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

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Abstract:	<p>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.</p>
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- A trial with 6.9, 5.2, and 3.8 pH levels of drinking water was conducted
- Ruminant 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.

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

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13 **Abstract**

14 Water available to livestock in the tropical lowlands region is generally high in
15 acidity. Therefore, the effects of acid water were investigated in this study. Nine Kacang
16 goats were stratified based on body weight (BW) and then assigned to three treatment groups:
17 6.9, 5.2, and 3.8 which were offered drinking water varying pH levels: 6.9, 5.2, and 3.8,
18 respectively. All goats were offered *ad libitum Asystasia gangetica* hay and dried cassava
19 chips at 1% of BW (dry matter (DM) basis) followed a crossover design with three treatments
20 tested in three periods. Total DM intake (%BW) was lowered ($P < 0.05$) as lower drinking
21 water intake (DWI) ($P = 0.09$) at the water pH of 5.2. Ruminant pH also declined (6.98, 6.94,
22 and 6.58 at the pH levels of 6.9, 5.2, and 3.8, respectively) ($P < 0.01$). Metabolizable energy
23 and daily gain tended to be higher at 6.9 and 3.8 pH levels compared to those at pH 5.2 level
24 ($P = 0.08$). There were no significant adverse effects of acid water on nutrient intake,
25 utilization, and growth. Moreover, elevated ambient temperature was followed by the

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

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

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31 **1. Introduction**

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

41 In humid tropical lowlands, most of the water is characterized by high acidity due to
42 natural oxidation processes of pyrite and ferric ion. The pH of the surface water could drop to
43 3 and the most potential contaminants are sulfate (SO_4), iron (Fe), manganese (Mn), and
44 aluminum (Al) (Manders et al., 2002; Sahrawat, 2004). Another water source in the lowland
45 region is groundwater where the water has less acidity and contaminants (Winkel et al.,
46 2008). Whilst recommended minimum pH levels for livestock is 5.5 (Bagley et al., 1997) or
47 6.0 (Olkowski, 2009), the effects of the acid water on ruminant animals have not been clearly
48 documented. Therefore, it is necessary to identify the effects of the water on the animal's
49 performance. The present study will have significant implications for water quality standards
50 and for intervention options for the animal particularly reared in the lowland region. Thus, the

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

53 **2. Materials and Methods**

54 **2.1. Study site**

55 Approval of the experiment was granted from the Faculty of Agriculture, Universitas
56 Sriwijaya, Indonesia. The site is situated at an altitude of ± 6 m above sea level and
57 $3^{\circ}11'38.4''S$, $104^{\circ}39'30.5''E$. The animals were cared for according to the Animal Welfare
58 Guidelines of the Indonesian Institute of Sciences. The environmental variables in the site
59 (indoor temperature and relative humidity, RH), rainfall, sunshine, and wind speed are shown
60 in Table 1.

61 **2.2. Experimental animal, treatments, and feeding management**

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

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

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

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

85 **2.3. Preparation of different pH levels of water**

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

94 **2.4. Sample collection, preparation, and analysis**

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

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

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

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

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

116 The dried feces, feed, and refusals were analyzed as follows: DM, ash (AOAC, 1990;
117 Method 924.05), N (AOAC, 1990; Method 988.05), ether extract (EE; Method 920.39), NDF,
118 and acid detergent fiber (ADF) with alpha-amylase and including residual ash (Van Soest et
119 al., 1991). The DM content of urine was determined by drying a 3 mL urine sample at 60 °C
120 for 12 h. Total N in urine samples was determined by the micro Kjeldahl method (AOAC,
121 1990; Method 988.05). Neutral detergent-insoluble N (NDIN) and Neutral detergent-
122 insoluble ash (NDIash) were estimated according to Licitra et al. (1996). Water samples were
123 analyzed for TDS (conductivity method, Orion Star A212, Thermo Scientific), Fe, Mn, Al
124 (spectrometric techniques, inductively coupled plasma atomic emission spectroscopy Varian

125 715-ES, Agilent), nitrate (NO₃), nitrite (NO₂), ammonia (NH₃), sulfate (SO₄), hydrogen
126 sulfide (H₂S) (spectrometric techniques, Spectrophotometer UV-VIS Lambda 45, Perkin
127 Elmer), organic substances (permanganometric titration method).

128 **2.5. Data calculation and statistical analysis**

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

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

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

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

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

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

158 Where Y_{ijk} is observed response at a particular ijk case; μ is overall mean; T_i is the fixed effect
159 of treatment i ; P_j is the fixed effect of period j ; TP_{ij} is the fixed effect of the interaction
160 between treatment i and period j ; a_k is the random effect of animal k ; and e_{ijk} is experimental
161 error.

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

166 3. Results

167 The composition of drinking water offered to animals in different treatment groups
168 showed increases in Fe, Mn, Al, NH_3 , SO_4 , and organic substances with the decrease in pH
169 level. Nitrate was the lowest at 5.2 pH level, whereas for NO_3 and NO_2 , the highest
170 concentrations were found at 3.8 pH level (Table 3). Table 4 presents feed intake, nutrients
171 digestibility, rumen pH, and daily gain of the goats. Total DM intake in the 5.2 group was
172 lower ($P < 0.05$) than those subjected to the other treatments that comparable to the lower (P
173 < 0.05) DM intake of hay (%BW) in the group. Metabolizable energy intake ($MJ/kg BW^{0.75}$)
174 and daily gain were only influenced by trends ($P = 0.06$). Rumen pH was lowered ($P < 0.01$)

175 as the decrease of pH level where the pH in the 3.8 group was lower than those in the 6.9 and
176 5.2 group. Apparent DM, OM, NDF, and ADF digestibility were not significantly different (P
177 > 0.05).

178 Drinking water intake and FWI (%BW) were tended to be lowered at the 5.2 group (P
179 $= 0.09$) but metabolic water and TWI were not influenced ($P > 0.05$). Fecal water excretion
180 (%BW) was lowered ($P < 0.05$) at the 5.2 group, which was not different from those on the
181 6.9 group ($P > 0.05$) but higher than those at the 3.8 group. Urinary water excretion and
182 apparent water retention were not significantly affected by the pH level ($P > 0.05$) (Table 5).
183 Intake of N (%BW) and fecal N excretion (g/d) were also lowered at 5.2 level. However, N
184 absorption, urinary N excretion, and N retention did not vary among the different groups ($P >$
185 0.05) (Table 6).

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

4. Discussion

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

200 (TDS), Fe, NO₃, NO₂, SO₄ were much lower (Table 3). Besides contaminants
201 concentrations, the intake level of DWI might be more related to the palatability of the water.
202 A Similar decrease in DWI at a lower level of contaminant was also reported (Sharma et al.,
203 2017) for buffalo calves on five TDS levels in drinking water where the DWI was lower at
204 557 levels than those at 2571 mg/L level.

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

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

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

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

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

237 **5. Conclusions**

238 In conclusions, the effect of lowering pH level in drinking water always relates to the
239 concentration of contaminants in the water. In the present study, the lowering pH level from
240 6.9 to 3.8 level did not result in adverse effects on the nutrient intake, balance, and growth
241 due to the minimum levels of the contaminants in the water and the animal's ability to
242 maintain the water range of normal rumen pH. However, the better ability of the animal in the
243 6.9 group to the high temperature was evidenced by the positive correlation between drinking
244 water intake and ambient temperature. A further study with a more extended period of acid
245 drinking water offering with thermoregulation and drinking behavior responses of the
246 animals on the different pH levels is needed.

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250 **Author contribution**

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

256 **Data availability**

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

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313 **Tables**314 **Table 1**

Environmental variables observed during the trial.

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

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315 **Table 2**Chemical composition (mean \pm standard error) of Chinese violet (*Asystasia gangetica*) hay and cassava chips offered during the trial

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

^a100-CP (%)-EE (%)-NDF (%)-NDICP (%)-TA (%).^bNeutral detergent fiber corrected for ash and crude protein.

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Table 3

Concentrations of contaminant substances (mg/L, mean \pm 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 \pm 2.31	48.3 \pm 2.96	87.7 \pm 8.67	4000 ^a , 3000 ^b
Iron	0.008 \pm 0.002	0.010 \pm 0.000	0.223 \pm 0.074	2
Manganese	0.001 \pm 0.001	0.004 \pm 0.003	0.027 \pm 0.003	0.3 ^b
Aluminum	0.014 \pm 0.003	0.036 \pm 0.001	2.870 \pm 0.067	NA
Nitrate	14.1 \pm 3.52	12.8 \pm 0.51	24.8 \pm 1.03	100 ^a , 77 ^b
Nitrite	0.01 \pm 0.011	0.02 \pm 0.022	0.02 \pm 0.02	33 ^a , 10 ^b
Ammonia	0.27 \pm 0.033	0.30 \pm 0.058	0.47 \pm 0.033	NA
Sulfate	3.3 \pm 1.67	5.4 \pm 2.11	25.6 \pm 5.66	500 ^a , 1000 ^b
Hydrogen sulfide	ND	ND	ND	NA
Organic substances	1.9 \pm 0.07	1.7 \pm 0.16	2.6 \pm 0.28	NA
pH	6.9 \pm 0.03	5.2 \pm 0.06	3.8 \pm 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

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 \pm standard error) of Kacang goats offered water having different pH levels

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

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Table 5

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

1	ml/d	1070 ± 132.1	960 ± 97.9	1087 ± 88.4	0.421
2	%BW	5.7 ± 0.45	5.2 ± 0.49	5.8 ± 0.27	0.406

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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 ^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 levels¹ 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



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Rumin-D-21-539 Revision Requested

RUMIN <em@editorialmanager.com>

25 Oktober 2021 pukul 15.13

Balas Ke: RUMIN <support@elsevier.com>

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

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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.

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We are looking forward to receiving the revised submission.

With kind regards,

Small Ruminant Research

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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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Please contact the Data in Brief editorial office at dib-me@elsevier.com or visit the Data in Brief homepage (www.journals.elsevier.com/data-in-brief/) if you have questions or need further information.

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 readability 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 befor 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 T_{av} to table 1.

L314 (Table 2): Table is missing units(!)

L314 (Table 2): NDF is lower than NDF_{cp} (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 ($0.05 < p < 10$).

#AU_RUMIN#

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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 --Manuscript Draft--

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Abstract:	<p>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 <i>ad libitum</i> <i>Asystasia gangetica</i> 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.</p>
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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			
<p>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?).</p> <p>Overall, it is appreciated that you measured variables in some breadth, while all appear justified. The overall readability 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).</p>		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 bevoor 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 NDF _{acp} (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) 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
- Ruminant 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

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

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29 **Abstract**

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

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

53 **2. Materials and Methods**

54 **2.1. Study site**

55 This study has been approved by the Faculty of Agriculture, Universitas Sriwijaya,
56 Indonesia. The site is situated at an altitude of ± 6 m above sea level and $3^{\circ}11'38.4''S$,
57 $104^{\circ}39'30.5''E$. Meanwhile, the animals were cared for according to the Animal Welfare
58 Guidelines of the Indonesian Institute of Sciences. The environmental variables in the site are
59 shown in Table 1.

60 **2.2. Experimental animal, treatments, and feeding management**

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

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

1 75 measurement period was followed by one week of recovery, where all animals received only
2 76 pH 6.9 drinking water.
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4 77 The diet consisted of *Asystasia gangetica* hay and dried cassava chips as shown in
5
6 78 Table 2. The hay was harvested at the pre blooming stage, chaffed to ± 5 cm particle length,
7
8 79 and sun-dried for 4 d while the cassava tubers were chopped to ± 2 cm particle size and sun-
9
10 80 dried for 5 d. Subsequently, the feeding and drinking were started at 9:00 after refusals from
11
12 81 the previous day had been removed and weighed. The hay was offered ad libitum, according
13
14 82 to 15% of the previous intake, while the number of cassava chips was referred to 1% of
15
16 83 individual BW and adjusted after each BW measurement. Animals always had *ad libitum*
17
18 84 access to drinking water and salt-mineral lick, which contained g/kg, DM basis: 730 NaCl, 34
19
20 85 Calcium, 15 Magnesium, 8 Phosphorous, and 1 trace minerals.
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26 86 **2.3. Preparation of different pH levels of water**

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28 87 Naturally available high acidity surface water was collected from non-tidal swamp
29
30 88 area (3°10'29.7"S, 104°41'34.5"E), while the underground water with pH = 5.2 was collected
31
32 89 from a well in the experimental site. The swamp water was manually collected using a 20-L
33
34 90 bucket, while the well water was pumped. Meanwhile, the swamp water had an acidulous
35
36 91 taste and a 3.8 pH level, which was checked using a portable pH meter (Hanna HI 98130). A
37
38 92 pH level of 6.9 water was prepared from the well water by aeration for 4 d in a 50-L bucket
39
40 93 using an aerator (Amara BS-410) and each of the water was stored in separate 50-L buckets
41
42 94 before the offering.
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48 95 **2.4. Sample collection, preparation, and analysis**

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50 96 The indoor temperature and relative humidity (RH) were recorded by a climate data
51
52 97 logger (Benetech G1365) at a 10-minutes intervals, while rainfall, sunshine, and wind speed
53
54 98 were taken at a meteorological station. The temperature humidity index (THI) values were
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56 99 calculated according to formula NRC (1971).
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2 101 Moreover, the samples of the offered feeds were taken and stored in paper bags at
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5 102 room temperature. After weighing, refusals were homogenized and a subsample (~100 g) was
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7 103 taken and stored. Total fecal and urinary excretion was determined by daily collection over 7
8
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10 104 d. Meanwhile, the total feces excreted by each animal was thoroughly mixed by hand,
11
12 105 weighed, and a subsample of approximately 100 g fresh matter was taken and dried at 45°C
13
14 106 for three consecutive days. The dried feed and fecal samples were ground to pass through a 1-
15
16
17 107 mm mesh. At the end of each period, the feed and fecal samples were pooled per animal
18
19 108 proportionally to the daily amount of each animal during the sampling week. The dried
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22 109 samples were stored in zipper plastic bags before laboratory analyses.

23
24 110 The dried feces, feed, and refusals were analyzed as follows: DM, ash (AOAC, 1990;
25
26 111 Method 924.05), N (AOAC, 1990; Method 988.05), ether extract (EE; Method 920.39),
27
28
29 112 neutral detergent fiber (NDF), and acid detergent fiber (ADF) with alpha-amylase and
30
31 113 including residual ash (Van Soest et al., 1991). Organic matter (OM) concentrations were
32
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34 114 calculated by subtracting the ash concentration from 100, while the CP content was
35
36 115 calculated as $N \times 6.25$. Neutral detergent-insoluble N (NDIN) and Neutral detergent-insoluble
37
38
39 116 ash (NDIash) were estimated according to Licitra et al. (1996). Furthermore, NDF corrected
40
41 117 for ash and crude protein (NDF_{acp}) was calculated by subtracting the NDIN and NDIash. Non
42
43
44 118 fibrous carbohydrates (NFC) were calculated by subtracting the concentration of NDF_{acp} , CP,
45
46 119 EE, and ash from 100 (Mertens, 1997).

47
48
49 120 Daily feed intake was calculated as the difference between the amount of feed offered
50
51 121 and the amount of feed refusals for each animal across the sampling week. Metabolizable
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54 122 energy (ME, MJ/kg) content was calculated as $0.0157 \times \text{digestible OM}$ (AFRC, 1993). Total
55
56 123 tract apparent digestibility of DM, OM, NDF, and ADF were obtained from the difference
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124 between the number of nutrient ingested and of nutrients excreted in feces over the 7 d of
125 sampling week.

126 Before the measurement of rumen fluid pH, the animals were not given drinking
127 water for two h (9:00 – 11:00). The fluid was collected using a stomach tube of 6 mm
128 diameter one h after the goats consumed the water. The drinking water sample was collected
129 every week and stored in a 250-mL bottle at 5 °C. At the end of each period, the samples
130 were pooled proportionally and then analyzed to determine total dissolved solids (TDS,
131 conductivity method, Orion Star A212, Thermo Scientific), Fe, Mn, Al (spectrometric
132 techniques, inductively coupled plasma atomic emission spectroscopy Varian 715-ES,
133 Agilent), nitrate (NO₃), nitrite (NO₂), ammonia (NH₃), sulfate (SO₄) (spectrometric
134 techniques, Spectrophotometer UV-VIS Lambda 45, Perkin Elmer), organic substances
135 (permanganometric titration method).

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

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

149 The DM content of urine was determined by drying a 3 mL urine sample at 60 °C for 12 h
150 and the total was determined using the micro Kjeldahl method (AOAC, 1990; Method
151 988.05). Nitrogen absorption was calculated by subtracting fecal N excretion from the
152 amount of N intake (feed and DWI), while N retention was calculated by subtracting the
153 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, T_i is the fixed effect of treatment i , P_j is the fixed effect of period j , TP_{ij} 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

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

180 Drinking water intake and FWI (%BW) tended to be lowered at the 5.2 group ($P =$
181 0.09) but metabolic water and TWI were not influenced ($P > 0.05$). Fecal water excretion
182 (%BW) was lowered ($P < 0.05$) in the 5.2 pH group, which was not significantly different
183 from those in the 6.9 group ($P > 0.05$), but higher than those in the 3.8 group. Meanwhile,
184 urinary water excretion and apparent water retention were not significantly affected by the
185 pH level ($P > 0.05$) (Table 5).

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

189 During the collection weeks, daily maximum temperature humidity index (THI_{max})
190 correlated positively with DWI of the 6.9 group but not of the 5.2 and 3.8 groups.
191 Furthermore, DMI did not significantly correlate with THI_{max} among all the groups ($P >$
192 0.05), while the ratio DWI/DMI correlated with THI_{max} in the 6.9 group ($P < 0.01$) (Table 7).

193 4. Discussion

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

199 in the drinking water, the concentrations of TDS, Fe, NO₃, NO₂, SO₄ were much lower (Table
200 3). Several studies have been conducted on the effect of high-contaminants water on DWI
201 and the performance of ruminants. Mdletshe et al. (2017) stated that reductions of DWI,
202 DMI, and daily gain in Nguni goats as TDS content of water exceeded the permissible limits.
203 Meanwhile, other studies also observed decreased DWI due to the higher levels of TDS in
204 sheep (Assad and El-Sherif, 2002), beef cattle (López et al., 2016), and buffalo (Sharma et
205 al., 2017). The water intake of beef cattle was also reduced when SO₄ was 1900 mg/L
206 (Lardner et al., 2013) due to the ability of the animals to protect their metabolism status from
207 the salt stress.

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

213 The rumen pH was declined by the acid drinking water in this study, however, it was
214 still within the normal range. Acid drinking water may cause rumen acidosis (Olkowski,
215 2009) when the rumen pH becomes less than 5 (Giger-Reverdin, 2018; Ribeiro et al., 2020).
216 However, the rumen pH values at the pH levels of 5.2 and 3.8 in this study increased to the
217 normal range at 1 h post-drinking (Table 4). During the experiment, the animals' normal
218 eating and ruminating behavior and the sufficiency of the minerals-salt supplement might
219 indicate a normal secretion of saliva to maintain the range of rumen pH when the animal
220 continuously consumed the acid drinking water. As a result, the nutrients' digestibility was
221 not affected. A similar OM and NDF digestibility was also reported when the ruminal pH was
222 decreased from 7.0 to 6.2 (Shriver et al., 1986).

223 The lowered fecal water excretion at the 5.5 level was associated with the lowered
1
2 224 DWI and feed water intake, while the insignificant effect on urinary water excretion and
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5 225 apparent water retention was due to the lower contaminants contents in the drinking water.
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7 226 When TDS level was higher, a greater urinary water excretion was reported in sheep (Assad
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10 227 and El-Sherif, 2002), beef cattle (López et al., 2016), and buffalo (Sharma et al., 2017) as an
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12 228 adaptive response of the animals to excrete the excess salts.

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14 229 There was a trend for an effect on daily gain ($P = 0.06$), although the gain of goats at
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17 230 the 5.2 level was 48 and 29% lower than those at the 6.9 and 3.8 levels, respectively.
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19 231 Similarly, a higher N retention of the goats at the 6.9 level was not significantly different
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22 232 from those on the 5.2 and 3.8 levels (Table 6). This means the positive gain, N retention, feed
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24 233 intake, and nutrient digestibility indicated that the acid water did not have detrimental effects
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27 234 on the goat performances.

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29 235 The positive correlation of $THI_{max} - DWI$ and $THI_{max} - DWI/DMI$ was due to an
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32 236 increase in demand for water by the goats under heat stress in response to a higher loss of
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34 237 water through evaporation and sweating, which was only applied for the 6.9 group.
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36 238 Furthermore, a positive correlation for daily maximum temperature and DWI was also
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39 239 reported for buffalo calves on five levels of TDS in drinking water (Sharma et al., 2017),
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41 240 lactating goats (Olsson and Dahlborn, 1989) and goat kids (Al-Tamimi, 2007).

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43 241 In tropical humid areas, goats continuously face high ambient temperature and
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46 242 humidity that affect their physiology, behavior, metabolism, and performances, which will
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49 243 become worse in the future due to the increase of climatic extreme events (Silanikove and
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51 244 Koluman, 2015). According to Salama et al. (2021), Murciano-Granadina goats exposed to
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53 245 heat stress at THI of 77, 30 °C, and 40% humidity showed a reduction in feed intake and
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56 246 higher water consumption than goats in the thermal neutral environment. During the
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58 247 experimental periods of this study, the means of THI were 79 to 80 (Table 1) which
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248 fluctuated daily from 75 in the dawn to 85 in the afternoon (data not shown). Furthermore,
249 the positive correlation $THI_{max} - DWI$ was in line with the result of a previous study, which
250 indicated that DWI also fluctuated at a higher value in the afternoon when THI was at a
251 maximum level. A higher daily THI fluctuation from 70 to 87 with a shift of feeding and
252 drinking frequency was also reported in the tropical humid region of India. This fluctuation
253 showed the influence of feeding management in minimizing the adverse effect of heat stress
254 on goat performances. Since the drinking water was offered at *ad libitum* level in this study,
255 the animals could freely fulfill the additional requirement of water for the thermoregulation
256 processes. The significant correlations in the 6.9 group showed the important aspect of clean
257 and good palatability water for maximum intake when the animals experience heat stress.

258 **5. Conclusions**

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

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272 **Author contribution**

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

278 **Data availability**

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

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367 **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 (T _{min}) (°C)	24.4 ± 0.10	24.8 ± 0.14	24.7 ± 0.17
Average temperature (T _{av}) (°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 = $(1.8 \times T^{\circ}\text{C} + 32) - [(0.55 - 0.0055 \times \text{RH} \%) \times (1.8 \times T^{\circ}\text{C} - 26)]$ (NRC, 1971), where T°C is air temperature and RH is the relative humidity.

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Table 2Chemical composition (mean ± standard error) of Chinese violet (*Asystasia gangetica*) hay and cassava chips offered during the experiment (% dry matter basis)

	Chinese violet 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

^a100-CP (%)-EE (%)-[NDF (%)-NDICP (%)]-Ash (%).

^bNeutral detergent fiber corrected for residual ash and crude protein.

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Table 3

Concentrations of contaminant substances (mg/L, mean \pm 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 \pm 2.31	48.3 \pm 2.96	87.7 \pm 8.67	4000 ^a , 3000 ^b
Iron	0.008 \pm 0.002	0.010 \pm 0.000	0.223 \pm 0.074	2 ^a
Manganese	0.001 \pm 0.001	0.004 \pm 0.003	0.027 \pm 0.003	0.3 ^b
Aluminum	0.014 \pm 0.003	0.036 \pm 0.001	2.870 \pm 0.067	NA
Nitrate	14.1 \pm 3.52	12.8 \pm 0.51	24.8 \pm 1.03	100 ^a , 77 ^b
Nitrite	0.01 \pm 0.011	0.02 \pm 0.022	0.02 \pm 0.02	33 ^a , 10 ^b
Ammonia	0.27 \pm 0.033	0.30 \pm 0.058	0.47 \pm 0.033	NA
Sulfate	3.3 \pm 1.67	5.4 \pm 2.11	25.6 \pm 5.66	500 ^a , 1000 ^b
Organic substances	1.9 \pm 0.07	1.7 \pm 0.16	2.6 \pm 0.28	NA
pH	6.9 \pm 0.03	5.2 \pm 0.06	3.8 \pm 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

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 \pm standard error) of Kacang goats offered water having different pH levels

Parameter	pH level			<i>P</i> -value
	6.9	5.2	3.8	
Chinese violet hay				
g DM/d	389 \pm 36.6	332 \pm 32.5	390 \pm 48.3	0.154
%BW	2.1 \pm 0.15 ^b	1.8 \pm 0.13 ^a	2.1 \pm 0.17 ^b	0.035
Cassava chips				
g DM/d	159 \pm 15.2	166 \pm 15.6	158 \pm 11.3	0.715
%BW	0.9 \pm 0.06	0.9 \pm 0.05	0.9 \pm 0.05	0.683
Total DM intake				
g/d	548 \pm 41.8	498 \pm 39.9	549 \pm 49.6	0.078
%BW	3.0 \pm 0.13 ^b	2.7 \pm 0.11 ^a	2.9 \pm 0.13 ^b	0.026
ME intake				
(MJ/d)	5.8 \pm 0.44	5.3 \pm 0.40	5.8 \pm 0.43	0.137
MJ/kg BW ^{0.75}	0.65 \pm 0.03	0.59 \pm 0.02	0.64 \pm 0.02	0.078
Digestibility (%)				
DM	68.1 \pm 0.94	68.5 \pm 0.99	67.7 \pm 1.21	0.379
OM	67.9 \pm 1.04	68.5 \pm 1.04	67.5 \pm 1.28	0.339
NDF	41.6 \pm 1.61	41.9 \pm 2.06	40.3 \pm 2.46	0.448
ADF	23.4 \pm 2.55	19.8 \pm 3.91	23.6 \pm 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 \pm 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 \pm 173	1218 \pm 118	1460 \pm 173	0.243
%BW	7.8 \pm 0.59	6.6 \pm 0.58	7.7 \pm 0.55	0.091
Feed water intake				
ml/d	83.9 \pm 6.64	73.6 \pm 5.54	82.4 \pm 7.07	0.091
%BW	0.45 \pm 0.02	0.40 \pm 0.02	0.44 \pm 0.02	0.056
Metabolic water				
ml/d	209.2 \pm 15.8	191.6 \pm 14.4	206.2 \pm 14.6	0.330
%BW	1.13 \pm 0.05	1.02 \pm 0.04	1.11 \pm 0.03	0.186
Total water intake				
ml/d	1750 \pm 192	1484 \pm 133	1749 \pm 192	0.231
%BW	9.4 \pm 0.63	8.0 \pm 0.63	9.3 \pm 0.58	0.187
Fecal water excretion				
ml/d	261 \pm 32.4	202 \pm 21.9	277 \pm 45.5	0.055
%BW	1.4 \pm 0.15 ^{ab}	1.1 \pm 0.08 ^a	1.4 \pm 0.17 ^b	0.034
Urinary water excretion				
ml/d	418 \pm 56.2	321 \pm 37.6	385 \pm 66.4	0.392
%BW	2.3 \pm 0.24	1.8 \pm 0.21	2.0 \pm 0.23	0.397
Apparent water retention				

1	ml/d	1070 ± 132.1	960 ± 97.9	1087 ± 88.4	0.421
2	%BW	5.7 ± 0.45	5.2 ± 0.49	5.8 ± 0.27	0.406

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

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Table 6

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

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

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

Table 7

Pearson correlation coefficients and significance levels¹ 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

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

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13 **Abstract**

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

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

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

30

31 **1. Introduction**

32 Water is one of the most important nutrients in the animal body due to its
33 physiological roles in nutrient transport, maintenance of proper fluid and ion balance,
34 biochemical reactions, as well as body thermoregulation. **Previous study showed that** a
35 sufficient supply of good quality water is a limiting factor for all animals to maintain good
36 health and optimal productivity (NRC, 2001). However, the supply of clean water resources
37 is **a decreasing** trend globally, driven by population and economic growth. In the following
38 decades, there is a possibility of additional pressure on water resources to **fulfill the high**
39 demand of agriculture, household use, and industry. Moreover, the adequate supply of clean
40 water is challenged by extreme weather events due to climate change (Boretti and Rosa,
41 2019).

42 In humid tropical lowlands, most of the water is characterized by high acidity due to
43 natural oxidation processes of pyrite and ferric ion. The pH of the surface water drop to 3,
44 where most of the contaminants are sulfate (SO_4), iron (Fe), manganese (Mn), and aluminum
45 (Al) (Manders et al., 2002; Sahrawat, 2004). Another water source in the lowland region is
46 groundwater, which has less acidity and contaminants (Winkel et al., 2008). Although the
47 recommended minimum pH **level** for livestock is 5.5 (Bagley et al., 1997) or 6.0 (Olkowski,
48 2009), the effects of the acid water on ruminant animals have not been fully studied. This
49 makes it is necessary to identify the influence of acid water on the animal's performance,
50 implications for water quality standards, and for intervention options for the animal in the

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

53 **2. Materials and Methods**

54 **2.1. Study site**

55 This study has been approved by the Faculty of Agriculture, Universitas Sriwijaya,
56 Indonesia. The site is situated at an altitude of ± 6 m above sea level and $3^{\circ}11'38.4''\text{S}$,
57 $104^{\circ}39'30.5''\text{E}$. Meanwhile, the animals were cared for according to the Animal Welfare
58 Guidelines of the Indonesian Institute of Sciences. The environmental variables in the site are
59 shown in Table 1.

60 **2.2. Experimental animal, treatments, and feeding management**

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

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

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

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

86 **2.3. Preparation of different pH levels of water**

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

95 **2.4. Sample collection, preparation, and analysis**

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

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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 $N \times 6.25$. 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 $0.0157 \times \text{digestible OM}$ (AFRC, 1993). Total tract apparent digestibility of DM, OM, NDF, and ADF were obtained from the difference

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

126 Before the measurement of rumen fluid pH, the animals were not given drinking
127 water for two h (9:00 – 11:00). The fluid was collected using a stomach tube of 6 mm
128 diameter one h after the goats consumed the water. The drinking water sample was collected
129 every week and stored in a 250-mL bottle at 5 °C. At the end of each period, the samples
130 were pooled proportionally and then analyzed to determine total dissolved solids (TDS,
131 conductivity method, Orion Star A212, Thermo Scientific), Fe, Mn, Al (spectrometric
132 techniques, inductively coupled plasma atomic emission spectroscopy Varian 715-ES,
133 Agilent), nitrate (NO₃), nitrite (NO₂), ammonia (NH₃), sulfate (SO₄) (spectrometric
134 techniques, Spectrophotometer UV-VIS Lambda 45, Perkin Elmer), organic substances
135 (permanganometric titration method).

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

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

149 The DM content of urine was determined by drying a 3 mL urine sample at 60 °C for 12 h
150 and the total was determined using the micro Kjeldahl method (AOAC, 1990; Method
151 988.05). Nitrogen absorption was calculated by subtracting fecal N excretion from the
152 amount of N intake (feed and DWI), while N retention was calculated by subtracting the
153 amount of urinary N loss from the absorbed N.

154 2.5. Statistical analysis

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

$$158 Y_{ijk} = \mu + T_i + P_j + TP_{ij} + a_k + e_{ijk};$$

159 Where Y_{ijk} is observed response at a particular ijk case, μ is overall mean, T_i is the fixed effect
160 of treatment i , P_j is the fixed effect of period j , TP_{ij} is the fixed effect of the interaction
161 between treatment i and period j , a_k is the random effect of animal k , and e_{ijk} is experimental
162 error.

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

167 3. Results

168 The composition of drinking water offered to animals in different treatment groups
169 increases in Fe, Mn, Al, NH₃, SO₄, and organic substances with the decrease in pH level.
170 Based on the results, nitrate was the lowest at 5.2 pH level, while the highest concentrations
171 of NO₃ and NO₂ were found at 3.8 pH level (Table 3). Meanwhile, the values of feed intake,
172 nutrient digestibility, rumen pH, and daily gain of the goats are shown in Table 4. In the 5.2
173 group with a 5.2 pH level, total DMI was lower ($P < 0.05$) than those subjected to the other

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

180 Drinking water intake and FWI (%BW) **tended** to be lowered at the 5.2 group ($P =$
181 0.09) but metabolic water and TWI were not influenced ($P > 0.05$). Fecal water excretion
182 (%BW) was lowered ($P < 0.05$) in the 5.2 pH group, which was not significantly different
183 from those **in** the 6.9 group ($P > 0.05$), but higher than those **in** the 3.8 group. Meanwhile,
184 urinary water excretion and apparent water retention were not significantly affected by the
185 pH level ($P > 0.05$) (Table 5).

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

189 During the collection weeks, daily maximum temperature humidity index (THI_{max})
190 correlated positively with DWI of the 6.9 group but not of the 5.2 and 3.8 groups.
191 Furthermore, DMI did not significantly correlate with THI_{max} among all the groups ($P >$
192 0.05), while **the** ratio DWI/DMI correlated with THI_{max} in the 6.9 group ($P < 0.01$) (Table 7).

193 **4. Discussion**

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

199 contaminants concentrations in the drinking water, the concentrations of TDS, Fe, NO₃, NO₂,
200 SO₄ were much lower (Table 3). Several studies have been conducted on the effect of high-
201 contaminants water on DWI and the performance of ruminants. Mdletshe et al. (2017) stated
202 that reductions of DWI, DMI, and daily gain in Nguni goats as TDS content of water
203 exceeded the permissible limits. Meanwhile, other studies also observed decreased DWI due
204 to the higher levels of TDS in sheep (Assad and El-Sherif, 2002), beef cattle (López et al.,
205 2016), and buffalo (Sharma et al., 2017). The water intake of beef cattle was also reduced
206 when SO₄ was 1900 mg/L (Lardner et al., 2013) due to the ability of the animals to protect
207 their metabolism status from the salt stress.

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

214 The rumen pH was declined by the acid drinking water in this study, however, it was
215 still within the normal range. Acid drinking water may cause rumen acidosis (Olkowski,
216 2009) when the rumen pH becomes less than 5 (Giger-Reverdin, 2018; Ribeiro et al., 2020).
217 However, the rumen pH values at the pH levels of 5.2 and 3.8 in this study increased to the
218 normal range at 1 h post-drinking (Table 4). During the experiment, the animals' normal
219 eating and ruminating behavior and the sufficiency of the minerals-salt supplement might
220 indicate a normal secretion of saliva to maintain the range of rumen pH when the animal
221 continuously consumed the acid drinking water. As a result, the nutrients' digestibility was
222 not affected. A similar OM and NDF digestibility was also reported when the ruminal pH was
223 decreased from 7.0 to 6.2 (Shriver et al., 1986).

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

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

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

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

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

259 5. Conclusions

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

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273 **Author contribution**

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

279 **Data availability**

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

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366

368 **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 (T _{min}) (°C)	24.4 ± 0.10	24.8 ± 0.14	24.7 ± 0.17
Average temperature (T_{av}) (°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 = $(1.8 \times T^{\circ}\text{C} + 32) - [(0.55 - 0.0055 \times \text{RH} \%) \times (1.8 \times T^{\circ}\text{C} - 26)]$ (NRC, 1971), where T°C is air temperature and RH is the relative humidity.

Table 2Chemical composition (mean ± standard error) of Chinese violet (*Asystasia gangetica*) hay and cassava chips offered during the **experiment (% dry matter basis)**

	Chinese violet 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

^a100-CP (%) - EE (%) - [NDF (%) - NDICP (%) - Ash (%).

^bNeutral detergent fiber corrected for **residual ash** and crude protein.

Table 3

Concentrations of contaminant substances (mg/L, mean \pm 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 \pm 2.31	48.3 \pm 2.96	87.7 \pm 8.67	4000 ^a , 3000 ^b
Iron	0.008 \pm 0.002	0.010 \pm 0.000	0.223 \pm 0.074	2 ^a
Manganese	0.001 \pm 0.001	0.004 \pm 0.003	0.027 \pm 0.003	0.3 ^b
Aluminum	0.014 \pm 0.003	0.036 \pm 0.001	2.870 \pm 0.067	NA
Nitrate	14.1 \pm 3.52	12.8 \pm 0.51	24.8 \pm 1.03	100 ^a , 77 ^b
Nitrite	0.01 \pm 0.011	0.02 \pm 0.022	0.02 \pm 0.02	33 ^a , 10 ^b
Ammonia	0.27 \pm 0.033	0.30 \pm 0.058	0.47 \pm 0.033	NA
Sulfate	3.3 \pm 1.67	5.4 \pm 2.11	25.6 \pm 5.66	500 ^a , 1000 ^b
Organic substances	1.9 \pm 0.07	1.7 \pm 0.16	2.6 \pm 0.28	NA
pH	6.9 \pm 0.03	5.2 \pm 0.06	3.8 \pm 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

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 \pm 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 \pm 36.6	332 \pm 32.5	390 \pm 48.3	0.154
%BW	2.1 \pm 0.15 ^b	1.8 \pm 0.13 ^a	2.1 \pm 0.17 ^b	0.035
Cassava chips				
g DM/d	159 \pm 15.2	166 \pm 15.6	158 \pm 11.3	0.715
%BW	0.9 \pm 0.06	0.9 \pm 0.05	0.9 \pm 0.05	0.683
Total DM intake				
g/d	548 \pm 41.8	498 \pm 39.9	549 \pm 49.6	0.078
%BW	3.0 \pm 0.13 ^b	2.7 \pm 0.11 ^a	2.9 \pm 0.13 ^b	0.026
ME intake				
(MJ/d)	5.8 \pm 0.44	5.3 \pm 0.40	5.8 \pm 0.43	0.137
MJ/kg BW ^{0.75}	0.65 \pm 0.03	0.59 \pm 0.02	0.64 \pm 0.02	0.078
Digestibility (%)				
DM	68.1 \pm 0.94	68.5 \pm 0.99	67.7 \pm 1.21	0.379
OM	67.9 \pm 1.04	68.5 \pm 1.04	67.5 \pm 1.28	0.339
NDF	41.6 \pm 1.61	41.9 \pm 2.06	40.3 \pm 2.46	0.448
ADF	23.4 \pm 2.55	19.8 \pm 3.91	23.6 \pm 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 \pm 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 \pm 173	1218 \pm 118	1460 \pm 173	0.243
%BW	7.8 \pm 0.59	6.6 \pm 0.58	7.7 \pm 0.55	0.091
Feed water intake				
ml/d	83.9 \pm 6.64	73.6 \pm 5.54	82.4 \pm 7.07	0.091
%BW	0.45 \pm 0.02	0.40 \pm 0.02	0.44 \pm 0.02	0.056
Metabolic water				
ml/d	209.2 \pm 15.8	191.6 \pm 14.4	206.2 \pm 14.6	0.330
%BW	1.13 \pm 0.05	1.02 \pm 0.04	1.11 \pm 0.03	0.186
Total water intake				
ml/d	1750 \pm 192	1484 \pm 133	1749 \pm 192	0.231
%BW	9.4 \pm 0.63	8.0 \pm 0.63	9.3 \pm 0.58	0.187
Fecal water excretion				
ml/d	261 \pm 32.4	202 \pm 21.9	277 \pm 45.5	0.055
%BW	1.4 \pm 0.15 ^{ab}	1.1 \pm 0.08 ^a	1.4 \pm 0.17 ^b	0.034
Urinary water excretion				
ml/d	418 \pm 56.2	321 \pm 37.6	385 \pm 66.4	0.392
%BW	2.3 \pm 0.24	1.8 \pm 0.21	2.0 \pm 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

378

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Table 6

Nitrogen (N) balance (mean \pm 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 \pm 0.907	9.0 \pm 0.812	10.3 \pm 1.112	0.074
%BW	0.056 \pm 0.003 ^b	0.048 \pm 0.003 ^a	0.055 \pm 0.004 ^{ab}	0.036
Fecal N				
g/d	4.41 \pm 0.403 ^b	3.84 \pm 0.357 ^a	4.43 \pm 0.527 ^b	0.037
%BW	0.024 \pm 0.002	0.020 \pm 0.001	0.024 \pm 0.002	0.062
N absorb				
g/day	5.98 \pm 0.526	5.18 \pm 0.487	5.87 \pm 0.620	0.313
%BW	0.03 \pm 0.002	0.03 \pm 0.002	0.03 \pm 0.002	0.240
Urinary N				
g/day	3.32 \pm 0.615	2.80 \pm 0.413	3.10 \pm 0.698	0.531
%BW	0.02 \pm 0.003	0.02 \pm 0.002	0.02 \pm 0.003	0.469
N retention				
g/day	2.66 \pm 0.542	2.38 \pm 0.465	2.78 \pm 0.439	0.789
%BW	0.01 \pm 0.003	0.01 \pm 0.002	0.02 \pm 0.002	0.728

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

Table 7

Pearson correlation coefficients and significance levels¹ 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

Conflict of Interest Statement

The authors declare that they have no competing interests.



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Rumin-D-21-539R1 Revision Requested

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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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We are looking forward to receiving the revised submission.

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 addressed:

- 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.

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Ms. No. Rumin-D-21-539R2

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 --Manuscript Draft--

Manuscript Number:	Rumin-D-21-539R2
Article Type:	Research Paper
Section/Category:	Nutrition and Feeding Systems
Keywords:	acid drinking water; ruminal pH; livestock; heat stress
Corresponding Author:	Asep Indra Munawar Ali, Ph.D Universitas Sriwijaya Fakultas Pertanian Ogan Ilir, Sumatera Selatan INDONESIA
First Author:	Asep Indra Munawar Ali, Ph.D
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Abstract:	<p>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.</p>
Suggested Reviewers:	Uta Dickhöfer, PhD Professor, University of Hohenheim: Universitat Hohenheim uta.dickhoefer@uni-hohenheim.de Komang Gede Wiryawan, PhD Professor, Institut Pertanian Bogor Fakultas Peternakan kgwiryawan@ipb.ac.id; kgwiryawan61@gmail.com Asep Sudarman, PhD Professor, Institut Pertanian Bogor Fakultas Peternakan asudarman@ipb.ac.id Idat Galih Permana, PhD Assistant Professor, Institut Pertanian Bogor Fakultas Peternakan permana@ipb.ac.id Riswandi Riswandi, PhD Assistant Professor, Universitas Sriwijaya Fakultas Pertanian riswandi@fp.unsri.ac.id

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
- Ruminant 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

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

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23
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29 13 **Abstract**

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

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

32 1. Introduction

33 Water is one of the most important nutrients in the animal body due to its
34 physiological roles in nutrient transport, maintenance of proper fluid and ion balance,
35 biochemical reactions, as well as body thermoregulation. Previous study showed that a
36 sufficient supply of good quality water is a limiting factor for all animals to maintain good
37 health and optimal productivity (NRC, 2001). However, the supply of clean water resources
38 is a decreasing trend globally, driven by population and economic growth. In the following
39 decades, there is a potential for additional pressure on water resources to fulfill the high
40 demand for agriculture, household use, and industry. Moreover, the adequate supply of clean
41 water is challenged by extreme weather events due to climate change (Boretti and Rosa,
42 2019).

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

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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 $3^{\circ}11'38.4''\text{S}$, $104^{\circ}39'30.5''\text{E}$. 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 $\text{BW}=14.8 \pm 1.0$ 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 \times 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

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

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

87 **2.3. Preparation of different pH levels of water**

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

96 **2.4. Sample collection, preparation, and analysis**

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

101 Moreover, the samples of the offered feeds were taken and stored in paper bags at
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2 room temperature. After weighing, refusals were homogenized and a subsample (~100 g) was
3
4 taken and stored. Total fecal and urinary excretion was determined by daily collection over 7
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7 d. Meanwhile, the total feces excreted by each animal was thoroughly mixed by hand,
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9 weighed, and a subsample of approximately 100 g fresh matter was taken and dried at 45°C
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11 for three consecutive days. The dried feed and fecal samples were ground to pass through a 1-
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13 mm mesh. At the end of each period, the feed and fecal samples were pooled per animal
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15 proportionally to the daily amount of each animal during the sampling week. The dried
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17 samples were stored in zipper plastic bags before laboratory analyses.
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22 The dried feces, feed, and refusals were analyzed as follows: DM, ash (AOAC, 1990;
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24 Method 924.05), N (AOAC, 1990; Method 988.05), ether extract (EE; Method 920.39),
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26 neutral detergent fiber (NDF, with alpha-amylase), and acid detergent fiber (ADF) including
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28 residual ash (Van Soest et al., 1991). Organic matter (OM) concentrations were calculated by
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30 subtracting the ash concentration from 100, while the crude protein (CP) content was
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32 calculated as $N \times 6.25$. Neutral detergent-insoluble N (NDIN) and Neutral detergent-insoluble
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34 ash (NDIash) were estimated according to Licitra et al. (1996). Furthermore, NDF corrected
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36 for ash and CP (NDF_{acp}) was calculated by subtracting the NDIN and NDIash. Non fibrous
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38 carbohydrates (NFC) were calculated by subtracting the concentration of NDF_{acp} , CP, EE,
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40 and ash from 100 (Mertens, 1997).
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46 Daily feed intake was calculated as the difference between the amount of feed offered
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48 and the amount of feed refusals for each animal across the sampling week. Metabolizable
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50 energy (ME, MJ/kg) content was calculated as $0.0157 \times \text{digestible OM}$ (AFRC, 1993). Total
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52 tract apparent digestibility of DM, OM, CP, NDF, and ADF were obtained from the
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54 difference between the amount of nutrient ingested and of nutrients excreted in feces over the
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126 Before the measurement of rumen fluid pH, the animals were not given drinking
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2 127 water for two h (9:00 – 11:00). The fluid was collected using a stomach tube of 6 mm
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5 128 diameter one h after the goats consumed the water. The drinking water sample was collected
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7 129 every week and stored in a 250-mL bottle at 5 °C. At the end of each period, the samples
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10 130 were pooled proportionally and then analyzed to determine total dissolved solids (TDS,
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12 131 conductivity method, Orion Star A212, Thermo Scientific), Fe, Mn, Al (spectrometric
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14 132 techniques, inductively coupled plasma atomic emission spectroscopy Varian 715-ES,
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17 133 Agilent), nitrate (NO₃), nitrite (NO₂), ammonia (NH₃), sulfate (SO₄) (spectrometric
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19 134 techniques, Spectrophotometer UV-VIS Lambda 45, Perkin Elmer), organic substances
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22 135 (permanganometric titration method).

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24 136 Individual drinking water intake (DWI) was calculated as the difference between the
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27 137 amount of water offered and refusals. Subsequently, three buckets with water were placed in
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29 138 the barn to estimate daily evaporative water loss, and then the daily DWI was corrected by
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31
32 139 the evaporative loss. The amount of water in the consumed feed (FWI) was calculated by the
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34 140 difference between the amount of water in the feed offered and refusals. Metabolic water was
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36 141 estimated using the factors 0.62, 0.42, and 1.10 for digestible carbohydrates, protein, and fat,
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39 142 respectively (Taylor, 1970). Apparent total water intake (TWI) was determined as the sum of
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41 143 DWI, FWI, and metabolic water, while the fecal water was estimated from the amount of
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44 144 fecal excretion and the content of water. The amount of urinary water was the amount of
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46 145 urine corrected by the DM content of urine. Meanwhile, the water retention was calculated by
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49 146 subtracting the amount of water in fecal and urinary excretion from TWI.

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51 147 After homogenizing and filtering with a surgical gaze, individual urine excretion was
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54 148 recorded. A sample of urine (~100 mL) was taken daily and stored at -20 °C for N analysis.
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56 149 The DM content of urine was determined by drying a 3 mL urine sample at 60 °C for 12 h
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58 150 and the total was determined using the micro Kjeldahl method (AOAC, 1990; Method
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151 988.05). Nitrogen absorption was calculated by subtracting fecal N excretion from the
152 amount of N intake (feed and DWI), while N retention was calculated by subtracting the
153 amount of urinary N loss from the absorbed N.

154 2.5. Statistical analysis

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

$$158 Y_{ijk} = \mu + T_i + P_j + TP_{ij} + a_k + e_{ijk};$$

159 Where Y_{ijk} is observed response at a particular ijk case, μ is overall mean, T_i is the fixed effect
160 of treatment i , P_j is the fixed effect of period j , TP_{ij} is the fixed effect of the interaction
161 between treatment i and period j , a_k is the random effect of animal k , and e_{ijk} is experimental
162 error.

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

167 3. Results

168 The composition of drinking water offered to animals in different treatment groups
169 increases in Fe, Mn, Al, NH_3 , SO_4 , and organic substances with the decrease in pH level. In
170 the 6.9 and 5.2 levels, the contaminant concentrations were not significantly different ($P >$
171 0.05) while the highest concentrations were found in the 3.8 pH level ($P < 0.05$; Table 3).

172 Meanwhile, the values of feed intake, nutrient digestibility, rumen pH, and daily gain
173 of the goats are shown in Table 4. In the group with a 5.2 pH level, total DMI was lower ($P <$
174 0.05) than those subjected to the other treatments that comparable to the lower ($P < 0.05$) DM
175 intake of hay (%BW) in the group. Furthermore, metabolizable energy intake ($MJ/kg BW^{0.75}$)

176 and daily gain were only influenced by trends ($P = 0.06$). As the pH level reduced, the rumen
177 pH was also decreasing ($P < 0.01$), where the pH in the 3.8 group was lower than those in the
178 6.9 and 5.2 groups. Meanwhile, the apparent DM, OM, CP, NDF, and ADF digestibility were
179 not significantly different ($P > 0.05$).

180 Drinking water intake and FWI (%BW) tended to be lowered at the 5.2 group ($P =$
181 0.09) but metabolic water and TWI were not influenced ($P > 0.05$). Fecal water excretion
182 (%BW) was lowered ($P < 0.05$) in the 5.2 pH group, which was not significantly different
183 from those in the 6.9 group ($P > 0.05$), but higher than those in the 3.8 group. Meanwhile,
184 urinary water excretion and apparent water retention were not significantly affected by the
185 pH level ($P > 0.05$) (Table 5).

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

188 During the collection weeks, the daily maximum temperature-humidity index
189 (THI_{max}) correlated positively with DWI of the 6.9 group but not of the 5.2 and 3.8 groups.
190 Furthermore, DMI did not significantly correlate with THI_{max} among all the groups ($P >$
191 0.05), while the ratio DWI/DMI correlated with THI_{max} in the 6.9 group ($P < 0.01$) (Table 7).

192 **4. Discussion**

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

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

204 Several studies have been conducted on the effect of high-contaminant water on DWI
205 and the performance of ruminants. Mdletshe et al. (2017) stated that reductions of DWI,
206 DMI, and daily gain in Nguni goats as the TDS content of water exceeded the permissible
207 limits. Meanwhile, other studies also observed decreased DWI due to the higher levels of
208 TDS in sheep (Assad and El-Sherif, 2002), beef cattle (López et al., 2016), and buffalo
209 (Sharma et al., 2017). The water intake of beef cattle was also reduced when SO₄ was 1900
210 mg/L (Lardner et al., 2013) due to the ability of the animals to protect their metabolism status
211 from salt stress.

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

217 The rumen pH was declined by the acid drinking water in this study, however, it was
218 still within the normal range. Acid drinking water may cause rumen acidosis (Olkowski,
219 2009) when the rumen pH becomes less than 5 (Giger-Reverdin, 2018; Ribeiro et al., 2020).
220 However, the rumen pH values at the pH levels of 5.2 and 3.8 in this study increased to the
221 normal range at one h post-drinking (Table 4). During the experiment, the animals' normal
222 eating and ruminating behavior and the sufficiency of the minerals-salt supplement might
223 indicate a normal secretion of saliva to maintain the range of rumen pH when the animal
224 continuously consumed the acid drinking water. As a result, the nutrients' digestibility was

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

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

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

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

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

1 250 higher water consumption than goats in the thermal neutral environment. During the
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3 251 experimental periods of this study, the means of THI were 79 to 80 (Table 1) which
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5 252 fluctuated daily from 75 in the dawn to 85 in the afternoon (data not shown). Furthermore,
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7 253 the positive correlation $THI_{max} - DWI$ was in line with the result of a previous study, which
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9 254 indicated that DWI also fluctuated at a higher value in the afternoon when THI was at a
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11 255 maximum level. A higher daily THI fluctuation from 70 to 87 with a shift of feeding and
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13 256 drinking frequency was also reported in the tropical humid region of India (Abhijith et al.,
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15 257 2021). This fluctuation showed the influence of feeding management in minimizing the
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17 258 adverse effect of heat stress on goat performances. Since the drinking water was offered at *ad*
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19 259 *libitum* level in this study, the animals could freely fulfill the additional requirement of water
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21 260 for the thermoregulation processes. The significant correlations in the 6.9 group showed the
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23 261 important aspect of clean and good palatability water for maximum intake when the animals
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25 262 experience heat stress.
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31 263 **5. Conclusions**

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34 264 The effect of lowering pH levels in drinking water depends on the concentration of
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36 265 contaminants in the water. In this study, the lowering of pH level from 6.9 to 3.8 did not lead
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38 266 to adverse effects on the nutrient intake, balance, and growth due to the minimum levels of
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40 267 the contaminants in the water and the animal's ability to maintain the normal range of the
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42 268 ruminal pH. However, the better ability of the animal in the 6.9 group to cope with the heat
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44 269 stress was shown by the positive correlation between DWI and THI_{max} . In addition, a further
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46 270 study with a more extended period of the acid drinking water is recommended to confirm the
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48 271 effects on rumen fermentation characteristics, thermoregulation, and drinking behavior
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50 272 responses.
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277 **Author contribution**

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

283 **Data availability**

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

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379 **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 (T _{min}) (°C)	24.4 ± 0.10	24.8 ± 0.14	24.7 ± 0.17
Average temperature (T _{av}) (°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 = $(1.8 \times T^{\circ}\text{C} + 32) - [(0.55 - 0.0055 \times \text{RH} \%) \times (1.8 \times T^{\circ}\text{C} - 26)]$ (NRC, 1971), where T°C is air temperature and RH is the relative humidity.

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Table 2Chemical composition (mean ± standard error) of Chinese violet (*Asystasia gangetica*) hay and cassava chips offered during the experiment (% dry matter basis)

	Chinese violet 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

^a100-CP (%)-EE (%)-[NDF (%)-NDICP (%)]-Ash (%).

^bNeutral detergent fiber corrected for residual ash and crude protein.

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Table 3

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

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

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 \pm standard error) of Kacang goats offered water having different pH levels

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

1	ADF	23.4 ± 2.55	19.8 ± 3.91	23.6 ± 2.95	0.866
2	Rumen pH	6.98 ± 0.06 ^b	6.94 ± 0.05 ^b	6.58 ± 0.08 ^a	0.002
3					
4	Daily gain (g/d)	73.4 ± 8.74	49.7 ± 8.42	64.2 ± 6.16	0.062
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7 Means with different superscripts are significantly different ($P < 0.05$); BW: body weight

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Table 5

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

1	ml/d	1070 ± 132.1	960 ± 97.9	1087 ± 88.4	0.421
2	%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 \pm standard error) of Kacang goats offered water having different pH levels

Parameter (%BW)	pH level			<i>P</i> -value
	6.9	5.2	3.8	
N intake	0.056 \pm 0.003 ^b	0.048 \pm 0.003 ^a	0.055 \pm 0.004 ^{ab}	0.036
Fecal N	0.024 \pm 0.002	0.020 \pm 0.001	0.024 \pm 0.002	0.062
N absorb	0.032 \pm 0.002	0.028 \pm 0.002	0.031 \pm 0.002	0.240
Urinary N	0.018 \pm 0.003	0.015 \pm 0.002	0.016 \pm 0.003	0.469
N retention	0.015 \pm 0.003	0.013 \pm 0.002	0.015 \pm 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 levels¹ 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

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

3
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11
12
13 **Abstract**

14 Water available ~~to-for~~ livestock in the tropical lowlands region is generally high in acidity.
15 ~~Thiserefore, this~~ study ~~aimeds to-determined~~ the effects of ~~the~~ acid water on nutrient intake,
16 water balance, and the growth of goats in the tropical environment. A total of nine Kacang
17 goats were stratified based on body weight (BW) and assigned to three treatment groups
18 which were offered drinking water at varying pH levels, namely 6.9, 5.2, and 3.8. All goats
19 were offered *ad libitum Asystasia gangetica* hay and dried cassava chips at 1% of BW (dry
20 matter (DM) basis) following a crossover design with three treatments tested in three periods.

21 At ~~the~~ 5.2 pH level, drinking water intake (DWI) tended to be lower ($P = 0.09$) while ~~t~~Total
22 DM intake (%BW) was decreased ($P < 0.05$). Ruminal pH ~~was significantly~~
23 ~~differencedeclined~~ ($P < 0.01$); 6.98, 6.94, and 6.58 at the 6.9, 5.2, and 3.8 pH levels,
24 ~~respectively~~ ($P < 0.01$). Metabolizable energy and daily gain tended to be higher at ~~the~~ 6.9
25 and 3.8 pH levels compared to those at ~~the pH~~-5.2 levels ($P = 0.08$). There were no

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

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

32

33 1. Introduction

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

44 In humid tropical lowlands, most of the water is characterized by high acidity due to
45 the natural oxidation processes of pyrite and ferric ion. The pH of the surface water could
46 ~~drop fall~~ to 3, where most of the contaminants are sulfate (SO₄), iron (Fe), manganese (Mn),
47 and aluminum (Al) (Ali et al., 2021a; Manders et al., 2002). Another ~~water source~~ of water in
48 the lowland region is groundwater, which has less acidity and contaminants (Winkel et al.,
49 2008). Although the ~~recommended~~ minimum recommended pH level for livestock is 5.5
50 (Bagley et al., 1997) or 6.0 (Olkowski, 2009), the effects of the acidic water on ruminants

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

56 **2. Materials and Methods**

57 **2.1. Study site**

58 This study has been approved by the Faculty of Agriculture, Universitas Sriwijaya,
59 Indonesia. The site is situated at an altitude of ± 6 m above sea level and $3^{\circ}11'38.4''S$,
60 $104^{\circ}39'30.5''E$. Meanwhile, the animals were cared for according to the Animal Welfare
61 Guidelines of the Indonesian Institute of Sciences. The environmental variables in the site are
62 shown in Table 1.

63 **2.2. Experimental animal, treatments, and feeding management**

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

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75 This study used a crossover design that consisted of three levels of pH ~~in-over~~ three
76 periods. Meanwhile, each experimental period lasted for three weeks of adaptation and one
77 week of ~~sample-collectionsampling~~, where feed intake, ~~feesfecal~~, and ~~urin~~ary excretion
78 were measured. Each measurement period was followed by one week of recovery, where all
79 animals received only pH 6.9 drinking water.

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

89 2.3. Preparation of different pH levels of water

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

98 **2.4. Sample collection, preparation, and analysis**

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

103
104 Moreover, the samples of the offered feeds were taken and stored in paper bags at
105 room temperature. After weighing, refusals were homogenized and a subsample (~100 g) was
106 taken and stored. Total fecal and urinary excretion was determined by daily collection over 7
107 d. Meanwhile, the total feces excreted by each animal was thoroughly mixed by hand,
108 weighed, and a subsample of approximately 100 g fresh matter was taken and dried at 45°C
109 for three consecutive days. The dried feed and fecal samples were ground to pass through a 1-
110 mm mesh. At the end of each period, the feed and fecal samples were pooled per animal
111 proportionally to the daily amount of each animal during the sampling week. The dried
112 samples were stored in zipper plastic bags before laboratory analyses.

113 The dried feces, feed, and refusals were analyzed as follows: DM, ash (AOAC, 1990;
114 Method 924.05), N (AOAC, 1990; Method 988.05), ether extract (EE; Method 920.39),
115 neutral detergent fiber (NDF, ~~.)~~-with alpha-amylase), and acid detergent fiber (ADF) ~~with~~
116 alpha amylase ~~and~~ including residual ash (Van Soest et al., 1991). Organic matter (OM)
117 concentrations were calculated by subtracting the ash concentration from 100, while the
118 crude protein ~~-(CP)~~ content was calculated as $N \times 6.25$. Neutral detergent-insoluble N (NDIN)
119 and Neutral detergent-insoluble ash (NDIash) were estimated according to Licitra et al.
120 (1996). Furthermore, NDF corrected for ash and crude proteinCP (NDF_{acp}) was calculated by
121 subtracting the NDIN and NDIash. Non fibrous carbohydrates (NFC) were calculated by
122 subtracting the concentration of NDF_{acp}, CP, EE, and ash from 100 (Mertens, 1997).

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

129 Before the measurement of rumen fluid pH, the animals were not given drinking
130 water for two h (9:00 – 11:00). The fluid was collected using a stomach tube of 6 mm
131 diameter one h after the goats consumed the water. The drinking water sample was collected
132 every week and stored in a 250-mL bottle at 5 °C. At the end of each period, the samples
133 were pooled proportionally and then analyzed to determine total dissolved solids (TDS,
134 conductivity method, Orion Star A212, Thermo Scientific), Fe, Mn, Al (spectrometric
135 techniques, inductively coupled plasma atomic emission spectroscopy Varian 715-ES,
136 Agilent), nitrate (NO₃), nitrite (NO₂), ammonia (NH₃), sulfate (SO₄) (spectrometric
137 techniques, Spectrophotometer UV-VIS Lambda 45, Perkin Elmer), organic substances
138 (permanganometric titration method).

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

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

150 After homogenizing and filtering with a surgical gaze, individual urine excretion was
151 recorded. A sample of urine (~100 mL) was taken daily and stored at -20 °C for N analysis.
152 The DM content of urine was determined by drying a 3 mL urine sample at 60 °C for 12 h
153 and the total was determined using the micro Kjeldahl method (AOAC, 1990; Method
154 988.05). Nitrogen absorption was calculated by subtracting fecal N excretion from the
155 amount of N intake (feed and DWI), while N retention was calculated by subtracting the
156 amount of urinary N loss from the absorbed N.

157 2.5. Statistical analysis

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

$$161 Y_{ijk} = \mu + T_i + P_j + TP_{ij} + a_k + e_{ijk};$$

162 Where Y_{ijk} is observed response at a particular ijk case, μ is overall mean, T_i is the fixed effect
163 of treatment i , P_j is the fixed effect of period j , TP_{ij} is the fixed effect of the interaction
164 between treatment i and period j , a_k is the random effect of animal k , and e_{ijk} is experimental
165 error.

166 Differences between means were determined using the Tukey test and the significance
167 level was declared at $P < 0.05$, where p-values of 0.05 to 0.10 were considered as a trend.

168 The relationship between daily maximum temperature-humidity index (THI_{max}), DWI, and
169 DM intake (DMI) during the collection weeks was tested by Pearson correlation analysis.

170 3. Results

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

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

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177 Meanwhile, the values of feed intake, nutrient digestibility, rumen pH, and daily gain
178 of the goats are shown in Table 4. In the group with a 5.2 pH level, total DMI was lower ($P <$
179 0.05) than those subjected to the other treatments that comparable to the lower ($P < 0.05$) DM
180 intake of hay (%BW) in the group. Furthermore, metabolizable energy intake (MJ/kg $\text{BW}^{0.75}$)
181 and daily gain were only influenced by trends ($P = 0.06$). As the pH level reduces, the
182 rumen pH was also decreasing ($P < 0.01$), where the pH in the 3.8 group was lower than
183 those in the 6.9 and 5.2 groups. Meanwhile, the apparent DM, OM, CP, NDF, and ADF
184 digestibility were not significantly different ($P > 0.05$).

185 Drinking water intake and FWI (%BW) tended to be lowered at the 5.2 group ($P =$
186 0.09) but metabolic water and TWI were not influenced ($P > 0.05$). Fecal water excretion
187 (%BW) was lowered ($P < 0.05$) in the 5.2 pH group, which was not significantly different
188 from those in the 6.9 group ($P > 0.05$), but higher than those in the 3.8 group. Meanwhile,
189 urinary water excretion and apparent water retention were not significantly affected by the
190 pH level ($P > 0.05$) (Table 5).

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191 Intake of N (%BW) and fecal N excretion (g/d) were also lowered at 5.2 level (P
192 < 0.05). However, N absorption, urinary N excretion, and N retention did not vary among the
193 different groups ($P > 0.05$) (Table 6).

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194 During the collection weeks, the daily maximum temperature-humidity index
195 (THI_{max}) correlated positively with DWI of the 6.9 group but not of the 5.2 and 3.8 groups.

196 Furthermore, DMI did not significantly correlate with THI_{max} among all the groups ($P >$
197 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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198 **4. Discussion**

199 The varied DM intake was not attributable to the DWI while water contaminant
200 concentrations were varied among the different pH levels of drinking water. The tendency of
201 lower DWI in the 5.2 pH group was also not related to the contaminant concentrations in the
202 water where the higher concentrations were found in the 3.8 pH group. Based on the
203 maximum limits of contaminant concentrations in the drinking water, the concentrations of
204 TDS, Fe, NO₃, NO₂, SO₄ were much lower (Table 3)~~The decreased in DM intake was due to~~
205 ~~the lower DWI at 5.2 pH level, while water contaminant concentrations were varied among~~
206 ~~the different pH levels of drinking water. However, the tendency of lower DWI in the 5.2 pH~~
207 ~~group was not related to the contaminant concentrations in the water where the higher~~
208 ~~concentrations were found in the 3.8 pH group compared to the 5.2 pH group. Based on the~~
209 ~~maximum limits of contaminants concentrations in the drinking water, the concentrations of~~
210 ~~TDS, Fe, NO₃, NO₂, SO₄ were much lower (Table 3). The oxidation process of contaminant~~
211 ~~ions could be relate to the lowered H⁺ concentration of the aerated water in the 6.9 pH group~~
212 (Lytle et al., 1998; Manders et al., 2002). Aeration followed by filtration treatment to remove
213 contaminants from water has been widely used (Lytle et al., 1998; Marsidi et al., 2018). The
214 non-significant differences of the contaminants concentrations in the 6.9 and 5.2 groups due
215 to the absence of the filtration process to remove the precipitates.

216 Several studies have been conducted on the effect of high-contaminants water on
217 DWI and the performance of ruminants. Mdletshe et al. (2017) stated that reductions of DWI,
218 DMI, and daily gain in Nguni goats as the TDS content of water exceeded the permissible
219 limits. Meanwhile, other studies also observed decreased DWI due to the higher levels of
220 TDS in sheep (Assad and El-Sherif, 2002), beef cattle (López et al., 2016), and buffalo
221 (Sharma et al., 2017). The water intake of beef cattle was also reduced when SO₄ was 1900

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

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

229 The rumen pH was declined by the acid drinking water in this study, however, it was
230 still within the normal range. Acid drinking water may cause rumen acidosis (Olkowski,
231 2009) when the rumen pH becomes less than 5 (Giger-Reverdin, 2018; Ribeiro et al., 2020).
232 However, the rumen pH values at the pH levels of 5.2 and 3.8 in this study increased to the
233 normal range at ~~1~~one h post-drinking (Table 4). During the experiment, the animals' normal
234 eating and ruminating behavior and the sufficiency of the minerals-salt supplement might
235 indicate a normal secretion of saliva to maintain the range of rumen pH when the animal
236 continuously consumed the acid drinking water. As a result, the nutrients' digestibility was
237 not affected. A similar OM and NDF digestibility was also reported when the ruminal pH was
238 decreased from 7.0 to 6.2 (Shriver et al., 1986).

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

245 The ~~re was a trend for an effect on~~ daily gain was only affected by a trend ($P = 0.06$),
246 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 °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

272 requirement of water for the thermoregulation processes. The significant correlations in the
273 6.9 group showed the important aspect of clean and good palatability water for maximum
274 intake when the animals experience heat stress.

275 **5. Conclusions**

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

285

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289 **Author contribution**

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

295 **Data availability**

296 The datasets analyzed during this study are available from the corresponding author
297 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 (T _{min}) (°C)	24.4 ± 0.10	24.8 ± 0.14	24.7 ± 0.17
Average temperature (T _{av}) (°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 = $(1.8 \times T^{\circ}\text{C} + 32) - [(0.55 - 0.0055 \times \text{RH} \%) \times (1.8 \times T^{\circ}\text{C} - 26)]$ (NRC, 1971), where T°C is air temperature and RH is the relative humidity.

392

Table 2Chemical composition (mean ± standard error) of Chinese violet (*Asystasia gangetica*) hay and cassava chips offered during the experiment (% dry matter basis)

	Chinese violet 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

^a100-CP (%)-EE (%)-[NDF (%)-NDICP (%)]-Ash (%).

^bNeutral detergent fiber corrected for residual ash and crude protein.

393

Table 3

Concentrations of contaminant substances (mg/L, mean \pm 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 \pm 2.31 ^a	48.3 \pm 2.96 ^a	87.7 \pm 8.67 ^b	0.004	4000 ¹ , 3000 ²
Iron	0.008 \pm 0.002 ^a	0.010 \pm 0.000 ^a	0.223 \pm 0.074 ^b	0.019	2 ¹
Manganese	0.001 \pm 0.001 ^a	0.004 \pm 0.003 ^a	0.027 \pm 0.003 ^b	0.001	0.3 ²
Aluminum	0.014 \pm 0.003 ^a	0.036 \pm 0.001 ^a	2.870 \pm 0.067 ^b	0.000	NA
Nitrate	14.1 \pm 3.52 ^a	12.8 \pm 0.51 ^a	24.8 \pm 1.03 ^b	0.014	100 ¹ , 77 ²
Nitrite	0.01 \pm 0.011	0.02 \pm 0.022	0.02 \pm 0.02	0.897	33 ¹ , 10 ²
Ammonia	0.27 \pm 0.033 ^a	0.30 \pm 0.058 ^{ab}	0.47 \pm 0.033 ^b	0.035	NA
Sulfate	3.3 \pm 1.67 ^a	5.4 \pm 2.11 ^a	25.6 \pm 5.66 ^b	0.009	500 ¹ , 1000 ²
Organic substances	1.9 \pm 0.07	1.7 \pm 0.16	2.6 \pm 0.28	0.053	NA
pH	6.9 \pm 0.03 ^c	5.2 \pm 0.06 ^b	3.8 \pm 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 \pm 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 \pm 36.6	332 \pm 32.5	390 \pm 48.3	0.154
%BW	2.1 \pm 0.15 ^b	1.8 \pm 0.13 ^a	2.1 \pm 0.17 ^b	0.035
Cassava chips				
g DM/d	159 \pm 15.2	166 \pm 15.6	158 \pm 11.3	0.715
%BW	0.9 \pm 0.06	0.9 \pm 0.05	0.9 \pm 0.05	0.683
Total DM intake				
g/d	548 \pm 41.8	498 \pm 39.9	549 \pm 49.6	0.078
%BW	3.0 \pm 0.13 ^b	2.7 \pm 0.11 ^a	2.9 \pm 0.13 ^b	0.026
ME intake				
(MJ/d)	5.8 \pm 0.44	5.3 \pm 0.40	5.8 \pm 0.43	0.137
MJ/kg BW ^{0.75}	0.65 \pm 0.03	0.59 \pm 0.02	0.64 \pm 0.02	0.078
Digestibility (%)				
DM	68.1 \pm 0.94	68.5 \pm 0.99	67.7 \pm 1.21	0.379
OM	67.9 \pm 1.04	68.5 \pm 1.04	67.5 \pm 1.28	0.339
<u>CP</u>	<u>57.7 \pm 0.95</u>	<u>57.3 \pm 1.29</u>	<u>56.9 \pm 0.62</u>	<u>0.722</u>
NDF	41.6 \pm 1.61	41.9 \pm 2.06	40.3 \pm 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

398

399

Table 5

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

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

<u>Parameter</u> <u>(%BW)</u>	<u>pH level</u>			<u>P-value</u>
	<u>6.9</u>	<u>5.2</u>	<u>3.8</u>	
<u>N intake</u>	<u>0.056 \pm 0.003^b</u>	<u>0.048 \pm 0.003^a</u>	<u>0.055 \pm 0.004^{ab}</u>	<u>0.036</u>
<u>Fecal N</u>	<u>0.024 \pm 0.002</u>	<u>0.020 \pm 0.001</u>	<u>0.024 \pm 0.002</u>	<u>0.062</u>
<u>N absorb</u>	<u>0.032 \pm 0.002</u>	<u>0.028 \pm 0.002</u>	<u>0.031 \pm 0.002</u>	<u>0.240</u>
<u>Urinary N</u>	<u>0.018 \pm 0.003</u>	<u>0.015 \pm 0.002</u>	<u>0.016 \pm 0.003</u>	<u>0.469</u>
<u>N retention</u>	<u>0.015 \pm 0.003</u>	<u>0.013 \pm 0.002</u>	<u>0.015 \pm 0.002</u>	<u>0.728</u>

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

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Table 7

Pearson correlation coefficients and significance levels¹ 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

Conflict of Interest Statement

The authors declare that they have no competing interests.



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