Original Article

The effects of alkaline water and rainwater on the production and health performance of commercial broilers under tropical conditions

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Abstract

Water consumption of a broiler is affected by temperature and the absence or presence of some minerals which could affect its production. Therefore, this study aimed to determine the impact of giving alkaline water and rainwater on the growth performance, carcass characteristics, meat quality and white blood cell changes of broilers under tropical condition. A total of 108 broiler birds (Cobb 500) were randomly divided into three treatment groups with six replicates consisting of six birds per replicate. Treatment 1 (control), 2 and 3 were provided with available tap water (pH 7.17), sodium bicarbonate alkaline water (pH 8.05) and chlorinated rainwater (pH 5.90), respectively. Throughout the 42-day study period, weekly body weight, feed intake and feed conversion ratio were calculated to determine their growth performance. A total of six broilers were randomly selected on day 21 and 42 from each treatment group for blood samples collection via the brachial vein into anticoagulant (EDTA) blood tubes for white blood cell analysis. On day 42, the same broilers were slaughtered for carcass characteristics and meat quality assessment after blood sampling. Based on the results obtained, there were significant differences ($p<0.05$) on the growth performance, carcass characteristics, meat quality and white blood cell responses among treatment groups. T2 broilers demonstrated a higher body weight gain, feed intake and feed efficiency. As a result, the carcass traits and meat quality were also improved due to better growth performance. The immune response of T2 broilers was also enhanced via the drinking of sodium bicarbonate alkaline water. Contrariwise, T3 broilers exhibited the worst growth performance, carcass characteristics, meat quality and white blood cell values compared to T2 and the control group. In conclusion, sodium bicarbonate alkaline water could be recommended as the main water source as it improves the overall production and health of the broilers.

Keywords: Carcass characteristics, drinking water, growth performance, meat quality, white blood cell response

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Introduction

In tropical regions, high environmental temperature cause heat stress in poultry that leads to detrimental effects on the growth rate, feed consumption, feed efficiency and mortality, subsequently resulting in a large economical loss in commercial poultry farms (Ramiah *et al.*, 2019). Heat stress is one of the most challenging environmental stressors in the livestock industries (Chung *et al.*, 2019a). Commercial broilers are more predisposed to heat stress due to their greater metabolic activities (Wen at al., 2020). Altering the nutrients requirement according to the environmental stressors is the current trend of mitigating the effects of heat stress on poultry which could help minimise feed cost and environmental impact (Zaman *et al.*, 2008). Water is one of the essential nutrients required by broiler chickens for optimum growth and production but often neglected during farm management. The general rule of thumb indicated that broilers consume 1.5 to 2 times as much water as it does for feed (Amaral, 2004). Thus, changes in the water content have a greater effects on the broiler performance, as compared to the feed contents (Manning *et al.*, 2007). A reduction in the performance of broiler has been observed when the birds are consuming low quality or contaminated water supply (Maharjan *et al.*, 2016). In addition, contaminated water also contributes to viral diseases outbreak like infectious bursal disease, avian encephalomyelitis as well as protozoal diseases like histomoniasis and coccidiosis. Hence, the usage of different water sources like alkaline water and rainwater could be an alternative drinking water source that could be beneficial for the production and health of broilers.

Alkaline water from water magnetisation has recently been utilised in different fields such as medical, engineering and agriculture particularly in plants, animals as well as in livestock production (Maheshwari and Grewal, 2009). It is believed that the properties of alkaline water which are more energized, active, soft and high pH toward slight alkaline and germs free could contribute to better livestock production performance (Alhassani, 2012). Feeding alkaline water could improve the production performance of farm animals such as chickens, turkeys, pigs, cows, calves and sheep. Agreeing to this, Jassim and Aqeel (2017), reported that the use of alkaline water was found to be beneficial to poultry health. Conversely, Al-Mufarrej *et al.* (2005), claimed that alkaline water did not significantly influence the body weight gain, feed intake, feed conversion ratio and carcass composition of broiler chickens. The differences in these studies warrant further investigation on the usage of alkaline water in the broiler industry and different method to produce alkaline water since the water magnetisation method is expensive and time consuming.

Rainwater, on the other hand, is often considered not suitable for drinking without treatment as it may be contaminated (Tobin, 2014). This is proven by the presence of *Escherichia coli* and other coliforms that are detected in rainwater but can be treated with ultraviolet (UV) light sterilisation or chlorination (Lee *et al.*, 2017). Mohajan (2018), also reported that rainwater is slightly acidic and very low in dissolved minerals. Rainwater mixed with carbon dioxide in the air to form the weak carbonic acid with a pH of 5.6 which is not harmful to the environment and plants (Mohajan, 2018). In most cases, rainwater has chemical concentrations that are within acceptable limits; however, elevated levels of zinc and lead have sometimes been reported that could be from leaching of metallic roofs, storage tanks or atmospheric pollution. Rainwater lacks minerals, but some minerals at appropriate concentrations are essential for health, such as calcium, magnesium, iron and fluoride (Lee *et al.*, 2017). In this circumstance, the implications of using rainwater as the primary source of drinking water can be considered during water shortages. At the same time, rainwater harvesting helps poultry farmers to reduce water dependency.

Generally, it is clear that the characteristics and taste of water can influence both feed and water consumption of a broiler bird which are affected by the absence and presence of some minerals. In theory, chickens prefer water that is cold and slightly acidic in taste rather than sweet. For that reason, the objective of this study was to elucidate the effects of alkaline water and rainwater on the growth performance, carcass characteristics, meat quality and white blood cell changes of commercial broilers.

Materials and Methods

Birds, husbandry and diets: All experimental procedures were conducted in accordance with the Institutional Animal Care and Use Committee of Research Policy at Universiti Putra Malaysia (UPM). A total of 108 day-old male broiler chicks (Cobb 500) obtained from a local hatchery were weighed and randomly allocated into 3 treatment groups placed on wired flooring battery cages in an open-sided house with the mean temperature of 29ºC and humidity of 79% during the time of the study. Each treatment consisted of 6 replications, with 6 broilers per replication. Newcastle disease (ND) and infectious bronchitis (IB) vaccines were used to vaccinate those broilers intraocularly on day 7 followed by infectious bursal disease (IBD) vaccine on day 14. Feed and water were provided ad libitum to the broilers. The broilers were fed with commercial starter and finisher diets from 0 to 21 and 22 to 42 days respectively but were provided with three types of drinking water via plastic water troughs. The nutritive value of the starter and finisher diets are presented in Table 1.

Drinking water preparation: In the current work, the pH of different types of drinking water was prepared and measured daily using a portable pH meter (Mettler Toledo, AG 8603, Switzerland) before provided to the broilers. The tap water source was collected from a tap available at the farm. The pH of the tap water was measured at a mean value of 7.17 which is widely used as the main drinking water source for broilers in Malaysia. On the contrary, the alkaline water source was obtained through the mixing of 200 mg/L feed grade sodium bicarbonate into available tap water to achieve the pH mean value of 8.05. On the other hand, rainwater was harvested in an open space into disinfected polyethylene water tanks to reduce zinc and lead contamination. The rainwater was treated with 0.5 mg/L of chlorine to prevent bacteriological contamination (Lee *et al.*, 2017). The chlorinated rainwater pH was measured at a mean value of 5.90 and was supplied to the broilers after a contact time of 30 minutes. After preparation, all water sources were supplied to the broilers at the same time with the mean temperature of 20ºC.

Table 1 Nutrient content of starter and finisher diets.

	Treatments					
	Starter (0-21 Days)			**Finisher (22-42 days)**		
Nutrient content (%)	**T1**	**T2**	**T3**	**T1**	**T2**	**T3**
Crude protein	22.00	22.00	22.00	20.00	20.00	20.00
Crude fibre	5.00	5.00	5.00	8.00	8.00	8.00
Crude fat	4.50	4.50	4.50	4.00	4.00	4.00
Moisture	13.00	13.00	13.00	13.00	13.00	13.00
Ash	8.00	8.00	8.00	8.00	8.00	8.00
Calcium	0.90	0.90	0.90	0.85	0.85	0.85
Phosphorus	0.45	0.45	0.45	0.42	0.42	0.42

Note: T1 (Tap water, control), T2 (Alkaline water) and T3 (Rainwater).

Experimental design: In this completely randomised design, the treatments consisted of Treatment 1 (T1) commercial broilers fed with tap water (control); Treatment 2 (T2) commercial broilers fed with alkaline water and Treatment 3 (T3) commercial broilers fed with rainwater. Throughout the 42 days study period, weekly body weight and feed intake were recorded per replicate for the calculation of body weight gain and feed conversion ratio (FCR) to determine the growth performance. A total of six broilers were randomly selected on day 21 and 42 from each replicate of each treatment group for blood samples collection via the brachial vein into anticoagulant (EDTA) blood tubes for white blood cell analysis (Chung *et al.*, 2020). On day 42, the same broilers were slaughtered for carcass characteristics and meat quality assessment after blood sampling. The birds were slaughtered at the Department of Animal Science abattoir, Faculty of Agriculture, UPM according to the Halal slaughter procedure as outlined in MS 1500: 2009 (Department of Standards Malaysia, 2009).

Carcass characteristics: The carcasses were dissected manually and the following parameters were recorded: final live weight, kill-out weight, de-feathered weight, dressing percentage, breast muscle weight, drumsticks weight, wings weight, neck weight, head weight, shank weight, full gizzard weight, empty gizzard weight, gastrointestinal tract weight, heart weight, and liver weight (Chung *et al.*, 2019b).

Meat quality analysis: The determination of pH, colour, drip loss, cooking loss, texture and tenderness were also evaluated according to the procedures described by Chung *et al.* (2019b):

Determination of pH: The right pectoralis major (breast muscle) was collected, snap-frozen in -195°C liquid nitrogen and stored at −80°C to preserve the pH properties of the meat. After 24 hours, the samples of each treatment group were crushed and homogenised for the measurement of pH using a portable pH meter (Mettler Toledo, AG 8603, Switzerland).

Determination of colour: A total of 30g of breast meat samples were bloomed at room temperature (27°C) 30 minutes prior to colour analysis. The meat samples of each treatment group were analysed using a colour flex spectrophotometer (Hunter Lab Reston, VA, USA). The colour flex spectrophotometer was standardised and properly set up as required. Once the meat samples were analysed, the colour flex spectrophotometer produced the lightness (L*), redness (a*) and yellowness (b*) results according to the gross appearance of the meat samples' colour.

Drip loss measurement: A total of 40g of breast meat from the samples of each treatment group were weighed and recorded as initial weight (W1). The meat samples were kept in vacuum-packed plastic bags and kept in the freezer at 4°C. After 24 hours, the samples were removed from the bags, gently blotted with a tissue to dry and final weight was taken (W2). The percentage of drip loss was calculated as (W1-W2)/ W1 x 100 (%).

Cooking loss measurement: A total of 30g of breast meat from the samples of each treatment group were weighed and recorded as initial weight (W1). The muscle samples were placed in vacuum-packed plastic bags and fully immersed in the water bath at 80°C for 20 minutes. After cooking, the samples were removed from the water bath and the bags were allowed to cool down to room temperature for 15 minutes. The cooked samples were blotted with tissue paper and weighed, the weight was recorded as final weight (W2). The cooking loss percentage was calculated using the formula (W1-W2)/ W1 x 100 (%).

Texture and tenderness: The same breast meat samples that have been cooked will be used to determine the texture and tenderness of each treatment group. The cooked breast meat samples were cut into a rectangular size chunk of 10 mm x 10 mm x 20 mm. The samples were cut perpendicularly to the fiber direction and placed on a texture analyzer an HDP/WBV Warner Bratzler blade set with V slot table (TA/XT Analyzer

Plus of Stable MicroSystems, Vienna Court, UK) to obtain the shear force value (kgF).

Analysis of white blood cell: Periphery blood smears stained with Wright Stain were performed to manually determine the estimated total white blood cell (TWBC) and differential white blood cell (WBC) counts. The counting process for TWBC was carried out by counting the WBC in 10 different fields of the slide at 40x objective and then the average of the number of the WBC was calculated. The average value obtained was then multiplied by 2000 to obtain an estimated TWBC count per microliter. The calculations were done by applying the formula: Estimated TWBC/µl = (Mean of 10 views) x 2000 (Carisch *et al.*, 2019). Additionally, the differential WBC counts were done by manually counting and identifying 200 WBC at 100x magnification with the aid of emulsion oil for a clearer focus on the white blood cells. The values obtained were then converted into percentages (Lilliehook *et al.*, 2004).

Statistical Analysis: JMP® Version 11. NC: SAS Institute Inc. software was used to analyse all the data collected. One-way analysis of variance (ANOVA) and Dunnett's multiple comparison post-hoc tests were used to compare means between treatment groups. The data were considered significant at $p<0.05$.

Results

Growth performance: The effects of alkaline water and rainwater on the growth performance of broilers are presented in Table 2. There were no significant differences ($p>0.05$) in the growth performance parameters during the starter phase among treatment groups. The differences in the growth performance of the birds were only observed during the finisher phase. There were significant differences ($p<0.05$) in the body weight, body weight gain and cumulative FCR among broilers fed with different water sources. T2 broilers supplied with alkaline water were found to have higher body weight and body weight gain as well as a lower cumulative FCR compared to T1 and T3 broilers consuming tap water and rainwater respectively indicating better growth performance.

Table 2 Effects of alkaline water and rainwater on the growth performance of broilers throughout the 42 days experiment period.

Parameters	Treatments			P value
	T1	T2	T3	
1-21 days (Starter phase)				
Initial body weight (kg)	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.18
Body weight (kg)	1.03 ± 0.02	1.03 ± 0.02	1.02 ± 0.02	0.09
Body weight gain (kg)	0.88 ± 0.02	0.88 ± 0.01	0.87 ± 0.02	0.36
Feed intake (kg)	1.28 ± 0.02	1.23 ± 0.02	1.28 ± 0.02	0.12
Cumulative FCR	1.45 ± 0.04	1.39 ± 0.01	1.47 ± 0.04	0.25
22-42 days (Finisher phase)				
Body weight (kg)	$2.54 \pm 0.04^{a,b}$	2.64 ± 0.09^{a}	2.39 ± 0.02^{b}	0.04
Body weight gain (kg)	$2.49 \pm 0.04^{a,b}$	2.59 ± 0.09^{a}	2.34 ± 0.02^{b}	0.04
Feed intake (kg)	4.91 ± 0.08	4.82 ± 0.02	4.65 ± 0.07	0.58
Cumulative FCR	1.97 ± 0.04^{a}	1.86 ± 0.05^{b}	1.98 ± 0.10^{a}	0.04

Note: All values were expressed as mean ± SE; $^{a, b, c}$ values with superscript within column are significantly different at $p< 0.05$.
T1 (Tap water, control), T2 (Alkaline water) and T3 (Rainwater). FCR: Feed conversion ratio.

Carcass characteristics: The carcass characteristics of broilers drinking tap water, alkaline water and rainwater are presented in Table 3. Among the treatment groups, there were significant changes ($p<0.05$) in the final live weight, kill-out weight, de-feathered weight, dressing weight, dressing percentage, heart, liver, full gizzard, empty gizzard, viscera fat, head, shank, drumsticks, wings, breast muscles and neck weight. In contrast, only the gastrointestinal tract weight between treatment groups showed no significant change. T2 broilers showed an overall better carcass characteristics, as compared to their counterparts.

Meat quality: The impact of alkaline water and rainwater on the meat quality of broilers are presented in Table 4. Cooking loss, drip loss including texture and tenderness showed significant differences ($p<0.05$) among treatments. Nonetheless, no significant differences ($p>0.05$) were observed for the muscle pH, colour L* (lightness), colour a* (redness) and colour b* (yellowness) among treatment groups. Similarly, feeding alkaline water was found to improve the meat quality of T2 broilers due to the lower cooking loss, drip loss as well as texture and tenderness values. T3 broilers have the lowest quality of meat attributable to the highest meat quality values compared to the control and T2 broilers.

White blood cell analysis: The WBC changes of broilers drinking tap water, alkaline water and rainwater are presented in Table 5. At the starter phase, the total WBC count, heterophils, lymphocytes and heterophils to lymphocytes (H/L) ratio were exhibiting significant differences ($p<0.05$) between treatment groups. At the finisher phase, there were significant differences ($p<0.05$) in the total WBC count, lymphocytes and H/L ratio. No significant differences ($p>0.05$) were shown for the monocytes, eosinophil and basophil percentages between treated groups for both starter and finisher phases. T2 broilers exhibited the highest total WBC count and the lowest H/L ratio throughout the 42 days study period demonstrating a better health performance followed by T1 and T3 broilers.

Table 3 Effects of alkaline water and rainwater on the carcass characteristics of broilers throughout the 42 days experiment period.

Parameters	Treatments T1	T2	T3	P value
Final Live Weight (g)	2319.00±52.90^{b}	2610.17±63.42^{a}	2223.17±63.79^{b}	0.01
Kill-out Weight(g)	2211.83±46.39^{b}	2594.33±88.31^{a}	2087.17±60.87^{b}	0.01
De-feathered Weight (g)	2097.67±49.81^{b}	2349.17±81.99^{a}	2030.50±57.36^{b}	0.01
Dressing Weight (g)	1598.67±52.04^{b}	1868.00±73.60^{a}	1620.83±55.53^{b}	0.01
Dressing Percentage (%)	68.88±1.01^{b}	71.78±0.77^{a}	72.85±0.71^{a}	0.01
Heart (g)	11.00±0.26^{b}	13.33±0.21^{a}	12.67±0.42^{a}	0.01
Liver (g)	48.33±4.10^{ab}	65.67±10.87^{a}	42.67±2.64^{b}	0.04
GIT (g)	150.67±8.60	154.00±3.81	142.00±18.99	0.77
Full Gizzard (g)	42.00±2.56^{ab}	48.67±2.95^{a}	34.67±4.90^{b}	0.04
Empty Gizzard (g)	29.33±1.82^{ab}	35.00±2.99^{a}	26.67±1.48^{b}	0.01
Viscera Fat (g)	45.00±4.07^{c}	91.33±3.06^{a}	56.33±3.06^{b}	0.01
Head (g)	59.67±1.38^{c}	72.00±0.97^{a}	66.67±0.56^{b}	0.01
Shank (g)	93.00±5.11^{b}	110.67±2.59^{a}	102.33±1.12^{ab}	0.01
Drumstick (g)	239.00±4.66^{b}	271.00±2.92^{a}	228.67±6.22^{b}	0.01
Wings (g)	214.33±5.80^{ab}	220.00±3.81^{a}	204.67±3.55^{b}	0.04
Breast Muscles (g)	551.33±12.44^{a}	585.67±32.52^{a}	465.33±11.69^{b}	0.01
Neck (g)	32.00±1.59^{b}	38.00±2.28^{a}	32.00±0.63^{b}	0.03

Note: All values were expressed as mean ± SE; $^{a, b, c}$ values with superscript within column are significantly different at $p< 0.05$. T1 (Tap water, control), T2 (Alkaline water) and T3 (Rainwater).

Table 4 Effects of alkaline water and rainwater on the meat quality of broilers throughout the 42 days experiment period.

Parameters	Treatments T1	T2	T3	P value
Cooking Loss (%)	25.87 ± 1.08^{ab}	20.53 ±1.48^{b}	28.36 ± 2.13^{a}	0.01
Muscle pH	5.89 ± 0.04	5.92 ± 0.04	5.80 ± 0.04	0.62
Colour L* (lightness)	58.73±0.73	58.45±0.81	58.27±2.18	0.97
Colour a* (redness)	3.15±0.08	3.22±0.67	3.12±0.28	0.85
Colour b* (yellowness)	15.04±1.52	14.60±1.11	15.82±0.79	0.15
Drip Loss (%)	4.11±0.68^{a}	2.53±1.03^{b}	4.34±0.67^{a}	0.02
Texture & Tenderness (kgF)	1477.60±37.58^{b}	833.30±12.02^{a}	1502.20±12.79^{c}	0.01

Note: All values were expressed as mean ± SE; $^{a, b, c}$ values with superscript within column are significantly different at $p< 0.05$. T1 (Tap water, control), T2 (Alkaline water) and T3 (Rainwater).

Table 5 Effects of alkaline water and rainwater on the white blood cell parameters of broilers throughout the 42 days experiment period.

Parameters	Treatments T1	T2	T3	P value
<u>1-21 days (Starter phase)</u>				
Total WBC count (Cell/ Cu mm)	22666 ± 240^{a}	23200 ± 503^{a}	19333 ± 546^{b}	0.02
Heterophils (%)	34.67 ± 1.45^{a,b}	30.00 ± 0.58^{b}	37.67 ± 0.56^{a}	0.02
Lymphocytes (%)	63.67 ± 1.85^{a,b}	68.00 ± 0.58^{a}	60.00 ± 0.56^{b}	0.04
H/L ratio	0.54 ± 0.03^{a,b}	0.44 ± 0.01^{b}	0.63 ± 0.01^{a}	0.02
Monocytes (%)	0.33 ± 0.33	0.33 ± 0.33	0.67 ± 0.21	0.73
Eosinophil (%)	0.67 ± 0.33	0.67± 0.33	1.00 ± 0.37	0.88
Basophil (%)	0.67 ± 0.33	0.67 ± 0.33	0.65 ± 0.21	0.92
<u>22-42 days (Finisher phase)</u>				
Total WBC count (Cell/ Cu mm)	21333 ± 381^{a}	23933 ± 578^{b}	18300 ± 395^{c}	0.01
Heterophils (%)	37.33 ± 2.65	35.33 ± 2.17	36.50 ± 3.21	0.85
Lymphocytes (%)	61.17 ± 2.50^{a}	64.00 ± 2.22^{b}	59.33 ± 3.72^{a}	0.04
H/L ratio	0.61 ± 0.06^{a}	0.55 ± 0.05^{b}	0.62 ± 0.06^{a}	0.04
Monocytes (%)	0.50 ± 0.22	0.50 ± 0.22	0.50 ± 0.22	0.85
Eosinophil (%)	1.17 ± 0.60	1.17 ± 0.60	1.38 ± 0.60	0.65
Basophil (%)	0.83 ± 0.40	0.97 ± 0.43	0.90 ± 0.21	0.58

Note: All values were expressed as mean ± SE; $^{a, b, c}$ values with superscript within column are significantly different at $p< 0.05$. T1 (Tap water, control), T2 (Alkaline water) and T3 (Rainwater). WBC: white blood cell; H/L ratio: heterophils to lymphocytes ratio.

Discussion

The production performance and health status of a broiler bird are affected by various factors ranging from genetic, environment, management, health and nutrition. In tropical regions, high environmental temperature and humidity cause heat stress in poultry that leads to detrimental effects on the growth rate, feed consumption, feed efficiency and mortality, subsequently resulting in a large economical loss in commercial poultry farms (Ramiah *et al.*, 2019). Therefore, there is a severe need to investigate different mitigation strategies to overcome the future challenges to the poultry industry. This study aimed to identify the effect of different water sources on the production and health performance of commercial broilers under tropical conditions. As a result, the husbandry and herd health management of each treatment group in this study was standardised to prevent the infliction of unnecessary stressors towards broiler birds during the rearing period. Water is an essential component of the bird's daily nutrient requirement but least appreciated by broiler producers (Amaral, 2004). As the bird grows, the efficiency of nutrient absorption along the gastrointestinal tract is challenged by the dynamic changes in the gut microbial populations, including the potentially pathogenic species. (Fortun-Lamothe and Boullier, 2007). Agreeing to Jin (2007), total digestive enzyme activity tends to increase in parallel to the rapid increase in the weight of the pancreas and intestines leading to better digestion plus absorption with water as the medium. As a result, the introduction of a novel supplement to the gastrointestinal tract could be either beneficial or detrimental to the growth performance and immune response of those broilers.

In the present study, T2 broiler fed with sodium bicarbonate alkaline water showed a significantly improved growth performance with higher body weight gain and improved FCR during the finisher phase. This could be attributable to the presence of sodium bicarbonate of the alkaline water. Sodium bicarbonate can help maintain the acid-base and electrolyte balance, and alleviate respiratory alkalosis following the exposure to high ambient temperatures (Mujahid, 2011). Furthermore, sodium bicarbonate can also neutralise the strong acidity of vitamin C without destroying its electron donor and antioxidant activities (Padayatty and Levine, 2016). Consequently, sodium bicarbonate supplementation in hens was used to improve shell quality and in broilers to enhance body weight which was consistent with the current work (Ahmad *et al.*, 2009). On the other hand, T3 broilers which consumed rainwater with a pH of 5.90, showed the lowest body weight gain, feed intake and the worst FCR. These observations indicate a lower absorption rate of nutrients in the intestine. Even though poultry can tolerate a wide range of pH (pH 4 to 8), broiler birds have a higher preference of water with pH ranging from 6.0 to 6.8. Drinking water with a pH less than 6, has been shown to negatively affect the chicken performance (Amaral, 2004). This acidic environment (pH 5) in the small intestine will decrease the digestibility and absorption of nutrients such as protein, leading to poor growth performance which was observed in the current study. Moreover, acidic water could affect the integrity of the intestinal wall and interrupt the absorption process. According to Huff *et al.* (1994) and Parker *et al.* (2019), continuous high acidic environment could lead to proventriculitis, inflammation of the intestine and consequent fragile intestinal wall. Hence, acidic drinking water like rainwater will negatively affect the growth performance of broilers and at the same time damages the gastrointestinal tract of the birds.

Broilers are expected to voluntarily reduce their feed intake when the quantity and quality of water are unsatisfactory, which will eventually affect the carcass characteristics. In a study conducted by Abdullah and Matarneh (2010), a strong relationship between body weight and carcass weight as well as between carcass weight and dressing percentage was described. Similar results were obtained in this study where T2 broilers drinking sodium bicarbonate alkaline water have the highest carcass characteristic values, attributable to a better growth performance, as compared to T1 and T3 broilers. These findings were in accord with Alhassani and Amin (2012), that feeding alkaline drinking water for chickens resulted in shortening of fattening period of broiler chickens, an increase in growth rate by 5-7%, improving meat quality flavour and tenderness, a decrease of mortality and diseases, as well as a decrease in feed consumption and an improvement in FCR. The higher breast meat and drumstick yielded from T2 broilers might be attributed to the increased water accumulation in muscle tissues as higher water intake was observed in high sodium diets which in this case from the sodium bicarbonate alkaline water (Rondon *et al.*, 2001). Water is thought to retain in the muscle tissues, which was also reflected from higher dressing weight observed in the previous study by Borgatti *et al.*, (2004). Besides, the increased weight of the gizzard and gastrointestinal tract reflects the increasing digestive or metabolic capacity of T2 broilers which resulted in the highest carcass characteristics values. In contrast, the feeding of rainwater will affect the growth performance and causing poor carcass characteristics value as presented in the current work. Prasad and Sen (1992) stated that too much lowering of the pH will not provide the benefit from acidification of drinking water, which explained the lower carcass characteristics observed in T3 broilers.

Meat quality is strongly influenced by the stress during the pre-slaughter and slaughter processes that lead to an accumulation of lactic acid in the muscle, resulting in a lower meat pH. Mir *et al.*, (2017), reported that broiler breast meat with a higher pH has a higher water binding capacity than meat with a lower pH. It is possible that the pre-slaughter stress may lower the muscle pH. Alkaline water has been shown to alleviate the stress by lowering the lactic acid production during slaughter. The lower level of lactic acid in muscle tissue will lead to a higher meat pH, which is associated with minimal protein denaturation and higher water-binding capacity of the myofibrillar proteins (Lonergan and Lonergan, 2005). The positive outcome from this could be related to the alkalinisation effect that moved the muscle pH away from the isoelectric point of myofibrillar proteins and increased the net negative charge. This will lead to muscle fiber expansion or swelling caused by electrostatic repulsion that allows more water to be immobilized in the myofibrillar lattice. Moreover, a recent study also found that bicarbonate was able to reduce shear force and improve the yield of enhanced pork and poultry meat by reducing the stress and lactic acid accumulation (Sheard and Tali, 2005). These studies support the findings in the present study, where T2 breast muscles demonstrated a higher pH parameter leading to better water holding capacity and tenderness which could be a value-added product. On the contrary, muscles at a lower pH undergo greater protein denaturation (Mir *et al.*, 2017). As a result of greater protein denaturation, there will be more damage in the muscle fibers, negatively affecting their functional properties to retain water and maintain texture. Based on the current results, T3 meat quality showed the lowest water holding capacity plus the highest texture and tenderness value attributable to the low pH value. According to Amaral (2004), chickens do not prefer water with a pH of less than 6 which will eventually affect the feed intake. The stress incurs to those chickens will then lead to higher lactic acid accumulation at slaughter causing lower meat quality as described earlier. Overall, the comparatively lower pH values in T3 broilers meat as compared to the other treatments was possibly caused by enhanced metabolic stress from the feeding of rainwater throughout the production period.

The health performance of broilers can be determined through blood sampling by measuring the H:L ratio in addition to other components of the blood (Chung *et al.*, 2020). Alkaline water has been shown to exert a suppressive effect on free radical levels in living organisms, thereby resulting in disease prevention. Various biological effects, such as antidiabetic and antioxidant actions, DNA protecting effects and growth-stimulation activities are some of the extra properties of alkaline water (Magro *et al.*, 2016). The gastrointestinal tract and the immune organs of broilers are developing throughout the six weeks of life which require optimum nutrients for growth. From this study, T2 broilers fed with sodium bicarbonate alkaline water exhibited the highest TWBC count and the lowest H/L ratio throughout the starter and finisher phases indicating an enhanced health performance (Zakaria *et al.*, 2009). This could be attributable to the characteristics of sodium bicarbonate alkaline water which causes elevation of metabolic activity and the immune system of those broilers (Magro *et al.*, 2016). It has been demonstrated that the addition of sodium bicarbonate in water gives positive effects to broilers raised above thermoneutral conditions. Sodium is the major cation in the extracellular fluid and is closely associated with bicarbonate in the management of acid-base balance for the synthesis of tissue proteins, enzymatic reactions and osmotic pressure that influence the host immunity status. If it is not properly treated, drinking contaminated rainwater plays an important role in the transmission of some bacterial, viral and protozoan diseases that are among the most common poultry diseases. The TWBC was significantly lower in T3 birds demonstrating a lower immune status. This is further confirmed with a higher H/L ratio which is a more reliable parameter for the measurement of immune function in broilers (Chung *et al.*, 2020). Davis (2008), described that corticosterone is positively correlated to the H/L ratio and in some cases provide an indication of circulating corticosterone over time. As stated previously, rainwater could contain a tremendous amount of heavy metals and other minerals that could affect the production and health of broilers. Acidic toxicants water which affects the immune system may contribute to an increased incidence of autoimmune diseases, infectious diseases and cancer (Misyara, 2009). This proved that acidic water may decrease the immunity of living organisms and may cause disease. Poor growth rate and disease outbreak will incur a significant cost to broiler production.

Generally, sodium bicarbonate alkaline water was found to be beneficial towards the production and health performance of broilers reared under tropical conditions. Nevertheless, the application of sodium bicarbonate should be used cautiously as the higher concentration will lead to an increase in pH causing chickens to reduce their water consumption due to the bitterness. Dey *et al.*, (2018), reported that younger chicks are more sensitive to bitterness as compared to older birds due to the higher presence of specific bitter taste receptors. Additionally, alkaline water was found to reduce the efficiency of antibiotic and vaccine, decrease the effectiveness of chlorine treatment as well as favours the growth of detrimental Gram-negative bacteria such as *Salmonella* and *Escherichia coli* (Amaral, 2004). Naturally, water sources with a high pH are often characterised by high levels of calcium and magnesium, which can build up over time and clog the water system leading to bacterial growth. Excessive consumption may also disturb the pH of the gastrointestinal tract which will ultimately affect the gut microflora, digestion process and other health problems (Abbas *et al.*, 2008). This is because the excessive calcium present in the water will be indigestible and it will change the intestinal pH to be more acidic. This acidic environment in the small intestine will then decrease the digestibility and absorption of nutrients (Mutucumarana *et al.*, 2014; Chung *et al.*, 2019b). Hence, home based and alkaline water sources should be prepared and used cautiously to prevent unnecessary production losses.

In summary, different water sources and pH will have varying effects on broiler production and health performance. Based on the results obtained, the addition of 200 mg/L feed grade sodium bicarbonate into available tap water to achieve the pH mean value of 8.05 was found to improve the growth performance, carcass characteristics, meat quality and immune status of broilers. Nevertheless, further research needs to be conducted to determine the suitability and availability of sodium bicarbonate alkaline water to substitute tap water as the main water source for broiler management without incurring extra cost to the farmer.

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