muhamad Nasir Rofiq, PhD
	Dahlanuddin
Abstract:	Water available to livestock in the tropical lowlands region is generally high in acidity. Therefore, this study aims to determine the effects of acid water on nutrient intake, water balance, and the growth of goats in the tropical environment. A total of nine Kacang goats were stratified based on body weight (BW) and assigned to three treatment groups which were offered drinking water at varying pH levels, namely 6.9, 5.2, and 3.8. All goats were offered ad libitum Asystasia gangetica hay and dried cassava chips at 1% of BW (dry matter (DM) basis) following a crossover design with three treatments tested in three periods. At 5.2 pH level, drinking water intake (DWI) tended to be lower ($P = 0.09$) while Total DM intake (%BW) was decreased ($P < 0.05$). Ruminal pH declined to 6.58 at 3.8 pH level ($P < 0.01$). Metabolizable energy and daily gain tended to be higher at 6.9 and 3.8 pH levels compared to those at pH 5.2 levels ($P = 0.08$). There were no significant adverse effects of acid water on nutrient intake, utilization, and growth of Kacang goats. Moreover, the increased in temperature humidity index was followed by the elevated DWI ($P < 0.01$) at 6.9 pH level, but no such significant relationship was found at other pH levels that indicated a better capability of thermoregulation response under heat stress exposure.
Suggested Reviewers:	Uta Dickhöfer, PhD Professor, University of Hohenheim: Universitat Hohenheim uta.dickhoefer@uni-hohenheim.de
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	Riswandi Riswandi, PhD Assistant Professor, Universitas Sriwijaya Fakultas Pertanian riswandi@fp.unsri.ac.id

Reviewer Comments		Authors Responses	
Comments	**Line**	**Comments/Correction**	**New line**
How was water acidified?		The water is naturally acid swamp water.	43/44 & 87
What means RH at l. 95?	95	RH: relative humidity. In first appearance, it has been abbreviated	96
In Table 2, what is the unit: DM or as fed?		DM basis. it has been added in table 2	Table 2
In Table 3, Statistics are unclear		Table 3 only compare the concentrations of contaminants with the permissible limits, not between treatments values.	
Reviewer #1			
The manuscript is considering a little investigated question: How does water quality (pH) influence water intake of ruminants (like goats). I found almost no literature considering this topic directly. In your approach, it may be doubted that the effect of pH and other, potentially not analysed constituents of water, can be distinguished. Not to be misunderstood: I do not think that this distinction can be done in a more appropriate easily, but you should be aware of this noteworthy, but inevitable shortcoming of your approach (and maybe point this out to the reader). You are looking at the overall palatability of water (as correlated to pH, but maybe pH is not the most direct influence?). Overall, it is appreciated that you measured variables in some breadth, while all appear justified. The overall readibility of the manuscript is ok, but it will still benefit from some language editing (although I am no native speaker by myself, this is encouraged; please see also some of the comments and suggestions below; sorry, this is not meant to be unpolite, but it will help to give your data the deserved frame).		Editing of English language has been conducted (Native-Proofreading.com)	
…is generally high in acidity." Why is that?	14	The reason had been explained	42/43
Avoid repeating the numbers	16/17; 63/64	The sentences have been revised	17/18;63
Rephrase the sentence	20/21	The sentence has been rephrased	
Please rephrase (No real hypothesis has been formulated; I would not consider a hypothesis necessary in your approach, but you simply investigate the influence of the water on several variables)	50-52	The sentence has been revised	51/52
Please add information on body weights of animals	61	The average body weight has been added	62
Comment (plus maybe short description; half a sentence) necessary that the housing allowed fecal and urine sampling; have animals been kept for 3 weeks under these conditions?	66	The sentence has been added	64/65
Important: Which origin/type of water was used in the recovery phases?	76	The origin of water (pH 6.9) had been informed	75/76

Give more details on salt-mineral lick (composition; ideal would be the amount used in the trials)	83	The composition has been added	84/85
More details possible on the swamp water? Humic acids present?	86	Based on the low concentration of total dissolved solid and organic substances. We assumed that humic acid was not present on the water	Table 3
Start with the sampling of the offered feeds, then mention the refusals.	98	The sentences have been revised	101
The explanations of sampling rumen fluid are contradictory (2 h of water deprivation bevor sampling, but sampling one hour after drinking?)	113-115	The sentence has been revised. 'prior to the fluid collection' has been deleted. Water bucket was taken at 9:00 h and then returned at 11:00 h. One hour after drinking, rumen fluid was then collected. Almost all the goats drink the water at 11:00-11:15 so the fluid was taken at 12:00 – 12:15, depend on the time of start of drink.	126/128
You mention that refusals were analysed, but have they been included in the calculations? (are nutrient intakes as used for digestibility calculations corrected for refusals?)	116	Yes. It has been explained	120/121 124/125
Amylase is only used for NDF	118	Amylase is only used for NDF fraction free from amylum	
All this information should be explained in the respective sections before (point 2.5 just statistical analysis, all other calculations directly when the method is outlined)	129-153	The sub section has been revised. The calculations was moved before statistical analysis.	
Maybe add the equation used for estimating ME from digestible nutrients	148	The equation has been added	122
„Nitrogen net absorption was calculated…" (be aware that fecal N represents considerable amounts of endogenous N, secretions into the gut, cell debris etc.)	151	Nitrogen absorption was calculated by subtracting fecal N excretion (including N Endogenous) by the amount of N intake (feed and DWI), whilst N retention was calculated by subtracting the amount of urinary N loss by the amount of absorbed N.	
Since you correlate average temperature with DMI or DWI/DMI (in table 7), please add values for Tav to table 1	313	The T_{av} value has been added to table 1	Table 1
Table is missing units (!)	314 (Table 2)	DM basis, has been added in table 2	Table 2
NDF is lower than NDFacp (does not make sense, must be lower)	314 (Table 2)	The value has been corrected	Table 2
a 100-CP (%)-EE (%)-[NDF (%)-NDICP (%)]-TA (%)"; if CP is already subtracted, NDICP should be subtracted from NDF (add square brackets)	314 (Table 2)	The brackets has been added and the calculation has been rechecked	Table 2
what does TA mean (ash is used in Tab 2)	314 (Table 2)	TA = total ash. It has been corrected	Table 2
best say: „bNeutral detergent fiber corrected for residual ash and crude protein" (to distinguish between „ND-residual ash" and „ash" as part of proximate/Weende analysis	314 (Table 2)	It has been corrected	
You may consider skipping „…within the same row…" (different variables in each row)	Tab 4- Tab 6:	"within the same row" has been deleted	Table 4- Table 6

following	19	It has been changed	20
Change sentence	20/21	The sentence has been changed	21/22
…a decreasing trend…	37	The sentence has been changed	37
… there will be additional…	38	The sentence has been changed to a possibility of….	38
level (singular)	46	The word has been corrected	47
Chopped?	79	The word has been corrected	79
rainfall	96	The word has been corrected	97
„…was measured then recorded after…" Please rephrase.	108/109	The sentence has been revised	147/148
TDS - abbreviation has not been explained before.	123	It has been explained	130
…on AFRC (1993).	147	It has been revised	122
…(% BW) tended to be lowered…	178	It has been revised	180
A similar…	202	It has been revised	210/211
…rumen pH becomes less than…	207	It has been revised	215
…digestibility was not affected.	212	It has been revised	220/221
„…was affected by a trend…" Please rephrase. (e.g. There was a trend for an effect on daily gain…)	215	It has been revised	229
„…that likely due to a higher standard deviation…" Please rephrase	218	The sentence has been deleted	233
„In group 3.8,…"	224	It has been revised	238
„…reported for lactating goats (Olsson…"	227	It has been revised	240
against	231	The sentences have been revised	
processes	234	It has been revised	256
…animals experience high…	236	It has been revised	257
…ability of the animal in the 6.9 group to cope with the high temperature…	242/243	It has been revised	263/264
Please rephrase the last sentence.	244-246	The sentence has been revised	264-267
(Table 2): violet	314		
Limits for pH (minimum) and other elements (maxima) for livestock drinking water based on Badgley et al. (1997)a or Olkowski (2009)	317	It has been revised	Table 3 370
Delete Hydrogen sulfite? (although interesting, but not measured and no limits included)	317	Hydrogen sulfite has been deleted	Table 3
(last line): body weight	328	The words has been corrected	
Reviewer #2			
The article could be of interest, however it needs a more thorough interpretation of the results. The discussion is too brief and does not fully explain all the major observations, particularly as related to the fact that the 5.2 treatment seems to stand out in its effects although the 3.6 is more acidic.		The discussion has been extended	
In addition, it is recommended to review the manuscript for language errors and structure.		Proof reading for language error, readability and structure has been conducted (Native-Proofreading.com)	
It is recommended to provide the average weather data for each of the three periods, not just the average and range for the whole experiment.		The data for each period has been added to table 1	Table 1
If possible to also calculate the THI as it gives a better reflection of the experienced heat stress, if any, by the animals during the three different periods.		The THI has been added to table 1 and 7 and also discussed in the discussion	Table 1, Table 7 and discussio n

It is also recommended to refer to newer publications, preferably on small ruminants, as applicable.		The newer publications (on small ruminants) has been referred: (Abhijit et al, 2021; Ali et al., 2021; Assad et al, 2002; Giger-Reverdin 2018; Lopez et al, 2016; Mdletshe et al 2017; Ribeiro, et al, 2020; Salama et al, 2021; Silanikove 2015	Reference list
I also recommend to remove the upper superscripts in the tables (0.05<p<10).		The upper superscripts have been removed	
		Thank you so much for your corrections and suggestions	

- A trial with 6.9, 5.2, and 3.8 pH levels of drinking water was conducted
- Ruminal pH was declined by acid drinking water
- No adverse effects of the acid water on nutrient intake, utilization, and growth
- Drinking water intake correlated with temperature humidity index at 6.9 pH level

Effects of acid drinking water on nutrient utilization, water balance, and growth of goats under hot-humid tropical environment

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Abstract

Water available to livestock in the tropical lowlands region is generally high in acidity. Therefore, this study aims to determine the effects of acid water on nutrient intake, water balance, and the growth of goats in the tropical environment. A total of nine Kacang goats were stratified based on body weight (BW) and assigned to three treatment groups which were offered drinking water at varying pH levels, namely 6.9, 5.2, and 3.8. All goats were offered *ad libitum Asystasia gangetica* hay and dried cassava chips at 1% of BW (dry matter (DM) basis) following a crossover design with three treatments tested in three periods. At 5.2 pH level, drinking water intake (DWI) tended to be lower ($P = 0.09$) while Total DM intake (%BW) was decreased ($P < 0.05$). Ruminal pH declined to 6.58 at 3.8 pH level ($P < 0.01$). Metabolizable energy and daily gain tended to be higher at 6.9 and 3.8 pH levels compared to those at pH 5.2 levels ($P = 0.08$). There were no significant adverse effects of acid water on nutrient intake, utilization, and growth of Kacang goats. Moreover, the increased in

temperature humidity index was followed by the elevated DWI ($P < 0.01$) at 6.9 pH level, but no such significant relationship was found at other pH levels that indicated a better capability of thermoregulation response under heat stress exposure.

Keyword acid drinking water, ruminal pH, livestock, heat stress

1. Introduction

Water is one of the most important nutrients in the animal body due to its physiological roles in nutrient transport, maintenance of proper fluid and ion balance, biochemical reactions, as well as body thermoregulation. Previous study showed that a sufficient supply of good quality water is a limiting factor for all animals to maintain good health and optimal productivity (NRC, 2001). However, the supply of clean water resources is a decreasing trend globally, driven by population and economic growth. In the following decades, there is a possibility of additional pressure on water resources to fulfill the high demand of agriculture, household use, and industry. Moreover, the adequate supply of clean water is challenged by extreme weather events due to climate change (Boretti and Rosa, 2019).

In humid tropical lowlands, most of the water is characterized by high acidity due to natural oxidation processes of pyrite and ferric ion. The pH of the surface water drop to 3, where most of the contaminants are sulfate (SO_4), iron (Fe), manganese (Mn), and aluminum (Al) (Manders et al., 2002; Sahrawat, 2004). Another water source in the lowland region is groundwater, which has less acidity and contaminants (Winkel et al., 2008). Although the recommended minimum pH level for livestock is 5.5 (Bagley et al., 1997) or 6.0 (Olkowski, 2009), the effects of the acid water on ruminant animals have not been fully studied. This makes it is necessary to identify the influence of acid water on the animal's performance, implications for water quality standards, and for intervention options for the animal in the

lowland region. Therefore, this study was conducted to assess the influence of acid drinking water on water consumption, nutrient intake, and growth goats under hot tropical climates.

2. Materials and Methods

2.1. Study site

This study has been approved by the Faculty of Agriculture, Universitas Sriwijaya, Indonesia. The site is situated at an altitude of ±6 m above sea level and 3°11'38.4"S, 104°39'30.5"E. Meanwhile, the animals were cared for according to the Animal Welfare Guidelines of the Indonesian Institute of Sciences. The environmental variables in the site are shown in Table 1.

2.2. Experimental animal, treatments, and feeding management

A total of nine Kacang goats, based on body weight (BW), were stratified and divided into three treatment groups with an average BW=14.8 ± 1.0 kg, which were offered drinking water at varying pH levels, namely 6.9, 5.2, and 3.8. The animals were housed in individual pens (1.5 m × 0.75 m) in an open-sided type of house which allowed a total collection of daily fecal and urine excretion (Ali et al., 2021). Each pen was equipped with two identical feed troughs and an individual water bucket of diameter 23 cm, 5 L capacity. Subsequently, the goats were treated orally with Oxfendazole (25 mg/5 kg BW), acclimatized to feeding and environmental conditions for 15 d, and subjected to their respective water treatments group. All animals were weighed at the beginning of the study as well as every Sunday and Thursday to determine changes in the BW on an electronic weighing balance before offering feed and water.

This study used a crossover design that consisted of three levels of pH in three periods. Meanwhile, each experimental period lasted for three weeks of adaptation and one week of sample collection, where feed intake, feces, and urine excretion were measured. Each

measurement period was followed by one week of recovery, where all animals received only pH 6.9 drinking water.

The diet consisted of *Asystasia gangetica* hay and dried cassava chips as shown in Table 2. The hay was harvested at the pre blooming stage, chaffed to ±5 cm particle length, and sun-dried for 4 d while the cassava tubers were chopped to ±2 cm particle size and sun-dried for 5 d. Subsequently, the feeding and drinking were started at 9:00 after refusals from the previous day had been removed and weighed. The hay was offered ad libitum, according to 15% of the previous intake, while the number of cassava chips was referred to 1% of individual BW and adjusted after each BW measurement. Animals always had *ad libitum* access to drinking water and salt-mineral lick, which contained g/kg, DM basis: 730 NaCl, 34 Calcium, 15 Magnesium, 8 Phosphorous, and 1 trace minerals.

2.3. Preparation of different pH levels of water

Naturally available high acidity surface water was collected from non-tidal swamp area (3°10'29.7"S, 104°41'34.5"E), while the underground water with pH = 5.2 was collected from a well in the experimental site. The swamp water was manually collected using a 20-L bucket, while the well water was pumped. Meanwhile, the swamp water had an acidulous taste and a 3.8 pH level, which was checked using a portable pH meter (Hanna HI 98130). A pH level of 6.9 water was prepared from the well water by aeration for 4 d in a 50-L bucket using an aerator (Amara BS-410) and each of the water was stored in separate 50-L buckets before the offering.

2.4. Sample collection, preparation, and analysis

The indoor temperature and relative humidity (RH) were recorded by a climate data logger (Benetech G1365) at a 10-minutes intervals, while rainfall, sunshine, and wind speed were taken at a meteorological station. The temperature humidity index (THI) values were calculated according to formula NRC (1971).

Moreover, the samples of the offered feeds were taken and stored in paper bags at room temperature. After weighing, refusals were homogenized and a subsample (~100 g) was taken and stored. Total fecal and urinary excretion was determined by daily collection over 7 d. Meanwhile, the total feces excreted by each animal was thoroughly mixed by hand, weighed, and a subsample of approximately 100 g fresh matter was taken and dried at 45°C for three consecutive days. The dried feed and fecal samples were ground to pass through a 1-mm mesh. At the end of each period, the feed and fecal samples were pooled per animal proportionally to the daily amount of each animal during the sampling week. The dried samples were stored in zipper plastic bags before laboratory analyses.

The dried feces, feed, and refusals were analyzed as follows: DM, ash (AOAC, 1990; Method 924.05), N (AOAC, 1990; Method 988.05), ether extract (EE; Method 920.39), neutral detergent fiber (NDF), and acid detergent fiber (ADF) with alpha-amylase and including residual ash (Van Soest et al., 1991). Organic matter (OM) concentrations were calculated by subtracting the ash concentration from 100, while the CP content was calculated as N×6.25. Neutral detergent-insoluble N (NDIN) and Neutral detergent-insoluble ash (NDIash) were estimated according to Licitra et al. (1996). Furthermore, NDF corrected for ash and crude protein (NDF_{acp}) was calculated by subtracting the NDIN and NDIash. Non fibrous carbohydrates (NFC) were calculated by subtracting the concentration of NDF_{acp}, CP, EE, and ash from 100 (Mertens, 1997).

Daily feed intake was calculated as the difference between the amount of feed offered and the amount of feed refusals for each animal across the sampling week. Metabolizable energy (ME, MJ/kg) content was calculated as 0.0157×digestible OM (AFRC, 1993). Total tract apparent digestibility of DM, OM, NDF, and ADF were obtained from the difference

between the number of nutrient ingested and of nutrients excreted in feces over the 7 d of sampling week.

Before the measurement of rumen fluid pH, the animals were not given drinking water for two h (9:00 – 11:00). The fluid was collected using a stomach tube of 6 mm diameter one h after the goats consumed the water. The drinking water sample was collected every week and stored in a 250-mL bottle at 5 °C. At the end of each period, the samples were pooled proportionally and then analyzed to determine total dissolved solids (TDS, conductivity method, Orion Star A212, Thermo Scientific), Fe, Mn, Al (spectrometric techniques, inductively coupled plasma atomic emission spectroscopy Varian 715-ES, Agilent), nitrate (NO_3), nitrite (NO_2), ammonia (NH_3), sulfate (SO_4) (spectrometric techniques, Spectrophotometer UV-VIS Lambda 45, Perkin Elmer), organic substances (permanganometric titration method).

Individual drinking water intake (DWI) was calculated as the difference between the amount of water offered and refusals. Subsequently, three buckets with water were placed in the barn to estimate daily evaporative water loss, and then the daily DWI was corrected by the evaporative loss. The amount of water in the consumed feed (FWI) was calculated by the difference between the amount of water in the feed offered and refusals. Metabolic water was estimated using the factors 0.62, 0.42, and 1.10 for digestible carbohydrates, protein, and fat, respectively (Taylor, 1970). Apparent total water intake (TWI) was determined as the sum of DWI, FWI, and metabolic water, while the fecal water was estimated from the amount of fecal excretion and the content of water. The amount of urinary water was the amount of urine corrected by the DM content of urine. Meanwhile, the water retention was calculated by subtracting the amount of water in fecal and urinary excretion from TWI.

After homogenizing and filtering with a surgical gaze, individual urine excretion was recorded. A sample of urine (~100 mL) was taken daily and stored at -20 °C for N analysis.

The DM content of urine was determined by drying a 3 mL urine sample at 60 °C for 12 h and the total was determined using the micro Kjeldahl method (AOAC, 1990; Method 988.05). Nitrogen absorption was calculated by subtracting fecal N excretion from the amount of N intake (feed and DWI), while N retention was calculated by subtracting the amount of urinary N loss from the absorbed N.

2.5. Statistical analysis

The data generated from 3 treatments, 3 periods, and 9 animals were analyzed using SAS 9.1 and presented as mean ± standard error. Meanwhile, the data were analyzed using the mixed model procedure as stated below:

$$Y_{ijk}=\mu + T_i + P_j + TP_{ij} + a_k + e_{ijk};$$

Where Y_{ijk} is observed response at a particular *ijk* case, μ is overall mean, *Ti* is the fixed effect of treatment *i*, *Pj* is the fixed effect of period *j*, *TPij* is the fixed effect of the interaction between treatment *i* and period *j*, a_k is the random effect of animal *k*, and e_{ijk} is experimental error.

Differences between means were determined using the Tukey test and the significance level was declared at $p < 0.05$, where p-values of 0.05 to 0.10 were considered as a trend. The relationship between daily maximum temperature humidity index (THI_{max}), DWI, and DM intake (DMI) during the collection weeks was tested by Pearson correlation analysis.

3. Results

The composition of drinking water offered to animals in different treatment groups increases in Fe, Mn, Al, NH_3, SO_4, and organic substances with the decrease in pH level. Based on the results, nitrate was the lowest at 5.2 pH level, while the highest concentrations of NO_3 and NO_2 were found at 3.8 pH level (Table 3). Meanwhile, the values of feed intake, nutrient digestibility, rumen pH, and daily gain of the goats are shown in Table 4. In the group with a 5.2 pH level, total DMI was lower ($P < 0.05$) than those subjected to the other

treatments that comparable to the lower ($P < 0.05$) DM intake of hay (%BW) in the group. Furthermore, metabolizable energy intake (MJ/kg $BW^{0.75}$) and daily gain were only influenced by trends ($P = 0.06$). As the pH level reduces, the rumen pH was also decreasing ($P < 0.01$), where the pH in the 3.8 group was lower than those in the 6.9 and 5.2 groups. Meanwhile, the apparent DM, OM, NDF, and ADF digestibility were not significantly different ($P > 0.05$).

Drinking water intake and FWI (%BW) tended to be lowered at the 5.2 group ($P = 0.09$) but metabolic water and TWI were not influenced ($P > 0.05$). Fecal water excretion (%BW) was lowered ($P < 0.05$) in the 5.2 pH group, which was not significantly different from those in the 6.9 group ($P > 0.05$), but higher than those in the 3.8 group. Meanwhile, urinary water excretion and apparent water retention were not significantly affected by the pH level ($P > 0.05$) (Table 5).

Intake of N (%BW) and fecal N excretion (g/d) were also lowered at 5.2 level. However, N absorption, urinary N excretion, and N retention did not vary among the different groups ($P > 0.05$) (Table 6).

During the collection weeks, daily maximum temperature humidity index (THI_{max}) correlated positively with DWI of the 6.9 group but not of the 5.2 and 3.8 groups. Furthermore, DMI did not significantly correlate with THI_{max} among all the groups ($P > 0.05$), while the ratio DWI/DMI correlated with THI_{max} in the 6.9 group ($P < 0.01$) (Table 7).

4. Discussion

The decreased in DM intake was due to the lower DWI at 5.2 pH level, while water contaminant concentrations were varied among the different pH levels of drinking water. However, the tendency of lower DWI in the 5.2 pH group was not related to the contaminant concentrations in the water where the higher concentrations were found in the 3.8 pH group compared to the 5.2 pH group. Based on the maximum limits of contaminants concentrations

in the drinking water, the concentrations of TDS, Fe, NO_3, NO_2, SO_4 were much lower (Table 3). Several studies have been conducted on the effect of high-contaminants water on DWI and the performance of ruminants. Mdletshe et al. (2017) stated that reductions of DWI, DMI, and daily gain in Nguni goats as TDS content of water exceeded the permissible limits. Meanwhile, other studies also observed decreased DWI due to the higher levels of TDS in sheep (Assad and El-Sherif, 2002), beef cattle (López et al., 2016), and buffalo (Sharma et al., 2017). The water intake of beef cattle was also reduced when SO_4 was 1900 mg/L (Lardner et al., 2013) due to the ability of the animals to protect their metabolism status from the salt stress.

Furthermore, the intake level of DWI might be more related to the palatability of the water. In this study, the tendency of lower DWI at 5.5 pH level (P=0.09) was due to the less palatability of the water for the goats. There was a significant decrease in DWI at a lower level of contaminant as reported by Sharma et al. (2017) for buffalo calves on five TDS levels in drinking water where DWI was lower at 557 levels than those at 2571 mg/L level.

The rumen pH was declined by the acid drinking water in this study, however, it was still within the normal range. Acid drinking water may cause rumen acidosis (Olkowski, 2009) when the rumen pH becomes less than 5 (Giger-Reverdin, 2018; Ribeiro et al., 2020). However, the rumen pH values at the pH levels of 5.2 and 3.8 in this study increased to the normal range at 1 h post-drinking (Table 4). During the experiment, the animals' normal eating and ruminating behavior and the sufficiency of the minerals-salt supplement might indicate a normal secretion of saliva to maintain the range of rumen pH when the animal continuously consumed the acid drinking water. As a result, the nutrients' digestibility was not affected. A similar OM and NDF digestibility was also reported when the ruminal pH was decreased from 7.0 to 6.2 (Shriver et al., 1986).

The lowered fecal water excretion at the 5.5 level was associated with the lowered DWI and feed water intake, while the insignificant effect on urinary water excretion and apparent water retention was due to the lower contaminants contents in the drinking water. When TDS level was higher, a greater urinary water excretion was reported in sheep (Assad and El-Sherif, 2002), beef cattle (López et al., 2016), and buffalo (Sharma et al., 2017) as an adaptive response of the animals to excrete the excess salts.

There was a trend for an effect on daily gain (P = 0.06), although the gain of goats at the 5.2 level was 48 and 29% lower than those at the 6.9 and 3.8 levels, respectively. Similarly, a higher N retention of the goats at the 6.9 level was not significantly different from those on the 5.2 and 3.8 levels (Table 6). This means the positive gain, N retention, feed intake, and nutrient digestibility indicated that the acid water did not have detrimental effects on the goat performances.

The positive correlation of THI_{max} – DWI and THI_{max} - DWI/DMI was due to an increase in demand for water by the goats under heat stress in response to a higher loss of water through evaporation and sweating, which was only applied for the 6.9 group. Furthermore, a positive correlation for daily maximum temperature and DWI was also reported for buffalo calves on five levels of TDS in drinking water (Sharma et al., 2017), lactating goats (Olsson and Dahlborn, 1989) and goat kids (Al-Tamimi, 2007).

In tropical humid areas, goats continuously face high ambient temperature and humidity that affect their physiology, behavior, metabolism, and performances, which will become worse in the future due to the increase of climatic extreme events (Silanikove and Koluman, 2015). According to Salama et al. (2021), Murciano-Granadina goats exposed to heat stress at THI of 77, 30 ^{o}C, and 40% humidity showed a reduction in feed intake and higher water consumption than goats in the thermal neutral environment. During the experimental periods of this study, the means of THI were 79 to 80 (Table 1) which

fluctuated daily from 75 in the dawn to 85 in the afternoon (data not shown). Furthermore, the positive correlation THI_{max} – DWI was in line with the result of a previous study, which indicated that DWI also fluctuated at a higher value in the afternoon when THI was at a maximum level. A higher daily THI fluctuation from 70 to 87 with a shift of feeding and drinking frequency was also reported in the tropical humid region of India. This fluctuation showed the influence of feeding management in minimizing the adverse effect of heat stress on goat performances. Since the drinking water was offered at *ad libitum* level in this study, the animals could freely fulfill the additional requirement of water for the thermoregulation processes. The significant correlations in the 6.9 group showed the important aspect of clean and good palatability water for maximum intake when the animals experience heat stress.

5. Conclusions

The effect of lowering pH levels in drinking water depends on to the concentration of contaminants in the water. In this study, the lowering of pH level from 6.9 to 3.8 level did not lead to adverse effects on the nutrient intake, balance, and growth due to the minimum levels of the contaminants in the water and the animal's ability to maintain the water range of normal rumen pH. However, the better ability of the animal in the 6.9 group to cope with the heat stress was shown by the positive correlation between DWI and THI_{max}. In addition, a further study with a more extended period of acid drinking water is recommended to confirm the effects on rumen fermentation characteristics, thermoregulation, and drinking behavior responses.

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Author contribution

Original intellectual concept and study design: A. I. M. Ali; Methodology: A. I. M. Ali, S. Sandi; Data curation, formal analysis, and investigation: E. Sahara, A. I. M. Ali; Writing - original draft preparation: A. I. M. Ali; Writing - review and editing: M. N. Rofiq, Dahlanuddin; Funding acquisition: A. I. M. Ali. All authors read and approved the final manuscript.

Data availability

The datasets analyzed during this study are available from the corresponding author on reasonable request.

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Tables

Table 1

Environmental variables observed during the experiment.

Variable	Experimental periods		
	1	2	3
Maximum temperature (T_{max}) (°C)	31.7 ± 0.27	32.7 ± 0.26	33.4 ± 0.29
Minimum temperature (Tmin) (°C)	24.4 ± 0.10	24.8 ± 0.14	24.7 ± 0.17
Average temperature (Tav) (°C)	26.9 ± 0.17	27.6 ± 0.22	27.8 ± 0.18
Average relative humidity (%)	86.0 ± 0.90	84.4 ± 1.07	80.4 ± 0.93
Temperature humidity index	78.7 ± 0.20	79.6 ± 0.29	79.3 ± 0.20
Rainfall (mm/d)	7.8 ± 2.92	2.3 ± 0.68	3.6 ± 2.16
Sunshine (h)	4.1 ± 0.54	5.3 ± 0.46	5.8 ± 0.55
Wind speed (m/s)	1.9 ± 0.11	1.6 ± 0.11	2.1 ± 0.14

Temperature humidity index = (1.8×T°C + 32) – [(0.55 – 0.0055×RH %) × (1.8×T°C - 26)] (NRC, 1971), where T°C is air temperature and RH is the relative humidity.

Table 2

Chemical composition (mean ± standard error) of Chinese violet (*Asystasia gangetica*) hay and cassava chips offered during the experiment (% dry matter basis)

	Chinese vioet hay	Cassava chips
Dry matter	88.4 ± 0.70	88.3 ± 1.06
Organic matter	89.8 ± 0.11	97.9 ± 0.13
Crude protein	14.3 ± 0.36	4.2 ± 0.25
Ether extract	1.7 ± 0.04	0.3 ± 0.02
Ash	10.2 ± 0.50	2.1 ± 0.13
Non fibrous carbohydrates[a]	27.6 ± 0.98	72.9 ± 1.50
Neutral detergent fiber	48.1 ± 0.75	22.2 ± 0.07
Neutral detergent fiber $_{acp}$[b]	46.2 ± 0.71	21.9 ± 0.08
Acid detergent fiber	30.5 ± 0.24	4.0 ± 0.18
Acid detergent lignin	14.9 ± 0.12	1.5 ± 0.07

[a]100-CP (%)-EE (%)-[NDF (%)-NDICP (%)]-Ash (%).

[b]Neutral detergent fiber corrected for residual ash and crude protein.

Table 3

Concentrations of contaminant substances (mg/L, mean ± standard error) in drinking water offered to treatment groups and their permissible limits

Element	Treatment groups			Permissible limits
	6.9	5.2	3.8	
Total dissolved solids	51.0 ± 2.31	48.3 ± 2.96	87.7± 8.67	4000[a], 3000[b]
Iron	0.008 ± 0.002	0.010 ± 0.000	0.223± 0.074	2[a]
Manganese	0.001 ± 0.001	0.004 ± 0.003	0.027± 0.003	0.3[b]
Aluminum	0.014 ± 0.003	0.036 ± 0.001	2.870± 0.067	NA
Nitrate	14.1 ± 3.52	12.8 ± 0.51	24.8± 1.03	100[a], 77[b]
Nitrite	0.01 ± 0.011	0.02 ± 0.022	0.02± 0.02	33[a], 10[b]
Ammonia	0.27 ± 0.033	0.30 ± 0.058	0.47± 0.033	NA
Sulfate	3.3 ± 1.67	5.4 ± 2.11	25.6± 5.66	500[a], 1000[b]
Organic substances	1.9 ± 0.07	1.7 ± 0.16	2.6± 0.28	NA
pH	6.9 ± 0.03	5.2 ± 0.06	3.8± 0.02	5.5[a], 6.0[b]

Limits for pH (minimum) and other elements (maxima) for livestock drinking water based on United States Environmental Protection Agency (Bagley et al., 1997)[a] and Canadian Council of Ministers of the Environment (Olkowski, 2009)[b];

ND: not detected;

NA: not available

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Table 4

Dry matter (DM) intake, metabolizable energy (ME) intake, digestibility of DM, organic matter (OM), neutral detergent fiber (NDF), and acid detergent fiber (ADF), as well as rumen pH, and daily gain (mean ± standard error) of Kacang goats offered water having different pH levels

Parameter	pH level			*P*-value
	6.9	5.2	3.8	
Chinese violet hay				
g DM/d	389 ± 36.6	332 ± 32.5	390 ± 48.3	0.154
%BW	2.1 ± 0.15[b]	1.8 ± 0.13[a]	2.1 ± 0.17[b]	0.035
Cassava chips				
g DM/d	159 ± 15.2	166 ± 15.6	158 ± 11.3	0.715
%BW	0.9 ± 0.06	0.9 ± 0.05	0.9 ± 0.05	0.683
Total DM intake				
g/d	548 ± 41.8	498 ± 39.9	549 ± 49.6	0.078
%BW	3.0 ± 0.13[b]	2.7 ± 0.11[a]	2.9 ± 0.13[b]	0.026
ME intake				
(MJ/d)	5.8 ± 0.44	5.3 ± 0.40	5.8 ± 0.43	0.137
MJ/kg $BW^{0.75}$	0.65 ± 0.03	0.59 ± 0.02	0.64± 0.02	0.078
Digestibility (%)				
DM	68.1 ± 0.94	68.5 ± 0.99	67.7 ± 1.21	0.379
OM	67.9 ± 1.04	68.5 ± 1.04	67.5 ± 1.28	0.339
NDF	41.6 ± 1.61	41.9 ± 2.06	40.3 ± 2.46	0.448
ADF	23.4 ± 2.55	19.8 ± 3.91	23.6 ± 2.95	0.866

Rumen pH	6.98 ± 0.06[b]	6.94 ± 0.05[b]	6.58 ± 0.08[a]	0.002
Daily gain (g/d)	73.4 ± 8.74	49.7 ± 8.42	64.2 ± 6.16	0.062

Means with different superscripts are significantly different ($P < 0.05$); BW: body weight

Table 5

Water balance (mean ± standard error) of Kacang goats offered water having different pH levels

Parameter	pH level			*P*-value
	6.9	5.2	3.8	
Drinking water intake				
ml/d	1456 ± 173	1218 ± 118	1460 ± 173	0.243
%BW	7.8 ± 0.59	6.6 ± 0.58	7.7 ± 0.55	0.091
Feed water intake				
ml/d	83.9 ± 6.64	73.6 ± 5.54	82.4 ± 7.07	0.091
%BW	0.45 ± 0.02	0.40 ± 0.02	0.44 ± 0.02	0.056
Metabolic water				
ml/d	209.2 ± 15.8	191.6 ± 14.4	206.2 ± 14.6	0.330
%BW	1.13 ± 0.05	1.02 ± 0.04	1.11 ± 0.03	0.186
Total water intake				
ml/d	1750 ± 192	1484 ± 133	1749 ± 192	0.231
%BW	9.4 ± 0.63	8.0 ± 0.63	9.3 ± 0.58	0.187
Fecal water excretion				
ml/d	261 ± 32.4	202 ± 21.9	277 ± 45.5	0.055
%BW	1.4 ± 0.15^{ab}	1.1 ± 0.08^{a}	1.4 ± 0.17^{b}	0.034
Urinary water excretion				
ml/d	418 ± 56.2	321 ± 37.6	385 ± 66.4	0.392
%BW	2.3 ± 0.24	1.8 ± 0.21	2.0 ± 0.23	0.397
Apparent water retention				

ml/d	1070 ± 132.1	960 ± 97.9	1087 ± 88.4	0.421
%BW	5.7 ± 0.45	5.2 ± 0.49	5.8 ± 0.27	0.406

Means with different superscripts are significantly different ($P < 0.05$); BW: body weight

Table 6

Nitrogen (N) balance (mean ± standard error) of Kacang goats offered water having different pH levels

Parameter	pH level			*P*-value
	6.9	5.2	3.8	
N intake				
g/day	10.4 ± 0.907	9.0 ± 0.812	10.3 ± 1.112	0.074
%BW	0.056 ± 0.003[b]	0.048 ± 0.003[a]	0.055 ± 0.004[ab]	0.036
Fecal N				
g/d	4.41 ± 0.403[b]	3.84 ± 0.357[a]	4.43 ± 0.527[b]	0.037
%BW	0.024 ± 0.002	0.020 ± 0.001	0.024 ± 0.002	0.062
N absorb				
g/day	5.98 ± 0.526	5.18 ± 0.487	5.87 ± 0.620	0.313
%BW	0.03 ± 0.002	0.03 ± 0.002	0.03 ± 0.002	0.240
Urinary N				
g/day	3.32 ± 0.615	2.80 ± 0.413	3.10 ± 0.698	0.531
%BW	0.02 ± 0.003	0.02 ± 0.002	0.02 ± 0.003	0.469
N retention				
g/day	2.66 ± 0.542	2.38 ± 0.465	2.78 ± 0.439	0.789
%BW	0.01 ± 0.003	0.01 ± 0.002	0.02 ± 0.002	0.728

Means with different superscripts are significantly different ($P < 0.05$); BW: body weight

Table 7

Pearson correlation coefficients and significance levels[1] of the relationship between daily maximum temperature humidity index (THI_{max}) as well as drinking water intake (DWI) and dry matter intake (DMI) in Kacang goats offered water having different pH levels

Parameter	pH level		
	6.9	5.2	3.8
THI_{max} - DWI			
ml/d	0.62 **	0.14 n.s.	-0.02 n.s.
%BW	0.54 *	-0.15 n.s.	-0.04 n.s.
THI_{max} - DMI			
g/d	0.04 n.s.	0.25 n.s.	-0.31 n.s.
%BW	-0.18 n.s.	-0.29 n.s.	-0.33 n.s.
THI_{max} - DWI/DMI	0.61 **	-0.06 n.s.	0.11 n.s.

[1] Significance levels: n.s., not significant, (*) $p \leq 0.10$, *$p \leq 0.05$, **$p \leq 0.01$; BW: body weight

Effects of acid drinking water on nutrient utilization, water balance, and growth of goats under hot-humid tropical environment

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Abstract

Water available to livestock in the tropical lowlands region is generally high in acidity. Therefore, this study aims to determine the effects of acid water on nutrient intake, water balance, and the growth of goats in the tropical environment. A total of nine Kacang goats were stratified based on body weight (BW) and assigned to three treatment groups which were offered drinking water at varying pH levels, namely 6.9, 5.2, and 3.8. All goats were offered *ad libitum Asystasia gangetica* hay and dried cassava chips at 1% of BW (dry matter (DM) basis) following a crossover design with three treatments tested in three periods. At 5.2 pH level, drinking water intake (DWI) tended to be lower ($P = 0.09$) while Total DM intake (%BW) was decreased ($P < 0.05$). Ruminal pH declined to 6.58 at 3.8 pH level ($P < 0.01$). Metabolizable energy and daily gain tended to be higher at 6.9 and 3.8 pH levels compared to those at pH 5.2 levels ($P = 0.08$). There were no significant adverse effects of acid water on nutrient intake, utilization, and growth of Kacang goats. Moreover, the increased in

temperature humidity index was followed by the elevated DWI ($P < 0.01$) at 6.9 pH level, but no such significant relationship was found at other pH levels that indicated a better capability of thermoregulation response under heat stress exposure.

Keyword acid drinking water, ruminal pH, livestock, heat stress

1. Introduction

Water is one of the most important nutrients in the animal body due to its physiological roles in nutrient transport, maintenance of proper fluid and ion balance, biochemical reactions, as well as body thermoregulation. Previous study showed that a sufficient supply of good quality water is a limiting factor for all animals to maintain good health and optimal productivity (NRC, 2001). However, the supply of clean water resources is a decreasing trend globally, driven by population and economic growth. In the following decades, there is a possibility of additional pressure on water resources to fulfill the high demand of agriculture, household use, and industry. Moreover, the adequate supply of clean water is challenged by extreme weather events due to climate change (Boretti and Rosa, 2019).

In humid tropical lowlands, most of the water is characterized by high acidity due to natural oxidation processes of pyrite and ferric ion. The pH of the surface water drop to 3, where most of the contaminants are sulfate (SO_4), iron (Fe), manganese (Mn), and aluminum (Al) (Manders et al., 2002; Sahrawat, 2004). Another water source in the lowland region is groundwater, which has less acidity and contaminants (Winkel et al., 2008). Although the recommended minimum pH level for livestock is 5.5 (Bagley et al., 1997) or 6.0 (Olkowski, 2009), the effects of the acid water on ruminant animals have not been fully studied. This makes it is necessary to identify the influence of acid water on the animal's performance, implications for water quality standards, and for intervention options for the animal in the

lowland region. Therefore, this study was conducted to assess the influence of acid drinking water on water consumption, nutrient intake, and growth goats under hot tropical climates.

2. Materials and Methods

2.1. Study site

This study has been approved by the Faculty of Agriculture, Universitas Sriwijaya, Indonesia. The site is situated at an altitude of ±6 m above sea level and 3°11'38.4"S, 104°39'30.5"E. Meanwhile, the animals were cared for according to the Animal Welfare Guidelines of the Indonesian Institute of Sciences. The environmental variables in the site are shown in Table 1.

2.2. Experimental animal, treatments, and feeding management

A total of nine Kacang goats, based on body weight (BW), were stratified and divided into three treatment groups with an average BW=14.8 ± 1.0 kg, which were offered drinking water at varying pH levels, namely 6.9, 5.2, and 3.8. The animals were housed in individual pens (1.5 m × 0.75 m) in an open-sided type of house which allowed a total collection of daily fecal and urine excretion (Ali et al., 2021). Each pen was equipped with two identical feed troughs and an individual water bucket of diameter 23 cm, 5 L capacity. Subsequently, the goats were treated orally with Oxfendazole (25 mg/5 kg BW), acclimatized to feeding and environmental conditions for 15 d, and subjected to their respective water treatments group. All animals were weighed at the beginning of the study as well as every Sunday and Thursday to determine changes in the BW on an electronic weighing balance before offering feed and water.

This study used a crossover design that consisted of three levels of pH in three periods. Meanwhile, each experimental period lasted for three weeks of adaptation and one week of sample collection, where feed intake, feces, and urine excretion were measured. Each

measurement period was followed by one week of recovery, where all animals received only pH 6.9 drinking water.

The diet consisted of *Asystasia gangetica* hay and dried cassava chips as shown in Table 2. The hay was harvested at the pre blooming stage, chaffed to ±5 cm particle length, and sun-dried for 4 d while the cassava tubers were chopped to ±2 cm particle size and sun-dried for 5 d. Subsequently, the feeding and drinking were started at 9:00 after refusals from the previous day had been removed and weighed. The hay was offered ad libitum, according to 15% of the previous intake, while the number of cassava chips was referred to 1% of individual BW and adjusted after each BW measurement. Animals always had *ad libitum* access to drinking water and salt-mineral lick, which contained g/kg, DM basis: 730 NaCl, 34 Calcium, 15 Magnesium, 8 Phosphorous, and 1 trace minerals.

2.3. Preparation of different pH levels of water

Naturally available high acidity surface water was collected from non-tidal swamp area (3°10'29.7"S, 104°41'34.5"E), while the underground water with pH = 5.2 was collected from a well in the experimental site. The swamp water was manually collected using a 20-L bucket, while the well water was pumped. Meanwhile, the swamp water had an acidulous taste and a 3.8 pH level, which was checked using a portable pH meter (Hanna HI 98130). A pH level of 6.9 water was prepared from the well water by aeration for 4 d in a 50-L bucket using an aerator (Amara BS-410) and each of the water was stored in separate 50-L buckets before the offering.

2.4. Sample collection, preparation, and analysis

The indoor temperature and relative humidity (RH) were recorded by a climate data logger (Benetech G1365) at a 10-minutes intervals, while rainfall, sunshine, and wind speed were taken at a meteorological station. The temperature humidity index (THI) values were calculated according to formula NRC (1971).

Moreover, the samples of the offered feeds were taken and stored in paper bags at room temperature. After weighing, refusals were homogenized and a subsample (~100 g) was taken and stored. Total fecal and urinary excretion was determined by daily collection over 7 d. Meanwhile, the total feces excreted by each animal was thoroughly mixed by hand, weighed, and a subsample of approximately 100 g fresh matter was taken and dried at 45°C for three consecutive days. The dried feed and fecal samples were ground to pass through a 1-mm mesh. At the end of each period, the feed and fecal samples were pooled per animal proportionally to the daily amount of each animal during the sampling week. The dried samples were stored in zipper plastic bags before laboratory analyses.

The dried feces, feed, and refusals were analyzed as follows: DM, ash (AOAC, 1990; Method 924.05), N (AOAC, 1990; Method 988.05), ether extract (EE; Method 920.39), neutral detergent fiber (NDF), and acid detergent fiber (ADF) with alpha-amylase and including residual ash (Van Soest et al., 1991). Organic matter (OM) concentrations were calculated by subtracting the ash concentration from 100, while the CP content was calculated as N×6.25. Neutral detergent-insoluble N (NDIN) and Neutral detergent-insoluble ash (NDIash) were estimated according to Licitra et al. (1996). Furthermore, NDF corrected for ash and crude protein (NDF_{acp}) was calculated by subtracting the NDIN and NDIash. Non fibrous carbohydrates (NFC) were calculated by subtracting the concentration of NDF_{acp}, CP, EE, and ash from 100 (Mertens, 1997).

Daily feed intake was calculated as the difference between the amount of feed offered and the amount of feed refusals for each animal across the sampling week. Metabolizable energy (ME, MJ/kg) content was calculated as 0.0157×digestible OM (AFRC, 1993). Total tract apparent digestibility of DM, OM, NDF, and ADF were obtained from the difference

between the number of nutrient ingested and of nutrients excreted in feces over the 7 d of sampling week.

Before the measurement of rumen fluid pH, the animals were not given drinking water for two h (9:00 – 11:00). The fluid was collected using a stomach tube of 6 mm diameter one h after the goats consumed the water. The drinking water sample was collected every week and stored in a 250-mL bottle at 5 °C. At the end of each period, the samples were pooled proportionally and then analyzed to determine total dissolved solids (TDS, conductivity method, Orion Star A212, Thermo Scientific), Fe, Mn, Al (spectrometric techniques, inductively coupled plasma atomic emission spectroscopy Varian 715-ES, Agilent), nitrate (NO_3), nitrite (NO_2), ammonia (NH_3), sulfate (SO_4) (spectrometric techniques, Spectrophotometer UV-VIS Lambda 45, Perkin Elmer), organic substances (permanganometric titration method).

Individual drinking water intake (DWI) was calculated as the difference between the amount of water offered and refusals. Subsequently, three buckets with water were placed in the barn to estimate daily evaporative water loss, and then the daily DWI was corrected by the evaporative loss. The amount of water in the consumed feed (FWI) was calculated by the difference between the amount of water in the feed offered and refusals. Metabolic water was estimated using the factors 0.62, 0.42, and 1.10 for digestible carbohydrates, protein, and fat, respectively (Taylor, 1970). Apparent total water intake (TWI) was determined as the sum of DWI, FWI, and metabolic water, while the fecal water was estimated from the amount of fecal excretion and the content of water. The amount of urinary water was the amount of urine corrected by the DM content of urine. Meanwhile, the water retention was calculated by subtracting the amount of water in fecal and urinary excretion from TWI.

After homogenizing and filtering with a surgical gaze, individual urine excretion was recorded. A sample of urine (~100 mL) was taken daily and stored at -20 °C for N analysis.

The DM content of urine was determined by drying a 3 mL urine sample at 60 °C for 12 h and the total was determined using the micro Kjeldahl method (AOAC, 1990; Method 988.05). Nitrogen absorption was calculated by subtracting fecal N excretion from the amount of N intake (feed and DWI), while N retention was calculated by subtracting the amount of urinary N loss from the absorbed N.

2.5. Statistical analysis

The data generated from 3 treatments, 3 periods, and 9 animals were analyzed using SAS 9.1 and presented as mean ± standard error. Meanwhile, the data were analyzed using the mixed model procedure as stated below:

$$Y_{ijk}=\mu + T_i + P_j + TP_{ij} + a_k + e_{ijk};$$

Where Y_{ijk} is observed response at a particular *ijk* case, μ is overall mean, *Ti* is the fixed effect of treatment *i*, *Pj* is the fixed effect of period *j*, *TPij* is the fixed effect of the interaction between treatment *i* and period *j*, a_k is the random effect of animal *k*, and e_{ijk} is experimental error.

Differences between means were determined using the Tukey test and the significance level was declared at $p < 0.05$, where p-values of 0.05 to 0.10 were considered as a trend. The relationship between daily maximum temperature humidity index (THI_{max}), DWI, and DM intake (DMI) during the collection weeks was tested by Pearson correlation analysis.

3. Results

The composition of drinking water offered to animals in different treatment groups increases in Fe, Mn, Al, NH_3, SO_4, and organic substances with the decrease in pH level. Based on the results, nitrate was the lowest at 5.2 pH level, while the highest concentrations of NO_3 and NO_2 were found at 3.8 pH level (Table 3). Meanwhile, the values of feed intake, nutrient digestibility, rumen pH, and daily gain of the goats are shown in Table 4. In the ~~5.2~~ group with a 5.2 pH level, total DMI was lower ($P < 0.05$) than those subjected to the other

treatments that comparable to the lower ($P < 0.05$) DM intake of hay (%BW) in the group. Furthermore, metabolizable energy intake (MJ/kg $BW^{0.75}$) and daily gain were only influenced by trends ($P = 0.06$). As the pH level reduces, the rumen pH was ~~lowered~~ also decreasing ($P < 0.01$~~) as the decrease of pH level~~), where the pH in the 3.8 group was lower than those in the 6.9 and 5.2 groups. Meanwhile, the apparent DM, OM, NDF, and ADF digestibility were not significantly different ($P > 0.05$).

Drinking water intake and FWI (%BW) tended to be lowered at the 5.2 group (P = 0.09) but metabolic water and TWI were not influenced (P > 0.05). Fecal water excretion (%BW) was lowered (P < 0.05) in the 5.2 pH group, which was not significantly different from those in the 6.9 group (P > 0.05), but higher than those in the 3.8 group. Meanwhile, urinary water excretion and apparent water retention were not significantly affected by the pH level (P > 0.05) (Table 5).

Intake of N (%BW) and fecal N excretion (g/d) were also lowered at 5.2 level. However, N absorption, urinary N excretion, and N retention did not vary among the different groups (P > 0.05) (Table 6).

During the collection weeks, daily maximum temperature humidity index (THI_{max}) correlated positively with DWI of the 6.9 group but not of the 5.2 and 3.8 groups. Furthermore, DMI did not significantly correlate with THI_{max} among all the groups (P > 0.05), while the ratio DWI/DMI correlated with THI_{max} in the 6.9 group (P < 0.01) (Table 7).

4. Discussion

The decreased in DM intake was due to the lower DWI at 5.2 pH level, while water contaminant concentrations were varied among the different pH levels of drinking water. However, the tendency of lower DWI in the 5.2 pH group was not ~~be associated with~~related to the contaminant concentrations in the water where the higher concentrations were found in the 3.8 pH group compared to the 5.2 pH group. Based on the maximum limits of

contaminants concentrations in the drinking water, the concentrations of TDS, Fe, NO_3, NO_2, SO_4 were much lower (Table 3). Several studies have been conducted on the effect of high-contaminants water on DWI and the performance of ruminants. Mdletshe et al. (2017) stated that reductions of DWI, DMI, and daily gain in Nguni goats as TDS content of water exceeded the permissible limits. Meanwhile, other studies also observed decreased DWI due to the higher levels of TDS in sheep (Assad and El-Sherif, 2002), beef cattle (López et al., 2016), and buffalo (Sharma et al., 2017). The water intake of beef cattle was also reduced when SO_4 was 1900 mg/L (Lardner et al., 2013) due to the ability of the animals to protect their metabolism status from the salt stress.

Furthermore, the intake level of DWI might be more related to the palatability of the water. In this study, the tendency of lower DWI at 5.5 pH level (P=0.09) was due to the less palatability of the water ~~was less palatable~~ for the goats. There was a significant decrease in DWI at a lower level of contaminant as reported by Sharma et al. (2017) for buffalo calves on five TDS levels in drinking water where DWI was lower at 557 levels than those at 2571 mg/L level.

The rumen pH was declined by the acid drinking water in this study, however, it was still within the normal range. Acid drinking water may cause rumen acidosis (Olkowski, 2009) when the rumen pH becomes less than 5 (Giger-Reverdin, 2018; Ribeiro et al., 2020). However, the rumen pH values at the pH levels of 5.2 and 3.8 in this study increased to the normal range at 1 h post-drinking (Table 4). During the experiment, the animals' normal eating and ruminating behavior and the sufficiency of the minerals-salt supplement might indicate a normal secretion of saliva to maintain the range of rumen pH when the animal continuously consumed the acid drinking water. As a result, the nutrients' digestibility was not affected. A similar OM and NDF digestibility was also reported when the ruminal pH was decreased from 7.0 to 6.2 (Shriver et al., 1986).

The lowered fecal water excretion at the 5.5 level was associated with the lowered DWI and feed water intake, while the insignificant effect on urinary water excretion and apparent water retention was due to the lower contaminants contents in the drinking water. When TDS level was higher, a greater urinary water excretion was reported in sheep (Assad and El-Sherif, 2002), beef cattle (López et al., 2016), and buffalo (Sharma et al., 2017) as an adaptive response of the animals to excrete the excess salts.

There was a trend for an effect on daily gain (P = 0.06), although the gain of goats at the 5.2 level was 48 and 29% lower than those at the 6.9 and 3.8 levels, respectively. Similarly, a higher N retention of the goats at the 6.9 level was not significantly different from those on the 5.2 and 3.8 levels (Table 6). This means the positive gain, N retention, feed intake, and nutrient digestibility indicated that the acid water did not have detrimental effects on the goat performances.

The positive correlation of THI_{max} – DWI and THI_{max} - DWI/DMI was due to an increase in demand for water by the goats under heat stress in response to a higher loss of water through evaporation and sweating, which was only applied for the 6.9 group. Furthermore, a positive correlation for daily maximum temperature and DWI was also reported for buffalo calves on five levels of TDS in drinking water (Sharma et al., 2017), lactating goats (Olsson and Dahlborn, 1989) and goat kids (Al-Tamimi, 2007).

In tropical humid areas, goats continuously face high ambient temperature and humidity that affect their physiology, behavior, metabolism, and performances, which will become worse in the future due to the increase of climatic extreme events (Silanikove and Koluman, 2015). According to Salama et al. (2021), Murciano-Granadina goats exposed to heat stress at THI of 77, 30 ^{o}C, and 40% humidity showed a reduction in feed intake and higher water consumption than goats in the thermal neutral environment. During the experimental periods of this study, the means of THI were 79 to 80 (Table 1) which

fluctuated daily from 75 in the dawn to 85 in the afternoon (data not shown). Furthermore, the positive correlation THI_{max} – DWI was in line with the result of a previous study, which indicated that DWI also fluctuated at a higher value in the afternoon when THI was at a maximum level. A higher daily THI fluctuation from 70 to 87 with a shift of feeding and drinking frequency was also reported in the tropical humid region of India. This fluctuation showed the influence of feeding management in minimizing the adverse effect of heat stress on goat performances. Since the drinking water was offered at *ad libitum* level in this study, the animals could freely fulfill the additional requirement of water for the thermoregulation processes. The significant correlations in the 6.9 group showed the important aspect of clean and good palatability water for maximum intake when the animals experience heat stress.

5. Conclusions

The effect of lowering pH levels in drinking water depends on to the concentration of contaminants in the water. In this study, the lowering of pH level from 6.9 to 3.8 level did not lead to adverse effects on the nutrient intake, balance, and growth due to the minimum levels of the contaminants in the water and the animal's ability to maintain the water range of normal rumen pH. However, the better ability of the animal in the 6.9 group to cope with the heat stress was shown by the positive correlation between DWI and THI_{max}. In addition, a further study with a more extended period of acid drinking water is recommended to confirm the effects on rumen fermentation characteristics, thermoregulation, and drinking behavior responses.

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Author contribution

Original intellectual concept and study design: A. I. M. Ali; Methodology: A. I. M. Ali, S. Sandi; Data curation, formal analysis, and investigation: E. Sahara, A. I. M. Ali; Writing - original draft preparation: A. I. M. Ali; Writing - review and editing: M. N. Rofiq, Dahlanuddin; Funding acquisition: A. I. M. Ali. All authors read and approved the final manuscript.

Data availability

The datasets analyzed during this study are available from the corresponding author on reasonable request.

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Tables

Table 1

Environmental variables observed during the experiment.

Variable	Experimental periods		
	1	2	3
Maximum temperature (T_{max}) (°C)	31.7 ± 0.27	32.7 ± 0.26	33.4 ± 0.29
Minimum temperature (Tmin) (°C)	24.4 ± 0.10	24.8 ± 0.14	24.7 ± 0.17
Average temperature (Tav) (°C)	26.9 ± 0.17	27.6 ± 0.22	27.8 ± 0.18
Average relative humidity (%)	86.0 ± 0.90	84.4 ± 1.07	80.4 ± 0.93
Temperature humidity index	78.7 ± 0.20	79.6 ± 0.29	79.3 ± 0.20
Rainfall (mm/d)	7.8 ± 2.92	2.3 ± 0.68	3.6 ± 2.16
Sunshine (h)	4.1 ± 0.54	5.3 ± 0.46	5.8 ± 0.55
Wind speed (m/s)	1.9 ± 0.11	1.6 ± 0.11	2.1 ± 0.14

Temperature humidity index = (1.8×T°C + 32) – [(0.55 – 0.0055×RH %) × (1.8×T°C - 26)] (NRC, 1971), where T°C is air temperature and RH is the relative humidity.

Table 2

Chemical composition (mean ± standard error) of Chinese violet (*Asystasia gangetica*) hay and cassava chips offered during the experiment (% dry matter basis)

	Chinese vioet hay	Cassava chips
Dry matter	88.4 ± 0.70	88.3 ± 1.06
Organic matter	89.8 ± 0.11	97.9 ± 0.13
Crude protein	14.3 ± 0.36	4.2 ± 0.25
Ether extract	1.7 ± 0.04	0.3 ± 0.02
Ash	10.2 ± 0.50	2.1 ± 0.13
Non fibrous carbohydrates[a]	27.6 ± 0.98	72.9 ± 1.50
Neutral detergent fiber	48.1 ± 0.75	22.2 ± 0.07
Neutral detergent fiber $_{acp}$[b]	46.2 ± 0.71	21.9 ± 0.08
Acid detergent fiber	30.5 ± 0.24	4.0 ± 0.18
Acid detergent lignin	14.9 ± 0.12	1.5 ± 0.07

[a]100-CP (%)-EE (%)-[NDF (%)-NDICP (%)]-Ash (%).

[b]Neutral detergent fiber corrected for residual ash and crude protein.

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Table 3

Concentrations of contaminant substances (mg/L, mean ± standard error) in drinking water offered to treatment groups and their permissible limits

Element	Treatment groups			Permissible limits
	6.9	5.2	3.8	
Total dissolved solids	51.0 ± 2.31	48.3 ± 2.96	87.7± 8.67	4000[a], 3000[b]
Iron	0.008 ± 0.002	0.010 ± 0.000	0.223± 0.074	2[a]
Manganese	0.001 ± 0.001	0.004 ± 0.003	0.027± 0.003	0.3[b]
Aluminum	0.014 ± 0.003	0.036 ± 0.001	2.870± 0.067	NA
Nitrate	14.1 ± 3.52	12.8 ± 0.51	24.8± 1.03	100[a], 77[b]
Nitrite	0.01 ± 0.011	0.02 ± 0.022	0.02± 0.02	33[a], 10[b]
Ammonia	0.27 ± 0.033	0.30 ± 0.058	0.47± 0.033	NA
Sulfate	3.3 ± 1.67	5.4 ± 2.11	25.6± 5.66	500[a], 1000[b]
Organic substances	1.9 ± 0.07	1.7 ± 0.16	2.6± 0.28	NA
pH	6.9 ± 0.03	5.2 ± 0.06	3.8± 0.02	5.5[a], 6.0[b]

Limits for pH (minimum) and other elements (maxima) for livestock drinking water based on United States Environmental Protection Agency (Bagley et al., 1997)[a] and Canadian Council of Ministers of the Environment (Olkowski, 2009)[b];

ND: not detected;

NA: not available

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Table 4

Dry matter (DM) intake, metabolizable energy (ME) intake, digestibility of DM, organic matter (OM), neutral detergent fiber (NDF), and acid detergent fiber (ADF), as well as rumen pH, and daily gain (mean ± standard error) of Kacang goats offered water having different pH levels

Parameter	pH level			*P*-value
	6.9	5.2	3.8	
Chinese violet hay				
g DM/d	389 ± 36.6	332 ± 32.5	390 ± 48.3	0.154
%BW	2.1 ± 0.15[b]	1.8 ± 0.13[a]	2.1 ± 0.17[b]	0.035
Cassava chips				
g DM/d	159 ± 15.2	166 ± 15.6	158 ± 11.3	0.715
%BW	0.9 ± 0.06	0.9 ± 0.05	0.9 ± 0.05	0.683
Total DM intake				
g/d	548 ± 41.8	498 ± 39.9	549 ± 49.6	0.078
%BW	3.0 ± 0.13[b]	2.7 ± 0.11[a]	2.9 ± 0.13[b]	0.026
ME intake				
(MJ/d)	5.8 ± 0.44	5.3 ± 0.40	5.8 ± 0.43	0.137
MJ/kg $BW^{0.75}$	0.65 ± 0.03	0.59 ± 0.02	0.64± 0.02	0.078
Digestibility (%)				
DM	68.1 ± 0.94	68.5 ± 0.99	67.7 ± 1.21	0.379
OM	67.9 ± 1.04	68.5 ± 1.04	67.5 ± 1.28	0.339
NDF	41.6 ± 1.61	41.9 ± 2.06	40.3 ± 2.46	0.448
ADF	23.4 ± 2.55	19.8 ± 3.91	23.6 ± 2.95	0.866

Rumen pH	6.98 ± 0.06^{b}	6.94 ± 0.05^{b}	6.58 ± 0.08^{a}	0.002
Daily gain (g/d)	73.4 ± 8.74	49.7 ± 8.42	64.2 ± 6.16	0.062

Means with different superscripts are significantly different ($P < 0.05$); BW: body weight

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Table 5

Water balance (mean ± standard error) of Kacang goats offered water having different pH levels

Parameter	pH level			*P*-value
	6.9	5.2	3.8	
Drinking water intake				
ml/d	1456 ± 173	1218 ± 118	1460 ± 173	0.243
%BW	7.8 ± 0.59	6.6 ± 0.58	7.7 ± 0.55	0.091
Feed water intake				
ml/d	83.9 ± 6.64	73.6 ± 5.54	82.4 ± 7.07	0.091
%BW	0.45 ± 0.02	0.40 ± 0.02	0.44 ± 0.02	0.056
Metabolic water				
ml/d	209.2 ± 15.8	191.6 ± 14.4	206.2 ± 14.6	0.330
%BW	1.13 ± 0.05	1.02 ± 0.04	1.11 ± 0.03	0.186
Total water intake				
ml/d	1750 ± 192	1484 ± 133	1749 ± 192	0.231
%BW	9.4 ± 0.63	8.0 ± 0.63	9.3 ± 0.58	0.187
Fecal water excretion				
ml/d	261 ± 32.4	202 ± 21.9	277 ± 45.5	0.055
%BW	1.4 ± 0.15^{ab}	1.1 ± 0.08^{a}	1.4 ± 0.17^{b}	0.034
Urinary water excretion				
ml/d	418 ± 56.2	321 ± 37.6	385 ± 66.4	0.392
%BW	2.3 ± 0.24	1.8 ± 0.21	2.0 ± 0.23	0.397
Apparent water retention				

ml/d	1070 ± 132.1	960 ± 97.9	1087 ± 88.4	0.421
%BW	5.7 ± 0.45	5.2 ± 0.49	5.8 ± 0.27	0.406

Means with different superscripts are significantly different ($P < 0.05$); BW: body weight

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Table 6

Nitrogen (N) balance (mean ± standard error) of Kacang goats offered water having different pH levels

Parameter	pH level			*P*-value
	6.9	5.2	3.8	
N intake				
g/day	10.4 ± 0.907	9.0 ± 0.812	10.3 ± 1.112	0.074
%BW	0.056 ± 0.003^{b}	0.048 ± 0.003^{a}	0.055 ± 0.004^{ab}	0.036
Fecal N				
g/d	4.41 ± 0.403^{b}	3.84 ± 0.357^{a}	4.43 ± 0.527^{b}	0.037
%BW	0.024 ± 0.002	0.020 ± 0.001	0.024 ± 0.002	0.062
N absorb				
g/day	5.98 ± 0.526	5.18 ± 0.487	5.87 ± 0.620	0.313
%BW	0.03 ± 0.002	0.03 ± 0.002	0.03 ± 0.002	0.240
Urinary N				
g/day	3.32 ± 0.615	2.80 ± 0.413	3.10 ± 0.698	0.531
%BW	0.02 ± 0.003	0.02 ± 0.002	0.02 ± 0.003	0.469
N retention				
g/day	2.66 ± 0.542	2.38 ± 0.465	2.78 ± 0.439	0.789
%BW	0.01 ± 0.003	0.01 ± 0.002	0.02 ± 0.002	0.728

Means with different superscripts are significantly different ($P < 0.05$); BW: body weight

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Table 7

Pearson correlation coefficients and significance levels[1] of the relationship between daily maximum temperature humidity index (THI_{max}) as well as drinking water intake (DWI) and dry matter intake (DMI) in Kacang goats offered water having different pH levels

Parameter	pH level		
	6.9	5.2	3.8
THI_{max} - DWI			
ml/d	0.62 **	0.14 n.s.	-0.02 n.s.
%BW	0.54 *	-0.15 n.s.	-0.04 n.s.
THI_{max} - DMI			
g/d	0.04 n.s.	0.25 n.s.	-0.31 n.s.
%BW	-0.18 n.s.	-0.29 n.s.	-0.33 n.s.
THI_{max} - DWI/DMI	0.61 **	-0.06 n.s.	0.11 n.s.

[1] Significance levels: n.s., not significant, (*) $p \leq 0.10$, *$p \leq 0.05$, **$p \leq 0.01$; BW: body weight

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Conflict of Interest Statement

The authors declare that they have no competing interests.

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Ms. No. Rumin-D-21-539R1
Effects of acid drinking water on nutrient utilization, water balance, and growth of goats under hot-humid tropical environment
Small Ruminant Research

Dear Dr. Ali,

I can now inform you that the Editorial Board has evaluated your manuscript. The Editor has advised that the manuscript will be reconsidered for publication after moderate revision.

The comments listed below should be taken into account when revising the manuscript. Along with your revision, you will need to supply a response letter ('Revision Note'), which is a thorough, detailed response to the referees' comments, specifically noting each comment made and describing all changes. Should you disagree with any comment(s), please explain why. In case the Associate Editor or a reviewer has supplied a detailed list of small changes please use red type in the text to signal the changes you have made.

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With kind regards,

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Reviewer #2: The improvements to the manuscript are appreciated. The below are a few remaining points that are suggested for consideration:
- Final review for English
- Suggested explanation for the different palatability of the water that did not correspond well with the pH or the contaminants level.

Reviewer #3: This paper falls within the scope of the Small Ruminant Research journal and brings some novelty. Nevertheless, it will be improved if the following points are adressed:
- statistical effects are lacking in Tables 1 and 3.
- water at pH = 6.9 is the water at pH 5.2 that has been aerated. You need to explain why there are differences in composition between these two sources of water and especially concerning water palatbility.

- when you claim an increase (or decrease), the difference must be statistically significant (L. 169?)
- if the groups were balanced for body weight, how can you explain that you have a decrease in DMI/kgBW and no effect on DMI?
- crude protein digestibility is missing. With an effect on DMI, it would be more relevant to express data in Table 6 as percentages.
- In the abstract, the values for the higher rumen pH are lacking
- what is the unit for digestible organic matter at L.122?
- as amylase treatment only concerns the NDF residue, please rephrase L. 110-113.

- do you think that the aeration of water might be of practical interest? If so, you might discuss it.

Data in Brief (optional):

We invite you to convert your supplementary data (or a part of it) into an additional journal publication in Data in Brief, a multi-disciplinary open access journal. Data in Brief articles are a fantastic way to describe supplementary data and associated metadata, or full raw datasets deposited in an external repository, which are otherwise unnoticed. A Data in Brief article (which will be reviewed, formatted, indexed, and given a DOI) will make your data easier to find, reproduce, and cite.

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Effects of acid drinking water on nutrient utilization, water balance, and growth of goats under hot-humid tropical environment

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Effects of acid drinking water on nutrient utilization, water balance, and growth of goats under hot-humid tropical environment

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Manuscript Number:	Rumin-D-21-539R2
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Abstract:	Water available for livestock in the tropical lowland region is generally high in acidity. This study determined the effects of the acid water on nutrient intake, water balance, and the growth of goats in the tropical environment. A total of nine Kacang goats were stratified based on body weight (BW) and assigned to three treatment groups which were offered drinking water at varying pH levels, namely 6.9, 5.2, and 3.8. All goats were offered ad libitum Asystasia gangetica hay and dried cassava chips at 1% of BW (dry matter (DM) basis) following a crossover design with three treatments tested in three periods. At the 5.2 pH level, drinking water intake (DWI) tended to be lower ($P = 0.09$) while total DM intake (%BW) was decreased ($P < 0.05$). Ruminal pH was significantly difference ($P < 0.01$); 6.98, 6.94, and 6.58 at the 6.9, 5.2, and 3.8 pH levels, respectively. Metabolizable energy and daily gain tended to be higher at the 6.9 and 3.8 pH levels compared to those at the 5.2 level ($P = 0.08$). There were no significant adverse effects of acid water on nutrient intake, utilization, and growth of Kacang goats. Moreover, the increase in temperature-humidity index was followed by the elevated DWI ($P < 0.01$) at 6.9 pH level, but no such significant relationship was found at other pH levels that indicated a better capability of thermoregulation response under heat stress exposure.
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Reviewer Comments		Authors Responses	
Comments	**Line**	**Comments/Correction**	**New line**
Reviewer #2			
Final review for English		Editing of English language has been conducted (Native-Proofreading.com)	
Suggested explanation for the different palatability of the water that did not correspond well with the pH or the contaminants level		We have explored some previous studies to find a logical reason for the difference and also tried to relate the water composition with the amount of water intake. For a comparison, a lower water intake was reported by Sharma et al. (2017) when TDS concentration was lowered (L 215). Though the difference was not significant (P = 0.091), it might cause the significant difference in the hay intake. However, we failed to explain the different palatability of the water	215
Reviewer #3			
Statistical effects are lacking in Tables 1 and 3.		Statistical differences in composition have been inserted in Table 3	Table 3
Water at pH = 6.9 is the water at pH 5.2 that has been aerated. You need to explain why there are differences in composition between these two sources of water and especially concerning water palatability.	91-94	The reason for the non-significant differences in composition has been added	198 - 203
When you claim an increase (or decrease), the difference must be statistically significant (L. 169?)	169	The probability values have been added	170/171
If the groups were balanced for body weight, how can you explain that you have a decrease in DMI/kgBW and no effect on DMI?		The balance for body weight was conducted once in the beginning of the experiment	
Crude protein digestibility is missing. With an effect on DMI, it would be more relevant to express data in Table 6 as percentages.		Crude protein digestibility has been added The means are presented as percentages	Table 4 Table 6
In the abstract, the values for the higher rumen pH are lacking	22	The values have been inserted	22/23
What is the unit for digestible organic matter at L.122?	122	The unit (MJ/kg) has been added	122
As amylase treatment only concerns the NDF residue, please rephrase L. 110-113.	110-113	The sentence has been rephrased	110-113
Do you think that the aeration of water might be of practical interest? If so, you might discuss it.		The discussion has been added	198 - 203
		Thank you so much for your corrections and suggestions	

- A trial with 6.9, 5.2, and 3.8 pH levels of drinking water was conducted
- Ruminal pH was declined by acid drinking water
- No adverse effects of the acid water on nutrient intake, utilization, and growth
- Drinking water intake correlated with temperature humidity index at 6.9 pH level

Effects of acid drinking water on nutrient utilization, water balance, and growth of goats under hot-humid tropical environment

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Abstract

Water available for livestock in the tropical lowland region is generally high in acidity. This study determined the effects of the acid water on nutrient intake, water balance, and the growth of goats in the tropical environment. A total of nine Kacang goats were stratified based on body weight (BW) and assigned to three treatment groups which were offered drinking water at varying pH levels, namely 6.9, 5.2, and 3.8. All goats were offered *ad libitum Asystasia gangetica* hay and dried cassava chips at 1% of BW (dry matter (DM) basis) following a crossover design with three treatments tested in three periods. At the 5.2 pH level, drinking water intake (DWI) tended to be lower (P = 0.09) while total DM intake (%BW) was decreased (P < 0.05). Ruminal pH was significantly difference (P < 0.01); 6.98, 6.94, and 6.58 at the 6.9, 5.2, and 3.8 pH levels, respectively. Metabolizable energy and daily gain tended to be higher at the 6.9 and 3.8 pH levels compared to those at the 5.2 level (P = 0.08). There were no significant adverse effects of acid water on nutrient intake, utilization,

and growth of Kacang goats. Moreover, the increase in temperature-humidity index was followed by the elevated DWI ($P < 0.01$) at 6.9 pH level, but no such significant relationship was found at other pH levels that indicated a better capability of thermoregulation response under heat stress exposure.

Keyword acid drinking water, ruminal pH, livestock, heat stress

1. Introduction

Water is one of the most important nutrients in the animal body due to its physiological roles in nutrient transport, maintenance of proper fluid and ion balance, biochemical reactions, as well as body thermoregulation. Previous study showed that a sufficient supply of good quality water is a limiting factor for all animals to maintain good health and optimal productivity (NRC, 2001). However, the supply of clean water resources is a decreasing trend globally, driven by population and economic growth. In the following decades, there is a potential for additional pressure on water resources to fulfill the high demand for agriculture, household use, and industry. Moreover, the adequate supply of clean water is challenged by extreme weather events due to climate change (Boretti and Rosa, 2019).

In humid tropical lowlands, most of the water is characterized by high acidity due to the natural oxidation processes of pyrite and ferric ion. The pH of the surface water could fall to 3, where most of the contaminants are sulfate (SO_4), iron (Fe), manganese (Mn), and aluminum (Al) (Ali et al., 2021a; Manders et al., 2002). Another source of water in the lowland region is groundwater, which has less acidity and contaminants (Winkel et al., 2008). Although the minimum recommended pH for livestock is 5.5 (Bagley et al., 1997) or 6.0 (Olkowski, 2009), the effects of the acidic water on ruminants have not been fully studied. It is necessary to identify the influence of acid water on the animal's performance, implications

for water quality standards, and intervention options for the animal in the lowland region. Therefore, this study was conducted to assess the influence of acid drinking water on water consumption, nutrient intake, and growth goats under hot tropical climates.

2. Materials and Methods

2.1. Study site

This study has been approved by the Faculty of Agriculture, Universitas Sriwijaya, Indonesia. The site is situated at an altitude of ±6 m above sea level and 3°11'38.4"S, 104°39'30.5"E. Meanwhile, the animals were cared for according to the Animal Welfare Guidelines of the Indonesian Institute of Sciences. The environmental variables in the site are shown in Table 1.

2.2. Experimental animal, treatments, and feeding management

A total of nine Kacang goats, based on body weight (BW), were stratified and divided into three treatment groups with an average BW=14.8 ± 1.0 kg, which were offered drinking water at varying pH levels, namely 6.9, 5.2, and 3.8. The animals were housed in individual pens (1.5 m × 0.75 m) in an open-sided type of house which allowed a total collection of daily fecal and urinary excretion (Ali et al., 2021b). Each pen was equipped with two identical feed troughs and an individual water bucket of diameter 23 cm, 5 L capacity. Subsequently, the goats were treated orally with Oxfendazole (25 mg/5 kg BW), acclimatized to feeding and environmental conditions for 15 d, and subjected to their respective water treatment group. All animals were weighed at the beginning of the study as well as every Sunday and Thursday to determine changes in the BW on a weighing scale before offering feed and water.

This study used a crossover design that consisted of three levels of pH over three periods. Meanwhile, each experimental period lasted for three weeks of adaptation and one week of sampling, where feed intake, fecal and urinary excretion were measured. Each

measurement period was followed by one week of recovery, where all animals received only pH 6.9 drinking water.

The diet consisted of *Asystasia gangetica* hay and dried cassava chips as shown in Table 2. The hay was harvested at the pre blooming stage, chaffed to ±5 cm particle length, and sun-dried for 4 d while the cassava tubers were chopped to ±2 cm particle size and sun-dried for 5 d. Subsequently, the feeding and drinking were started at 9:00 after refusals from the previous day had been removed and weighed. The hay was offered ad libitum, according to 15% of the previous intake, while the amount of cassava chips was referred to 1% of individual BW and adjusted after each BW measurement. Animals always had *ad libitum* access to drinking water and salt-mineral lick, which contained g/kg, DM basis: 730 NaCl, 34 Calcium, 15 Magnesium, 8 Phosphorous, and 1 trace minerals.

2.3. Preparation of different pH levels of water

Naturally available high-acidity surface water was collected from non-tidal swamp area (3°10'29.7"S, 104°41'34.5"E), while the underground water with pH = 5.2 was collected from a well in the experimental site. The swamp water was manually collected using a 20-L bucket, while the well water was pumped. Meanwhile, the swamp water had an acidulous taste and a 3.8 pH level, which was checked using a portable pH meter (Hanna HI 98130). A pH level of 6.9 water was prepared from the well water by aeration for 4 d in a 50-L bucket using an aerator (Amara BS-410) and each of the water was stored in separate 50-L buckets before the offering.

2.4. Sample collection, preparation, and analysis

The indoor temperature and relative humidity (RH) were recorded by a climate data logger (Benetech G1365) at 10-minutes intervals, while rainfall, sunshine, and wind speed were taken at a meteorological station. The temperature-humidity index (THI) values were calculated according to NRC (1971).

Moreover, the samples of the offered feeds were taken and stored in paper bags at room temperature. After weighing, refusals were homogenized and a subsample (~100 g) was taken and stored. Total fecal and urinary excretion was determined by daily collection over 7 d. Meanwhile, the total feces excreted by each animal was thoroughly mixed by hand, weighed, and a subsample of approximately 100 g fresh matter was taken and dried at 45°C for three consecutive days. The dried feed and fecal samples were ground to pass through a 1-mm mesh. At the end of each period, the feed and fecal samples were pooled per animal proportionally to the daily amount of each animal during the sampling week. The dried samples were stored in zipper plastic bags before laboratory analyses.

The dried feces, feed, and refusals were analyzed as follows: DM, ash (AOAC, 1990; Method 924.05), N (AOAC, 1990; Method 988.05), ether extract (EE; Method 920.39), neutral detergent fiber (NDF, with alpha-amylase), and acid detergent fiber (ADF) including residual ash (Van Soest et al., 1991). Organic matter (OM) concentrations were calculated by subtracting the ash concentration from 100, while the crude protein (CP) content was calculated as N×6.25. Neutral detergent-insoluble N (NDIN) and Neutral detergent-insoluble ash (NDIash) were estimated according to Licitra et al. (1996). Furthermore, NDF corrected for ash and CP (NDF_{acp}) was calculated by subtracting the NDIN and NDIash. Non fibrous carbohydrates (NFC) were calculated by subtracting the concentration of NDF_{acp}, CP, EE, and ash from 100 (Mertens, 1997).

Daily feed intake was calculated as the difference between the amount of feed offered and the amount of feed refusals for each animal across the sampling week. Metabolizable energy (ME, MJ/kg) content was calculated as 0.0157×digestible OM (AFRC, 1993). Total tract apparent digestibility of DM, OM, CP, NDF, and ADF were obtained from the difference between the amount of nutrient ingested and of nutrients excreted in feces over the 7 d of sampling week.

Before the measurement of rumen fluid pH, the animals were not given drinking water for two h (9:00 – 11:00). The fluid was collected using a stomach tube of 6 mm diameter one h after the goats consumed the water. The drinking water sample was collected every week and stored in a 250-mL bottle at 5 °C. At the end of each period, the samples were pooled proportionally and then analyzed to determine total dissolved solids (TDS, conductivity method, Orion Star A212, Thermo Scientific), Fe, Mn, Al (spectrometric techniques, inductively coupled plasma atomic emission spectroscopy Varian 715-ES, Agilent), nitrate (NO_3), nitrite (NO_2), ammonia (NH_3), sulfate (SO_4) (spectrometric techniques, Spectrophotometer UV-VIS Lambda 45, Perkin Elmer), organic substances (permanganometric titration method).

Individual drinking water intake (DWI) was calculated as the difference between the amount of water offered and refusals. Subsequently, three buckets with water were placed in the barn to estimate daily evaporative water loss, and then the daily DWI was corrected by the evaporative loss. The amount of water in the consumed feed (FWI) was calculated by the difference between the amount of water in the feed offered and refusals. Metabolic water was estimated using the factors 0.62, 0.42, and 1.10 for digestible carbohydrates, protein, and fat, respectively (Taylor, 1970). Apparent total water intake (TWI) was determined as the sum of DWI, FWI, and metabolic water, while the fecal water was estimated from the amount of fecal excretion and the content of water. The amount of urinary water was the amount of urine corrected by the DM content of urine. Meanwhile, the water retention was calculated by subtracting the amount of water in fecal and urinary excretion from TWI.

After homogenizing and filtering with a surgical gaze, individual urine excretion was recorded. A sample of urine (~100 mL) was taken daily and stored at -20 °C for N analysis. The DM content of urine was determined by drying a 3 mL urine sample at 60 °C for 12 h and the total was determined using the micro Kjeldahl method (AOAC, 1990; Method

988.05). Nitrogen absorption was calculated by subtracting fecal N excretion from the amount of N intake (feed and DWI), while N retention was calculated by subtracting the amount of urinary N loss from the absorbed N.

2.5. Statistical analysis

The data generated from 3 treatments, 3 periods, and 9 animals were analyzed using SAS 9.1 and presented as mean ± standard error. Meanwhile, the data were analyzed using the mixed model procedure as stated below:

$$Y_{ijk}=\mu + T_i + P_j + TP_{ij} + a_k + e_{ijk};$$

Where Y_{ijk} is observed response at a particular *ijk* case, μ is overall mean, *Ti* is the fixed effect of treatment *i*, *Pj* is the fixed effect of period *j*, *TPij* is the fixed effect of the interaction between treatment *i* and period *j*, a_k is the random effect of animal *k*, and e_{ijk} is experimental error.

Differences between means were determined using the Tukey test and the significance level was declared at $P < 0.05$, where p-values of 0.05 to 0.10 were considered as a trend. The relationship between daily maximum temperature-humidity index (THI_{max}), DWI, and DM intake (DMI) during the collection weeks was tested by Pearson correlation analysis.

3. Results

The composition of drinking water offered to animals in different treatment groups increases in Fe, Mn, Al, NH_3, SO_4, and organic substances with the decrease in pH level. In the 6.9 and 5.2 levels, the contaminant concentrations were not significantly different ($P > 0.05$) while the highest concentrations were found in the 3.8 pH level ($P < 0.05$; Table 3).

Meanwhile, the values of feed intake, nutrient digestibility, rumen pH, and daily gain of the goats are shown in Table 4. In the group with a 5.2 pH level, total DMI was lower ($P < 0.05$) than those subjected to the other treatments that comparable to the lower ($P < 0.05$) DM intake of hay (%BW) in the group. Furthermore, metabolizable energy intake (MJ/kg $BW^{0.75}$)

and daily gain were only influenced by trends ($P = 0.06$). As the pH level reduced, the rumen pH was also decreasing ($P < 0.01$), where the pH in the 3.8 group was lower than those in the 6.9 and 5.2 groups. Meanwhile, the apparent DM, OM, CP, NDF, and ADF digestibility were not significantly different ($P > 0.05$).

Drinking water intake and FWI (%BW) tended to be lowered at the 5.2 group ($P = 0.09$) but metabolic water and TWI were not influenced ($P > 0.05$). Fecal water excretion (%BW) was lowered ($P < 0.05$) in the 5.2 pH group, which was not significantly different from those in the 6.9 group ($P > 0.05$), but higher than those in the 3.8 group. Meanwhile, urinary water excretion and apparent water retention were not significantly affected by the pH level ($P > 0.05$) (Table 5).

Intake of N was also lowered at 5.2 level ($P < 0.05$). However, N absorption, urinary N excretion, and N retention did not vary among the different groups ($P > 0.05$) (Table 6).

During the collection weeks, the daily maximum temperature-humidity index (THI_{max}) correlated positively with DWI of the 6.9 group but not of the 5.2 and 3.8 groups. Furthermore, DMI did not significantly correlate with THI_{max} among all the groups ($P > 0.05$), while the ratio DWI/DMI correlated with THI_{max} in the 6.9 group ($P < 0.01$) (Table 7).

4. Discussion

The varied DM intake was not attributable to the DWI while water contaminant concentrations were varied among the different pH levels of drinking water. The tendency of lower DWI in the 5.2 pH group was also not related to the contaminant concentrations in the water where the higher concentrations were found in the 3.8 pH group. Based on the maximum limits of contaminant concentrations in the drinking water, the concentrations of TDS, Fe, NO_3, NO_2, SO_4 were much lower (Table 3). The oxidation process of contaminant ions could relate to the lowered H^+ concentration of the aerated water in the 6.9 pH group (Lytle et al., 1998; Manders et al., 2002). Aeration followed by filtration treatment to remove

contaminants from water has been widely used (Lytle et al., 1998; Marsidi et al., 2018). The non-significant differences of the contaminant concentrations in the 6.9 and 5.2 groups due to the absence of the filtration process to remove the precipitates.

Several studies have been conducted on the effect of high-contaminant water on DWI and the performance of ruminants. Mdletshe et al. (2017) stated that reductions of DWI, DMI, and daily gain in Nguni goats as the TDS content of water exceeded the permissible limits. Meanwhile, other studies also observed decreased DWI due to the higher levels of TDS in sheep (Assad and El-Sherif, 2002), beef cattle (López et al., 2016), and buffalo (Sharma et al., 2017). The water intake of beef cattle was also reduced when SO_4 was 1900 mg/L (Lardner et al., 2013) due to the ability of the animals to protect their metabolism status from salt stress.

Furthermore, the intake level of DWI might be more related to the palatability of the water. In this study, the tendency of lower DWI at 5.5 pH level (P=0.09) was due to the less palatability of the water for the goats. There was a significant decrease in DWI at a lower level of contaminant reported by Sharma et al. (2017) for buffalo calves on five TDS levels in drinking water where DWI was lower at 557 than those at 2571 mg/L level.

The rumen pH was declined by the acid drinking water in this study, however, it was still within the normal range. Acid drinking water may cause rumen acidosis (Olkowski, 2009) when the rumen pH becomes less than 5 (Giger-Reverdin, 2018; Ribeiro et al., 2020). However, the rumen pH values at the pH levels of 5.2 and 3.8 in this study increased to the normal range at one h post-drinking (Table 4). During the experiment, the animals' normal eating and ruminating behavior and the sufficiency of the minerals-salt supplement might indicate a normal secretion of saliva to maintain the range of rumen pH when the animal continuously consumed the acid drinking water. As a result, the nutrients' digestibility was

not affected. A similar OM and NDF digestibility was also reported when the ruminal pH was decreased from 7.0 to 6.2 (Shriver et al., 1986).

The lowered fecal water excretion at the 5.5 level was associated with the lowered DWI and feed water intake, while the insignificant effect on urinary water excretion and apparent water retention was due to the lower contaminants contents in the drinking water. When TDS level was higher, a greater urinary water excretion was reported in sheep (Assad and El-Sherif, 2002), beef cattle (López et al., 2016), and buffalo (Sharma et al., 2017) as an adaptive response of the animals to excrete the excess salts.

The daily gain was only affected by a trend (P = 0.06), although the gain of goats at the 5.2 level was 48 and 29% lower than those at the 6.9 and 3.8 levels, respectively. Similarly, a higher N retention of the goats at the 6.9 level was not significantly different from those on the 5.2 and 3.8 levels (Table 6). This means the positive gain, N retention, feed intake, and nutrient digestibility indicated that the acid water did not have detrimental effects on the goat performances.

The positive correlation of THI_{max} – DWI and THI_{max} - DWI/DMI was due to an increase in demand for water by the goats under heat stress in response to a higher loss of water through evaporation and sweating, which was only applied for the 6.9 group. Furthermore, a positive correlation for daily maximum temperature and DWI was also reported for buffalo calves on five levels of TDS in drinking water (Sharma et al., 2017), lactating goats (Olsson and Dahlborn, 1989) and goat kids (Al-Tamimi, 2007).

In tropical humid areas, goats continuously face high ambient temperature and humidity that affect their physiology, behavior, metabolism, and performances, which will become worse in the future due to the increase of climatic extreme events (Silanikove and Koluman, 2015). According to Salama et al. (2021), Murciano-Granadina goats exposed to heat stress at THI of 77, 30 ^{o}C, and 40% humidity showed a reduction in feed intake and

higher water consumption than goats in the thermal neutral environment. During the experimental periods of this study, the means of THI were 79 to 80 (Table 1) which fluctuated daily from 75 in the dawn to 85 in the afternoon (data not shown). Furthermore, the positive correlation THI_{max} – DWI was in line with the result of a previous study, which indicated that DWI also fluctuated at a higher value in the afternoon when THI was at a maximum level. A higher daily THI fluctuation from 70 to 87 with a shift of feeding and drinking frequency was also reported in the tropical humid region of India (Abhijith et al., 2021). This fluctuation showed the influence of feeding management in minimizing the adverse effect of heat stress on goat performances. Since the drinking water was offered at *ad libitum* level in this study, the animals could freely fulfill the additional requirement of water for the thermoregulation processes. The significant correlations in the 6.9 group showed the important aspect of clean and good palatability water for maximum intake when the animals experience heat stress.

5. Conclusions

The effect of lowering pH levels in drinking water depends on the concentration of contaminants in the water. In this study, the lowering of pH level from 6.9 to 3.8 did not lead to adverse effects on the nutrient intake, balance, and growth due to the minimum levels of the contaminants in the water and the animal's ability to maintain the normal range of the ruminal pH. However, the better ability of the animal in the 6.9 group to cope with the heat stress was shown by the positive correlation between DWI and THI_{max}. In addition, a further study with a more extended period of the acid drinking water is recommended to confirm the effects on rumen fermentation characteristics, thermoregulation, and drinking behavior responses.

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Author contribution

Original intellectual concept and study design: A. I. M. Ali; Methodology: A. I. M. Ali, S. Sandi; Data curation, formal analysis, and investigation: E. Sahara, A. I. M. Ali; Writing - original draft preparation: A. I. M. Ali; Writing - review and editing: M. N. Rofiq, Dahlanuddin; Funding acquisition: A. I. M. Ali. All authors read and approved the final manuscript.

Data availability

The datasets analyzed during this study are available from the corresponding author on reasonable request.

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Tables

Table 1

Environmental variables observed during the experiment.

Variable	Experimental periods		
	1	2	3
Maximum temperature (T_{max}) (°C)	31.7 ± 0.27	32.7 ± 0.26	33.4 ± 0.29
Minimum temperature (Tmin) (°C)	24.4 ± 0.10	24.8 ± 0.14	24.7 ± 0.17
Average temperature (Tav) (°C)	26.9 ± 0.17	27.6 ± 0.22	27.8 ± 0.18
Average relative humidity (%)	86.0 ± 0.90	84.4 ± 1.07	80.4 ± 0.93
Temperature humidity index	78.7 ± 0.20	79.6 ± 0.29	79.3 ± 0.20
Rainfall (mm/d)	7.8 ± 2.92	2.3 ± 0.68	3.6 ± 2.16
Sunshine (h)	4.1 ± 0.54	5.3 ± 0.46	5.8 ± 0.55
Wind speed (m/s)	1.9 ± 0.11	1.6 ± 0.11	2.1 ± 0.14

Temperature humidity index = (1.8×T°C + 32) – [(0.55 – 0.0055×RH %) × (1.8×T°C - 26)] (NRC, 1971), where T°C is air temperature and RH is the relative humidity.

Table 2

Chemical composition (mean ± standard error) of Chinese violet (*Asystasia gangetica*) hay and cassava chips offered during the experiment (% dry matter basis)

	Chinese vioet hay	Cassava chips
Dry matter	88.4 ± 0.70	88.3 ± 1.06
Organic matter	89.8 ± 0.11	97.9 ± 0.13
Crude protein (CP)	14.3 ± 0.36	4.2 ± 0.25
Ether extract (EE)	1.7 ± 0.04	0.3 ± 0.02
Ash	10.2 ± 0.50	2.1 ± 0.13
Non fibrous carbohydrates[a]	27.6 ± 0.98	72.9 ± 1.50
Neutral detergent fiber (NDF)	48.1 ± 0.75	22.2 ± 0.07
Neutral detergent fiber $_{acp}$[b]	46.2 ± 0.71	21.9 ± 0.08
Acid detergent fiber	30.5 ± 0.24	4.0 ± 0.18
Acid detergent lignin	14.9 ± 0.12	1.5 ± 0.07

[a]100-CP (%)-EE (%)-[NDF (%)-NDICP (%)]-Ash (%).

[b]Neutral detergent fiber corrected for residual ash and crude protein.

Table 3

Concentrations of contaminant substances (mg/L, mean ± standard error) in drinking water offered to treatment groups and their permissible limits

Element	Treatment groups			*P*-value	Permissible limits
	6.9	5.2	3.8		
Total dissolved solids	51.0 ± 2.31[a]	48.3 ± 2.96[a]	87.7 ± 8.67[b]	0.004	4000[1], 3000[2]
Iron	0.008 ± 0.002[a]	0.010 ± 0.000[a]	0.223 ± 0.074[b]	0.019	2[1]
Manganese	0.001 ± 0.001[a]	0.004 ± 0.003 [a]	0.027 ± 0.003[b]	0.001	0.3[2]
Aluminum	0.014 ± 0.003[a]	0.036 ± 0.001[a]	2.870 ± 0.067[b]	0.000	NA
Nitrate	14.1 ± 3.52 [a]	12.8 ± 0.51[a]	24.8 ± 1.03[b]	0.014	100[1], 77[2]
Nitrite	0.01 ± 0.011	0.02 ± 0.022	0.02 ± 0.02	0.897	33[1], 10[2]
Ammonia	0.27 ± 0.033[a]	0.30 ± 0.058[ab]	0.47 ± 0.033[b]	0.035	NA
Sulfate	3.3 ± 1.67[a]	5.4 ± 2.11[a]	25.6 ± 5.66[b]	0.009	500[1], 1000[2]
Organic substances	1.9 ± 0.07	1.7 ± 0.16	2.6 ± 0.28	0.053	NA
pH	6.9 ± 0.03[c]	5.2 ± 0.06[b]	3.8 ± 0.02[a]	0.000	5.5[1], 6.0[2]

Means with different superscripts are significantly different ($P < 0.05$);

Limits for pH (minimum) and other elements (maxima) for livestock drinking water based on United States Environmental Protection Agency (Bagley et al., 1997)[1] and Canadian Council of Ministers of the Environment (Olkowski, 2009)[2];

ND: not detected;

NA: not available

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Table 4

Dry matter (DM) intake, metabolizable energy (ME) intake, digestibility of DM, organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF), as well as rumen pH, and daily gain (mean ± standard error) of Kacang goats offered water having different pH levels

Parameter	pH level			*P*-value
	6.9	5.2	3.8	
Chinese violet hay				
g DM/d	389 ± 36.6	332 ± 32.5	390 ± 48.3	0.154
%BW	2.1 ± 0.15[b]	1.8 ± 0.13[a]	2.1 ± 0.17[b]	0.035
Cassava chips				
g DM/d	159 ± 15.2	166 ± 15.6	158 ± 11.3	0.715
%BW	0.9 ± 0.06	0.9 ± 0.05	0.9 ± 0.05	0.683
Total DM intake				
g/d	548 ± 41.8	498 ± 39.9	549 ± 49.6	0.078
%BW	3.0 ± 0.13[b]	2.7 ± 0.11[a]	2.9 ± 0.13[b]	0.026
ME intake				
(MJ/d)	5.8 ± 0.44	5.3 ± 0.40	5.8 ± 0.43	0.137
MJ/kg $BW^{0.75}$	0.65 ± 0.03	0.59 ± 0.02	0.64± 0.02	0.078
Digestibility (%)				
DM	68.1 ± 0.94	68.5 ± 0.99	67.7 ± 1.21	0.379
OM	67.9 ± 1.04	68.5 ± 1.04	67.5 ± 1.28	0.339
CP	57.7 ± 0.95	57.3 ± 1.29	56.9 ± 0.62	0.722
NDF	41.6 ± 1.61	41.9 ± 2.06	40.3 ± 2.46	0.448

ADF	23.4 ± 2.55	19.8 ± 3.91	23.6 ± 2.95	0.866
Rumen pH	6.98 ± 0.06[b]	6.94 ± 0.05[b]	6.58 ± 0.08[a]	0.002
Daily gain (g/d)	73.4 ± 8.74	49.7 ± 8.42	64.2 ± 6.16	0.062

Means with different superscripts are significantly different ($P < 0.05$); BW: body weight

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Table 5

Water balance (mean ± standard error) of Kacang goats offered water having different pH levels

Parameter	pH level			*P*-value
	6.9	5.2	3.8	
Drinking water intake				
ml/d	1456 ± 173	1218 ± 118	1460 ± 173	0.243
%BW	7.8 ± 0.59	6.6 ± 0.58	7.7 ± 0.55	0.091
Feed water intake				
ml/d	83.9 ± 6.64	73.6 ± 5.54	82.4 ± 7.07	0.091
%BW	0.45 ± 0.02	0.40 ± 0.02	0.44 ± 0.02	0.056
Metabolic water				
ml/d	209.2 ± 15.8	191.6 ± 14.4	206.2 ± 14.6	0.330
%BW	1.13 ± 0.05	1.02 ± 0.04	1.11 ± 0.03	0.186
Total water intake				
ml/d	1750 ± 192	1484 ± 133	1749 ± 192	0.231
%BW	9.4 ± 0.63	8.0 ± 0.63	9.3 ± 0.58	0.187
Fecal water excretion				
ml/d	261 ± 32.4	202 ± 21.9	277 ± 45.5	0.055
%BW	1.4 ± 0.15^{ab}	1.1 ± 0.08^{a}	1.4 ± 0.17^{b}	0.034
Urinary water excretion				
ml/d	418 ± 56.2	321 ± 37.6	385 ± 66.4	0.392
%BW	2.3 ± 0.24	1.8 ± 0.21	2.0 ± 0.23	0.397
Apparent water retention				

ml/d	1070 ± 132.1	960 ± 97.9	1087 ± 88.4	0.421
%BW	5.7 ± 0.45	5.2 ± 0.49	5.8 ± 0.27	0.406

Means with different superscripts are significantly different ($P < 0.05$); BW: body weight

Table 6

Nitrogen (N) balance (mean ± standard error) of Kacang goats offered water having different pH levels

Parameter (%BW)	pH level			*P*-value
	6.9	5.2	3.8	
N intake	0.056 ± 0.003[b]	0.048 ± 0.003[a]	0.055 ± 0.004[ab]	0.036
Fecal N	0.024 ± 0.002	0.020 ± 0.001	0.024 ± 0.002	0.062
N absorb	0.032 ± 0.002	0.028 ± 0.002	0.031 ± 0.002	0.240
Urinary N	0.018 ± 0.003	0.015 ± 0.002	0.016 ± 0.003	0.469
N retention	0.015 ± 0.003	0.013 ± 0.002	0.015 ± 0.002	0.728

Means with different superscripts are significantly different ($P < 0.05$); BW: body weight

Table 7

Pearson correlation coefficients and significance levels[1] of the relationship between daily maximum temperature humidity index (THI_{max}) as well as drinking water intake (DWI) and dry matter intake (DMI) in Kacang goats offered water having different pH levels

Parameter	pH level		
	6.9	5.2	3.8
THI_{max} - DWI			
ml/d	0.62 **	0.14 n.s.	-0.02 n.s.
%BW	0.54 *	-0.15 n.s.	-0.04 n.s.
THI_{max} - DMI			
g/d	0.04 n.s.	0.25 n.s.	-0.31 n.s.
%BW	-0.18 n.s.	-0.29 n.s.	-0.33 n.s.
THI_{max} - DWI/DMI	0.61 **	-0.06 n.s.	0.11 n.s.

[1] Significance levels: n.s., not significant, (*) $p \leq 0.10$, *$p \leq 0.05$, **$p \leq 0.01$; BW: body weight

Effects of acid drinking water on nutrient utilization, water balance, and growth of goats under hot-humid tropical environment

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Abstract

Water available ~~to~~ for livestock in the tropical lowland~~s~~ region is generally high in acidity. Thi~~s~~ere~~fore, this~~ study ~~aimed~~s ~~to~~ determined the effects of the acid water on nutrient intake, water balance, and the growth of goats in the tropical environment. A total of nine Kacang goats were stratified based on body weight (BW) and assigned to three treatment groups which were offered drinking water at varying pH levels, namely 6.9, 5.2, and 3.8. All goats were offered *ad libitum Asystasia gangetica* hay and dried cassava chips at 1% of BW (dry matter (DM) basis) following a crossover design with three treatments tested in three periods. At the 5.2 pH level, drinking water intake (DWI) tended to be lower (P = 0.09) while t~~T~~otal DM intake (%BW) was decreased (P < 0.05). Ruminal pH was significantly difference~~declined~~ (P < 0.01); 6.98, 6.94, and 6.58 at the 6.9, 5.2, and 3.8 pH levels, respectively ~~(P < 0.01)~~. Metabolizable energy and daily gain tended to be higher at the 6.9 and 3.8 pH levels compared to those at the ~~pH~~ 5.2 level~~s~~ (P = 0.08). There were no

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significant adverse effects of acid water on nutrient intake, utilization, and growth of Kacang goats. Moreover, the increased in temperature--humidity index was followed by the elevated DWI ($P < 0.01$) at 6.9 pH level, but no such significant relationship was found at other pH levels that indicated a better capability of thermoregulation response under heat stress exposure.

Keyword acid drinking water, ruminal pH, livestock, heat stress

1. Introduction

Water is one of the most important nutrients in the animal body due to its physiological roles in nutrient transport, maintenance of proper fluid and ion balance, biochemical reactions, as well as body thermoregulation. Previous studiesy showed that a sufficient supply of good quality water is a limiting factor for all animals to maintain good health and optimal productivity (NRC, 2001). However, the supply of clean water resources is a decreasing trend globally, driven by population and economic growth. In the following decades, there is a possibility ofpotential for additional pressure on water resources to fulfill the high demand of for agriculture, household use, and industry. Moreover, the adequate supply of clean water is challenged by extreme weather events due to climate change (Boretti and Rosa, 2019).

In humid tropical lowlands, most of the water is characterized by high acidity due to the natural oxidation processes of pyrite and ferric ion. The pH of the surface water could drop fall to 3, where most of the contaminants are sulfate (SO_4), iron (Fe), manganese (Mn), and aluminum (Al) (Ali et al., 2021a; Manders et al., 2002). Another water source of water in the lowland region is groundwater, which has less acidity and contaminants (Winkel et al., 2008). Although the recommended minimum recommended pH level for livestock is 5.5 (Bagley et al., 1997) or 6.0 (Olkowski, 2009), the effects of the acidic water on ruminants

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animals have not been fully studied. This makes it is necessary to identify the influence of acid water on the animal's performance, implications for water quality standards, and for intervention options for the animal in the lowland region. Therefore, this study was conducted to assess the influence of acid drinking water on water consumption, nutrient intake, and growth goats under hot tropical climates.

2. Materials and Methods

2.1. Study site

This study has been approved by the Faculty of Agriculture, Universitas Sriwijaya, Indonesia. The site is situated at an altitude of ±6 m above sea level and 3°11'38.4"S, 104°39'30.5"E. Meanwhile, the animals were cared for according to the Animal Welfare Guidelines of the Indonesian Institute of Sciences. The environmental variables in the site are shown in Table 1.

2.2. Experimental animal, treatments, and feeding management

A total of nine Kacang goats, based on body weight (BW), were stratified and divided into three treatment groups with an average BW=14.8 ± 1.0 kg, which were offered drinking water at varying pH levels, namely 6.9, 5.2, and 3.8. The animals were housed in individual pens (1.5 m × 0.75 m) in an open-sided type of house which allowed a total collection of daily fecal and urinarye excretion (Asep I M Ali et al., 2021b). Each pen was equipped with two identical feed troughs and an individual water bucket of diameter 23 cm, 5 L capacity. Subsequently, the goats were treated orally with Oxfendazole (25 mg/5 kg BW), acclimatized to feeding and environmental conditions for 15 d, and subjected to their respective water treatments group. All animals were weighed at the beginning of the study as well as every Sunday and Thursday to determine changes in the BW on a n electronic weighing scale before offering feed and water.

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This study used a crossover design that consisted of three levels of pH ~~in~~ over three periods. Meanwhile, each experimental period lasted for three weeks of adaptation and one week of ~~sample collection~~sampling, where feed intake, ~~feces~~fecal~~,~~ and urin~~ary~~e excretion were measured. Each measurement period was followed by one week of recovery, where all animals received only pH 6.9 drinking water.

The diet consisted of *Asystasia gangetica* hay and dried cassava chips as shown in Table 2. The hay was harvested at the pre blooming stage, chaffed to ±5 cm particle length, and sun-dried for 4 d while the cassava tubers were chopped to ±2 cm particle size and sun-dried for 5 d. Subsequently, the feeding and drinking were started at 9:00 after refusals from the previous day had been removed and weighed. The hay was offered ad libitum, according to 15% of the previous intake, while the ~~number~~ amount of cassava chips was referred to 1% of individual BW and adjusted after each BW measurement. Animals always had *ad libitum* access to drinking water and salt-mineral lick, which contained g/kg, DM basis: 730 NaCl, 34 Calcium, 15 Magnesium, 8 Phosphorous, and 1 trace minerals.

2.3. Preparation of different pH levels of water

Naturally available high~~-~~-acidity surface water was collected from non-tidal swamp area (3°10'29.7"S, 104°41'34.5"E), while the underground water with pH = 5.2 was collected from a well in the experimental site. The swamp water was manually collected using a 20-L bucket, while the well water was pumped. Meanwhile, the swamp water had an acidulous taste and a 3.8 pH level, which was checked using a portable pH meter (Hanna HI 98130). A pH level of 6.9 water was prepared from the well water by aeration for 4 d in a 50-L bucket using an aerator (Amara BS-410) and each of the water was stored in separate 50-L buckets before the offering.

2.4. Sample collection, preparation, and analysis

The indoor temperature and relative humidity (RH) were recorded by a climate data logger (Benetech G1365) at ~~a~~ 10-minutes intervals, while rainfall, sunshine, and wind speed were taken at a meteorological station. The temperature-~~-~~humidity index (THI) values were calculated according to ~~formula~~ NRC (1971).

Moreover, the samples of the offered feeds were taken and stored in paper bags at room temperature. After weighing, refusals were homogenized and a subsample (~100 g) was taken and stored. Total fecal and urinary excretion was determined by daily collection over 7 d. Meanwhile, the total feces excreted by each animal was thoroughly mixed by hand, weighed, and a subsample of approximately 100 g fresh matter was taken and dried at 45°C for three consecutive days. The dried feed and fecal samples were ground to pass through a 1-mm mesh. At the end of each period, the feed and fecal samples were pooled per animal proportionally to the daily amount of each animal during the sampling week. The dried samples were stored in zipper plastic bags before laboratory analyses.

The dried feces, feed, and refusals were analyzed as follows: DM, ash (AOAC, 1990; Method 924.05), N (AOAC, 1990; Method 988.05), ether extract (EE; Method 920.39), neutral detergent fiber (NDF, ~~),~~ with alpha-amylase), and acid detergent fiber (ADF) ~~with alpha-amylase and~~ including residual ash (Van Soest et al., 1991). Organic matter (OM) concentrations were calculated by subtracting the ash concentration from 100, while the crude protein ~~(~~CP) content was calculated as N×6.25. Neutral detergent-insoluble N (NDIN) and Neutral detergent-insoluble ash (NDIash) were estimated according to Licitra et al. (1996). Furthermore, NDF corrected for ash and ~~crude protein~~CP (NDF_{acp}) was calculated by subtracting the NDIN and NDIash. Non fibrous carbohydrates (NFC) were calculated by subtracting the concentration of NDF_{acp}, CP, EE, and ash from 100 (Mertens, 1997).

Daily feed intake was calculated as the difference between the amount of feed offered and the amount of feed refusals for each animal across the sampling week. Metabolizable energy (ME, MJ/kg) content was calculated as 0.0157×digestible OM (AFRC, 1993). Total tract apparent digestibility of DM, OM, CP, NDF, and ADF were obtained from the difference between the ~~number~~ amount of nutrient ingested and of nutrients excreted in feces over the 7 d of sampling week.

Before the measurement of rumen fluid pH, the animals were not given drinking water for two h (9:00 – 11:00). The fluid was collected using a stomach tube of 6 mm diameter one h after the goats consumed the water. The drinking water sample was collected every week and stored in a 250-mL bottle at 5 °C. At the end of each period, the samples were pooled proportionally and then analyzed to determine total dissolved solids (TDS, conductivity method, Orion Star A212, Thermo Scientific), Fe, Mn, Al (spectrometric techniques, inductively coupled plasma atomic emission spectroscopy Varian 715-ES, Agilent), nitrate (NO_3), nitrite (NO_2), ammonia (NH_3), sulfate (SO_4) (spectrometric techniques, Spectrophotometer UV-VIS Lambda 45, Perkin Elmer), organic substances (permanganometric titration method).

Individual drinking water intake (DWI) was calculated as the difference between the amount of water offered and refusals. Subsequently, three buckets with water were placed in the barn to estimate daily evaporative water loss, and then the daily DWI was corrected by the evaporative loss. The amount of water in the consumed feed (FWI) was calculated by the difference between the amount of water in the feed offered and refusals. Metabolic water was estimated using the factors 0.62, 0.42, and 1.10 for digestible carbohydrates, protein, and fat, respectively (Taylor, 1970). Apparent total water intake (TWI) was determined as the sum of DWI, FWI, and metabolic water, while the fecal water was estimated from the amount of fecal excretion and the content of water. The amount of urinary water was the amount of

urine corrected by the DM content of urine. Meanwhile, the water retention was calculated by subtracting the amount of water in fecal and urinary excretion from TWI.

After homogenizing and filtering with a surgical gaze, individual urine excretion was recorded. A sample of urine (~100 mL) was taken daily and stored at -20 °C for N analysis. The DM content of urine was determined by drying a 3 mL urine sample at 60 °C for 12 h and the total was determined using the micro Kjeldahl method (AOAC, 1990; Method 988.05). Nitrogen absorption was calculated by subtracting fecal N excretion from the amount of N intake (feed and DWI), while N retention was calculated by subtracting the amount of urinary N loss from the absorbed N.

2.5. Statistical analysis

The data generated from 3 treatments, 3 periods, and 9 animals were analyzed using SAS 9.1 and presented as mean ± standard error. Meanwhile, the data were analyzed using the mixed model procedure as stated below:

$$Y_{ijk}=\mu + T_i + P_j + TP_{ij} + a_k + e_{ijk};$$

Where Y_{ijk} is observed response at a particular *ijk* case, μ is overall mean, *Ti* is the fixed effect of treatment *i*, *Pj* is the fixed effect of period *j*, *TPij* is the fixed effect of the interaction between treatment *i* and period *j*, a_k is the random effect of animal *k*, and e_{ijk} is experimental error.

Differences between means were determined using the Tukey test and the significance level was declared at P < 0.05, where p-values of 0.05 to 0.10 were considered as a trend. The relationship between daily maximum temperature-humidity index (THI_{max}), DWI, and DM intake (DMI) during the collection weeks was tested by Pearson correlation analysis.

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3. Results

The composition of drinking water offered to animals in different treatment groups increases in Fe, Mn, Al, NH_3, SO_4, and organic substances with the decrease in pH level. In

the 6.9 and 5.2 levels, t~~Based on the results,~~ he contaminant concentrations were not significantly different ($P > 0.05$) while the highest concentrations were found in the 3.8 pH level ~~nitrate was the lowest at 5.2 pH level, while the highest concentrations of NO_3 and NO_2 were found at 3.8 pH level~~ ($P < 0.05$; Table 3).

Meanwhile, the values of feed intake, nutrient digestibility, rumen pH, and daily gain of the goats are shown in Table 4. In the group with a 5.2 pH level, total DMI was lower ($P < 0.05$) than those subjected to the other treatments that comparable to the lower ($P < 0.05$) DM intake of hay (%BW) in the group. Furthermore, metabolizable energy intake (MJ/kg $BW^{0.75}$) and daily gain were only influenced by trends ($P = 0.06$). As the pH level reduce~~s~~d, the rumen pH was also decreasing ($P < 0.01$), where the pH in the 3.8 group was lower than those in the 6.9 and 5.2 groups. Meanwhile, the apparent DM, OM, CP, NDF, and ADF digestibility were not significantly different ($P > 0.05$).

Drinking water intake and FWI (%BW) tended to be lowered at the 5.2 group ($P = 0.09$) but metabolic water and TWI were not influenced ($P > 0.05$). Fecal water excretion (%BW) was lowered ($P < 0.05$) in the 5.2 pH group, which was not significantly different from those in the 6.9 group ($P > 0.05$), but higher than those in the 3.8 group. Meanwhile, urinary water excretion and apparent water retention were not significantly affected by the pH level ($P > 0.05$) (Table 5).

Intake of N ~~(%BW) and fecal N excretion (g/d) were~~was also lowered at 5.2 level ($P < 0.05$). However, N absorption, urinary N excretion, and N retention did not vary among the different groups ($P > 0.05$) (Table 6).

During the collection weeks, the daily maximum temperature~~–~~humidity index (THI_{max}) correlated positively with DWI of the 6.9 group but not of the 5.2 and 3.8 groups. Furthermore, DMI did not significantly correlate with THI_{max} among all the groups ($P > 0.05$), while the ratio DWI/DMI correlated with THI_{max} in the 6.9 group ($P < 0.01$) (Table 7).

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4. Discussion

The varied DM intake was not attributable to the DWI while water contaminant concentrations were varied among the different pH levels of drinking water. The tendency of lower DWI in the 5.2 pH group was also not related to the contaminant concentrations in the water where the higher concentrations were found in the 3.8 pH group. Based on the maximum limits of contaminant concentrations in the drinking water, the concentrations of TDS, Fe, NO_3, NO_2, SO_4 were much lower (Table 3)~~The decreased in DM intake was due to the lower DWI at 5.2 pH level, while water contaminant concentrations were varied among the different pH levels of drinking water. However, the tendency of lower DWI in the 5.2 pH group was not related to the contaminant concentrations in the water where the higher concentrations were found in the 3.8 pH group compared to the 5.2 pH group. Based on the maximum limits of contaminants concentrations in the drinking water, the concentrations of TDS, Fe, NO_3, NO_2, SO_4 were much lower (Table 3)~~. The oxidation process of contaminant ions could ~~be~~ relate to the lowered H^+ concentration of the aerated water in the 6.9 pH group (Lytle et al., 1998; Manders et al., 2002). Aeration followed by filtration treatment to remove contaminants from water has been widely used (Lytle et al., 1998; Marsidi et al., 2018). The non-significant differences of the contaminant~~s~~ concentrations in the 6.9 and 5.2 groups due to the absence of the filtration process to remove the precipitates.

Several studies have been conducted on the effect of high-contaminant~~s~~ water on DWI and the performance of ruminants. Mdletshe et al. (2017) stated that reductions of DWI, DMI, and daily gain in Nguni goats as the TDS content of water exceeded the permissible limits. Meanwhile, other studies also observed decreased DWI due to the higher levels of TDS in sheep (Assad and El-Sherif, 2002), beef cattle (López et al., 2016), and buffalo (Sharma et al., 2017). The water intake of beef cattle was also reduced when SO_4 was 1900

mg/L (Lardner et al., 2013) due to the ability of the animals to protect their metabolism status from ~~the~~ salt stress.

Furthermore, the intake level of DWI might be more related to the palatability of the water. In this study, the tendency of lower DWI at 5.5 pH level (P=0.09) was due to the less palatability of the water for the goats. There was a significant decrease in DWI at a lower level of contaminant ~~as~~ reported by ~~(~~Sharma et al. (~~,~~ 2017) for buffalo calves on five TDS levels in drinking water where DWI was lower at 557 ~~levels~~ than those at 2571 mg/L level~~.~~.

The rumen pH was declined by the acid drinking water in this study, however, it was still within the normal range. Acid drinking water may cause rumen acidosis (Olkowski, 2009) when the rumen pH becomes less than 5 (Giger-Reverdin, 2018; Ribeiro et al., 2020). However, the rumen pH values at the pH levels of 5.2 and 3.8 in this study increased to the normal range at ~~1~~ one h post-drinking (Table 4). During the experiment, the animals' normal eating and ruminating behavior and the sufficiency of the minerals-salt supplement might indicate a normal secretion of saliva to maintain the range of rumen pH when the animal continuously consumed the acid drinking water. As a result, the nutrients' digestibility was not affected. A similar OM and NDF digestibility was also reported when the ruminal pH was decreased from 7.0 to 6.2 (Shriver et al., 1986).

The lowered fecal water excretion at the 5.5 level was associated with the lowered DWI and feed water intake, while the insignificant effect on urinary water excretion and apparent water retention was due to the lower contaminants contents in the drinking water. When TDS level was higher, a greater urinary water excretion was reported in sheep (Assad and El-Sherif, 2002), beef cattle (López et al., 2016), and buffalo (Sharma et al., 2017) as an adaptive response of the animals to excrete the excess salts.

The ~~re was a trend for an effect on~~ daily gain was only affected by a trend (P = 0.06), although the gain of goats at the 5.2 level was 48 and 29% lower than those at the 6.9 and 3.8

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247 levels, respectively. Similarly, a higher N retention of the goats at the 6.9 level was not
248 significantly different from those on the 5.2 and 3.8 levels (Table 6). This means the positive
249 gain, N retention, feed intake, and nutrient digestibility indicated that the acid water did not
250 have detrimental effects on the goat performances.

251 The positive correlation of THI_{max} – DWI and THI_{max} - DWI/DMI was due to an
252 increase in demand for water by the goats under heat stress in response to a higher loss of
253 water through evaporation and sweating, which was only applied for the 6.9 group.
254 Furthermore, a positive correlation for daily maximum temperature and DWI was also
255 reported for buffalo calves on five levels of TDS in drinking water (Sharma et al., 2017),
256 lactating goats (Olsson and Dahlborn, 1989) and goat kids (Al-Tamimi, 2007).

257 In tropical humid areas, goats continuously face high ambient temperature and
258 humidity that affect their physiology, behavior, metabolism, and performances, which will
259 become worse in the future due to the increase of climatic extreme events (Silanikove and
260 Koluman, 2015). According to Salama et al. (2021), -Murciano-Granadina goats exposed to
261 heat stress at THI of 77, 30 ^{o}C, and 40% humidity showed a reduction in feed intake and
262 higher water consumption than goats in the thermal neutral environment. During the
263 experimental periods of this study, the means of THI were 79 to 80 (Table 1) which
264 fluctuated daily from 75 in the dawn to 85 in the afternoon (data not shown). Furthermore,
265 the positive correlation THI_{max} – DWI was in line with the result of a previous study, which
266 indicated that DWI also fluctuated at a higher value in the afternoon when THI was at a
267 maximum level. A higher daily THI fluctuation from 70 to 87 with a shift of feeding and
268 drinking frequency was also reported in the tropical humid region of India (Abhijith et al.,
269 2021) ~~(Abhijith et al., 2021)~~. This fluctuation showed the influence of feeding management in
270 minimizing the adverse effect of heat stress on goat performances. Since the drinking water
271 was offered at *ad libitum* level in this study, the animals could freely fulfill the additional

requirement of water for the thermoregulation processes. The significant correlations in the 6.9 group showed the important aspect of clean and good palatability water for maximum intake when the animals experience heat stress.

5. Conclusions

The effect of lowering pH levels in drinking water depends on ~~to~~ the concentration of contaminants in the water. In this study, the lowering of pH level from 6.9 to 3.8 ~~level~~ did not lead to adverse effects on the nutrient intake, balance, and growth due to the minimum levels of the contaminants in the water and the animal's ability to maintain the normal ~~water~~ range of the ~~normal~~ ruminal~~en~~ pH. However, the better ability of the animal in the 6.9 group to cope with the heat stress was shown by the positive correlation between DWI and THI_{max}. In addition, a further study with a more extended period of the acid drinking water is recommended to confirm the effects on rumen fermentation characteristics, thermoregulation, and drinking behavior responses.

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Author contribution

Original intellectual concept and study design: A. I. M. Ali; Methodology: A. I. M. Ali, S. Sandi; Data curation, formal analysis, and investigation: E. Sahara, A. I. M. Ali; Writing - original draft preparation: A. I. M. Ali; Writing - review and editing: M. N. Rofiq, Dahlanuddin; Funding acquisition: A. I. M. Ali. All authors read and approved the final manuscript.

Data availability

The datasets analyzed during this study are available from the corresponding author on reasonable request.

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391 **Tables**

Table 1

Environmental variables observed during the experiment.

Variable	Experimental periods		
	1	2	3
Maximum temperature (T_{max}) (°C)	31.7 ± 0.27	32.7 ± 0.26	33.4 ± 0.29
Minimum temperature (Tmin) (°C)	24.4 ± 0.10	24.8 ± 0.14	24.7 ± 0.17
Average temperature (Tav) (°C)	26.9 ± 0.17	27.6 ± 0.22	27.8 ± 0.18
Average relative humidity (%)	86.0 ± 0.90	84.4 ± 1.07	80.4 ± 0.93
Temperature humidity index	78.7 ± 0.20	79.6 ± 0.29	79.3 ± 0.20
Rainfall (mm/d)	7.8 ± 2.92	2.3 ± 0.68	3.6 ± 2.16
Sunshine (h)	4.1 ± 0.54	5.3 ± 0.46	5.8 ± 0.55
Wind speed (m/s)	1.9 ± 0.11	1.6 ± 0.11	2.1 ± 0.14

Temperature humidity index = (1.8×T°C + 32) – [(0.55 – 0.0055×RH %) × (1.8×T°C - 26)] (NRC, 1971), where T°C is air temperature and RH is the relative humidity.

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Table 2

Chemical composition (mean ± standard error) of Chinese violet (*Asystasia gangetica*) hay and cassava chips offered during the experiment (% dry matter basis)

	Chinese vioet hay	Cassava chips
Dry matter	88.4 ± 0.70	88.3 ± 1.06
Organic matter	89.8 ± 0.11	97.9 ± 0.13
Crude protein (CP)	14.3 ± 0.36	4.2 ± 0.25
Ether extract (EE)	1.7 ± 0.04	0.3 ± 0.02
Ash	10.2 ± 0.50	2.1 ± 0.13
Non fibrous carbohydrates[a]	27.6 ± 0.98	72.9 ± 1.50
Neutral detergent fiber (NDF)	48.1 ± 0.75	22.2 ± 0.07
Neutral detergent fiber $_{acp}$[b]	46.2 ± 0.71	21.9 ± 0.08
Acid detergent fiber	30.5 ± 0.24	4.0 ± 0.18
Acid detergent lignin	14.9 ± 0.12	1.5 ± 0.07

[a]100-CP (%)-EE (%)-[NDF (%)-NDICP (%)]-Ash (%).

[b]Neutral detergent fiber corrected for residual ash and crude protein.

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Table 3

Concentrations of contaminant substances (mg/L, mean ± standard error) in drinking water offered to treatment groups and their permissible limits

Element	Treatment groups			*P*-value	Permissible limits
	6.9	5.2	3.8		
Total dissolved solids	51.0 ± 2.31[a]	48.3 ± 2.96[a]	87.7 ± 8.67[b]	0.004	4000[1], 3000[2]
Iron	0.008 ± 0.002[a]	0.010 ± 0.000[a]	0.223 ± 0.074[b]	0.019	2[1]
Manganese	0.001 ± 0.001[a]	0.004 ± 0.003 [a]	0.027 ± 0.003[b]	0.001	0.3[2]
Aluminum	0.014 ± 0.003[a]	0.036 ± 0.001[a]	2.870 ± 0.067[b]	0.000	NA
Nitrate	14.1 ± 3.52 [a]	12.8 ± 0.51[a]	24.8 ± 1.03[b]	0.014	100[1], 77[2]
Nitrite	0.01 ± 0.011	0.02 ± 0.022	0.02 ± 0.02	0.897	33[1], 10[2]
Ammonia	0.27 ± 0.033[a]	0.30 ± 0.058[ab]	0.47 ± 0.033[b]	0.035	NA
Sulfate	3.3 ± 1.67[a]	5.4 ± 2.11[a]	25.6 ± 5.66[b]	0.009	500[1], 1000[2]
Organic substances	1.9 ± 0.07	1.7 ± 0.16	2.6 ± 0.28	0.053	NA
pH	6.9 ± 0.03[c]	5.2 ± 0.06[b]	3.8 ± 0.02[a]	0.000	5.5[1], 6.0[2]

Means with different superscripts are significantly different ($P < 0.05$);

-Limits for pH (minimum) and other elements (maxima) for livestock drinking water based on United States Environmental Protection Agency (Bagley et al., 1997)[1] and Canadian Council of Ministers of the Environment (Olkowski, 2009)[2];

ND: not detected;

NA: not available

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Table 4

Dry matter (DM) intake, metabolizable energy (ME) intake, digestibility of DM, organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF), as well as rumen pH, and daily gain (mean ± standard error) of Kacang goats offered water having different pH levels

Parameter	pH level			*P*-value
	6.9	5.2	3.8	
Chinese violet hay				
g DM/d	389 ± 36.6	332 ± 32.5	390 ± 48.3	0.154
%BW	2.1 ± 0.15[b]	1.8 ± 0.13[a]	2.1 ± 0.17[b]	0.035
Cassava chips				
g DM/d	159 ± 15.2	166 ± 15.6	158 ± 11.3	0.715
%BW	0.9 ± 0.06	0.9 ± 0.05	0.9 ± 0.05	0.683
Total DM intake				
g/d	548 ± 41.8	498 ± 39.9	549 ± 49.6	0.078
%BW	3.0 ± 0.13[b]	2.7 ± 0.11[a]	2.9 ± 0.13[b]	0.026
ME intake				
(MJ/d)	5.8 ± 0.44	5.3 ± 0.40	5.8 ± 0.43	0.137
MJ/kg $BW^{0.75}$	0.65 ± 0.03	0.59 ± 0.02	0.64± 0.02	0.078
Digestibility (%)				
DM	68.1 ± 0.94	68.5 ± 0.99	67.7 ± 1.21	0.379
OM	67.9 ± 1.04	68.5 ± 1.04	67.5 ± 1.28	0.339
CP	57.7 ± 0.95	57.3 ± 1.29	56.9 ± 0.62	0.722
NDF	41.6 ± 1.61	41.9 ± 2.06	40.3 ± 2.46	0.448

ADF	23.4 ± 2.55	19.8 ± 3.91	23.6 ± 2.95	0.866
Rumen pH	6.98 ± 0.06[b]	6.94 ± 0.05[b]	6.58 ± 0.08[a]	0.002
Daily gain (g/d)	73.4 ± 8.74	49.7 ± 8.42	64.2 ± 6.16	0.062

Means with different superscripts are significantly different ($P < 0.05$); BW: body weight

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Table 5

Water balance (mean ± standard error) of Kacang goats offered water having different pH levels

Parameter	pH level			*P*-value
	6.9	5.2	3.8	
Drinking water intake				
ml/d	1456 ± 173	1218 ± 118	1460 ± 173	0.243
%BW	7.8 ± 0.59	6.6 ± 0.58	7.7 ± 0.55	0.091
Feed water intake				
ml/d	83.9 ± 6.64	73.6 ± 5.54	82.4 ± 7.07	0.091
%BW	0.45 ± 0.02	0.40 ± 0.02	0.44 ± 0.02	0.056
Metabolic water				
ml/d	209.2 ± 15.8	191.6 ± 14.4	206.2 ± 14.6	0.330
%BW	1.13 ± 0.05	1.02 ± 0.04	1.11 ± 0.03	0.186
Total water intake				
ml/d	1750 ± 192	1484 ± 133	1749 ± 192	0.231
%BW	9.4 ± 0.63	8.0 ± 0.63	9.3 ± 0.58	0.187
Fecal water excretion				
ml/d	261 ± 32.4	202 ± 21.9	277 ± 45.5	0.055
%BW	1.4 ± 0.15^{ab}	1.1 ± 0.08^{a}	1.4 ± 0.17^{b}	0.034
Urinary water excretion				
ml/d	418 ± 56.2	321 ± 37.6	385 ± 66.4	0.392
%BW	2.3 ± 0.24	1.8 ± 0.21	2.0 ± 0.23	0.397
Apparent water retention				

ml/d	1070 ± 132.1	960 ± 97.9	1087 ± 88.4	0.421
%BW	5.7 ± 0.45	5.2 ± 0.49	5.8 ± 0.27	0.406

Means with different superscripts are significantly different ($P < 0.05$); BW: body weight

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Table 6

Nitrogen (N) balance (mean ± standard error) of Kacang goats offered water having different pH levels

Parameter (%BW)	pH level			*P*-value
	6.9	5.2	3.8	
N intake	0.056 ± 0.003[b]	0.048 ± 0.003[a]	0.055 ± 0.004[ab]	0.036
Fecal N	0.024 ± 0.002	0.020 ± 0.001	0.024 ± 0.002	0.062
N absorb	0.032 ± 0.002	0.028 ± 0.002	0.031 ± 0.002	0.240
Urinary N	0.018 ± 0.003	0.015 ± 0.002	0.016 ± 0.003	0.469
N retention	0.015 ± 0.003	0.013 ± 0.002	0.015 ± 0.002	0.728

Means with different superscripts are significantly different ($P < 0.05$); BW: body weight

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Table 7

Pearson correlation coefficients and significance levels[1] of the relationship between daily maximum temperature humidity index (THI_{max}) as well as drinking water intake (DWI) and dry matter intake (DMI) in Kacang goats offered water having different pH levels

Parameter	pH level		
	6.9	5.2	3.8
THI_{max} - DWI			
ml/d	0.62 **	0.14 n.s.	-0.02 n.s.
%BW	0.54 *	-0.15 n.s.	-0.04 n.s.
THI_{max} - DMI			
g/d	0.04 n.s.	0.25 n.s.	-0.31 n.s.
%BW	-0.18 n.s.	-0.29 n.s.	-0.33 n.s.
THI_{max} - DWI/DMI	0.61 **	-0.06 n.s.	0.11 n.s.

[1] Significance levels: n.s., not significant, (*) $p \leq 0.10$, *$p \leq 0.05$, **$p \leq 0.01$; BW: body weight

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Conflict of Interest Statement

The authors declare that they have no competing interests.

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