Physiological and haematological responses of broiler chickens offered cold water and vitamin C during hot-dry season

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Abstract

The effects of cold water and vitamin C on broilers' rectal temperature (RT), respiratory rate (RR) and haematological parameters were evaluated during dry season. Two hundred and sixteen 4-week-old Anak 2000 broilers fed ad libitum were offered either water at ambient temperature (29.5°C; ORD) or cold water (8.0°C; COLD) to which either 0 (-C) or 500 mg vitamin C per litre water (+C) was added to give four treatment groups (ORD-C, ORD+C, COLD-C and COLD+C) from week 5 to 8 of age. There were 3 replicates with 18 birds per replicate. RT and RR were taken for three days weekly at 16.00 h. Blood was sampled weekly for haematological, plasma biochemical and tri-iodothyronine analyses. Data on RT, RR, packed cell volume (PCV), red blood cell (RBC), haemoglobin concentration (Hb), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), white blood cell (WBC), heterophil (HET), lymphocyte (LYM), eosinophil (EOS), monocyte (MON), heterophil:lymphocyte ratio (H:L), plasma K^+, Na^+, glucose (GLU), protein (PRO) and tri-iodothyronine (T_3) were subjected to ANOVA using least squares method. Water temperature had significant ($P<0.01$) effect on RT and RR. Cold water lowered RT and RR of the birds compared with water at ambient temperature. Similarly, vitamin C in drinking water caused decrease in RT ($P<0.001$) and RR ($P<0.01$) compared to ordinary water. Water temperature had no significant ($P>0.05$) effect on MCHC, MCH, MCV, LMY, EOS, plasma K^+, Na^+, GLU, PRO and T_3. But cold water significantly lowered MON ($P<0.05$) and increased PCV ($P<0.001$), RBC ($P<0.001$), Hb ($P<0.001$), WBC ($P<0.001$), HET ($P<0.01$) and H: L ($P<0.05$) when compared with water at ambient temperature. Addition of vitamin C significantly increased LYM ($P<0.05$) and H:L ($P<0.05$) but decreased HET ($P<0.05$) when compared with birds that received no vitamin C. Vitamin C had no significant ($P>0.05$) effect on PCV, RBC, Hb, WBC, MCHC, MCH, MCV, plasma K^+, Na^+, GLU, PRO and T_3. There were significant ($P<0.01$) interactions between water temperature and vitamin C on HET, LYM and H: L. Broiler chickens offered ORD+C had significantly lower HET and H: L than those offered ORD-C, COLD-C and COLD+C. However, the LYM was highest in ORD+C birds. High HET and H:L, and low LYM are indicators of heat stress in poultry. In conclusion, cold water and vitamin C were effective in reducing broilers' RT and RR in the afternoon during hot-dry season. Either of the two may improve the well-being of broilers during dry season.

Keywords: cold water; vitamin C; rectal temperature; respiratory rate; broilers

Introduction

Heat stress stands out as a major problem facing broiler production in the humid tropics (Sunil Kumar *et al.*, 2011). Broiler chickens are highly susceptible to heat stress. This can be attributed to the inferior development of their cardiovascular and respiratory systems compared to their growth rate (Yahav, 2000). Their high growth rate also depends on high feed intake which results in high metabolic heat production (Teeter, 1994), which is not easily dissipated especially under hot and humid environmental condition found in the humid tropics. This is commonly encountered in broiler farms in south-western Nigeria during hot-dry season, when the environmental temperature is reported to be greater than 30°C (Abioja, 2005; Abioja *et al.*, 2010). Optimum temperature for broiler chickens is 18-22°C (Charles, 2002). Heat stress remains one of the major challenges facing poultry production in the tropics and has adverse effects on productivity of broiler chickens (Howlider and Rose, 1987). In order to maintain body temperature, birds exhibit panting behaviour. Increase in body temperature and rate of respiration are indicators of heat stress in broiler chickens (Altan *et al.*, 2000), which may lead to a cascade of irreversible thermoregulatory events that may be lethal for the birds if the stressor is not removed (Yahav, 2000).

Optimal productivity in hot season requires an adequate and appropriate management system that can reduce the effects of heat stress to the minimum. Wilson and Edward (1952) proposed that offering of cooled drinking water to birds could reduce the effects in chickens. This was later confirmed in the report of van Kampen (1988). Many poultry managers in Nigeria have been supplying their birds with water cooled with ice cubes during dry season. However, this has not been experimentally investigated in the country. Recent works by Abioja *et al.* (2011) revealed that chilled water increased weight gain in broilers compared with water at ambient temperature. Puma *et al.* (2001) reported that birds prefer cold to warm water during both winter and summer.

Dietary supplementation with vitamin C has proved to be effective in combating detrimental effects of heat stress in broilers (Sayed and Shoieb, 1996). However, the use in water is not common. Deyhim and Teeter (1991) stated that any substance proposed for reduction of heat stress may be use in water as well. Therefore, this study is aimed at evaluating the combining effects of cold water and vitamin C in drinking water on rectal temperature, respiratory rate, haematology, plasma biochemistry and tri-iodothyronine of finishing broiler chickens during hot-dry season in south-western Nigeria.

Materials and methods

Experimental location

The research was carried out at the Poultry Unit of the University Teaching and Research Farm, Federal University of Agriculture, Alabata Road, Abeokuta (latitude 7° 13' 49.46" N; longitude 3° 26' 11.98" E (Google Earth, 2006) and altitude 76 m above sea level).

Meteorological observation

Daily minimum, maximum and mean ambient temperatures, relative humidity and wet- and dry-bulb temperatures at the level of the birds in the pen at 08.00 h and 16.00 h were monitored throughout the experimental period.

Experimental birds and their management

Day-old unsexed broiler chicks of *Anak*

2000 strain were purchased from a local hatchery for this study. The brooder house and equipment were thoroughly disinfected before the arrival of the chicks. The birds were floor-brooded for three weeks on wood-shaving and then transferred into the experimental pens where they were allowed a week for adjustment. During these four weeks, they were fed *ad libitum* with standard starter diet (contains: maize 46%, soybean meal 18.5%, groundnut cake 15%, fish meal 2%, wheat offal 12.45%, bone meal 2%, oyster shell 3%, salt 0.25%, methionine 0.3%, lysine 0.25% and premix 0.25%) and thereafter with finisher diet (contains: maize 50%, soybean meal 12%, groundnut cake 11%, fish meal 2%, wheat offal 19.05%, bone meal 2%, oyster shell 3%, salt 0.25%, methionine 0.25%, lysine 0.2% and premix 0.25%). The diets were fed in mash forms Water at ambient temperature was supplied *ad libitum* throughout the brooding and adjustment periods in plastic drinkers. Experimental protocols:

The study was carried out during the hot-dry season (February and March). Two hundred and sixteen (216) four weeks old broiler birds were randomly allotted to four treatment groups. Each group consists of 3 replicates and 18 birds per replicate. The treatments were:

I. Supply of water at ambient temperature (25-30°C) with no vitamin C supplement (ORD-C)

II. Water at ambient temperature (25-30°C) supplemented with 500 mg vitamin C per litre of water (ORD+C)

III. Cold water of 5-10°C, with no vitamin C supplement (COLD-C)

IV. Cold water 5-10°C, supplemented with 500 mg vitamin C per litre of water (COLD+C).

Water for the experiment was solely sourced from the well on the farm. The water was supplied in nipple drinker lines linked either to water reservoir (to supply water at 25-30°C) or a fabricated refrigerating unit (to supply water at 5-10°C). The birds were kept on deep litter floor in an open-sided poultry house. The stocking density was 18 kg per $2m^2$. The temperature of water was recorded with aid of suitable thermometers. Water in the lines supplying cold water was returned into the refrigerating unit to maintain water temperature with the aid of pumps.

Data collection

Physiological responses: The rectal temperature (RT) of three birds randomly selected out of each replicate was measured with a digital thermometer (0.1°C accuracy) inserted into the rectum (colon) of the birds for 1 minute as previously described by Yahav and McMurtry (2001). Respiratory rate (RR) of birds was taken as the number of breaths per minute. Data on RT and RR were collected on the last three consecutive days of each week by 16.00 h throughout the experimental period.

Haematological parameters: Blood samples were collected from three randomly picked birds from each replicate weekly throughout experimental period via brachial vein. Samples were analyzed for packed cell volume (PCV), red blood cell count (RBC), haemoglobin concentration (Hb), white blood cell count (WBC), differential counts, blood glucose, total protein and plasma K and Na concentrations (Lamb, 1991; Yalcin *et al.*, 2005). The mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) were calculated.

T_3 Assay: Tri-iodothyronine (T_3) concentrations in plasma samples were determined using a commercially available double antibody RIA kit as described by Tona *et al.* (2003). All samples were run together in the assay in order to avoid inter-assay variability.

Statistical design and analyses:

Data collected were analyzed by method of least squares of SYSTAT (1992) using the model:

$Y_{ijk}=\mu+T_i+C_j+TC_{ij}+\Sigma_{ijk}$

where Y_{ijk}=yield;

μ=population mean;

T_i=i^{th} effect due to water temperature,

i=1,2; C_j=j^{th} effect due to vitamin C,

j=1,2; TC_{ij}=ij^{th} effect due to interaction between water temperature and vitamin C;

Σ_{ijk}=residual error.

Results

The summary of the climatic conditions of the experimental location during the experiment is presented in Table 1. The daily mean temperature, relative humidity and temperature-humidity index during the experimental period were 27.6°C, 72.6% and 165.7 respectively. The dry-bulb temperature in the morning (08.00 h, 27.9°C) was lower than corresponding value for afternoon (16.00 h, 34.7°C). Reverse was the case for relative humidity (85.2 and 60.1% for 08.00 and 16.00 h respectively). The average temperature of the water used in the experiment is summarised in Table 2. Cold water (COLD) averaged 8.0°C while ordinary water supplied at ambient temperature (ORD) averaged 29.5°C.

The effects of water temperature and vitamin C on RT and RR of broiler chickens are shown in Tables 3-4. Effect of water temperature was significant (P<0.001) on RT (Table 3). The average RT of birds offered ORD water was higher than that of COLD water. Addition of vitamin C to broilers' drinking water lowered significantly (P<0.001) the average RT (Table 3). In Table 4, overall RR was significantly affected by water temperature (P<0.01) and vitamin C (P<0.01). COLD water and addition of vitamin C lowered RR in broilers.

The results of effects of water temperature and vitamin C on some haematological parameters and metabolites in broiler chickens are presented in Table 5. Water temperature significantly influenced PCV (P<0.001), RBC (P<0.001), Hb concentration (P<0.001), WBC (P<0.001), HET (P<0.01), MON (P<0.05) and H:L ratio (P<0.05) but had no effect on MCHC, MCH, MCV, LYM, EOS, plasma K^+, Na^+, GLU, PRO and T_3 (P>0.05). Birds offered COLD water had higher PCV, RBC, Hb concentration, WBC, HET and H:L ratio than birds on ORD water treatment. However, birds on COLD water had lower MON count than those offered ORD water.

HET, LYM and H:L ratio were significantly (P<0.05) affected by vitamin C. Vitamin C in drinking water lowered HET and H:L ratio but increased LYM compared with birds offered water without vitamin C. There were significant interactions between water temperature and vitamin C on HET, LYM and H:L ratio (P<0.01). Birds on ORD+C treatment had a significant lower HET compared to the other 3 treatments. HET was similar for birds on ORD-C, COLD-C and COLD+C treatments. Addition of vitamin C to ORD water decreased the H:L from 2.09 to 1.80. However, no difference was observed when vitamin C was added to COLD water. Vitamin C in ORD water increased LYM significantly (P<0.01). Water temperature and vitamin C did not affect T_3 (P>0.05) and there was no interaction between water temperature and vitamin C (P>0.05).

Discussion

The average temperature of ordinary water

Table 1: Average daily values for meteorological parameters observed during the experimental period

Week	Min. temp. (°C)	Max. temp. (°C)	Mean temp. (°C)	Relative humidity (%)	Temp.-humidity index
			Average		
5	20.1	36.6	28.4	68.4	163.7
6	19.1	36.6	27.9	70.1	165.3
7	18.7	34.9	26.8	73.2	167.1
8	19.7	35.4	27.6	78.9	166.6
Average	19.4	35.8	27.6	72.6	165.7
			08.00 h		
Week	Wet-bulb temp. (°C)	Dry-bulb temp. (°C)	Relative humidity (%)	Temp.-humidity index	
5	25.9	27.9	83.0	185.0	
6	25.9	27.7	84.1	185.7	
7	26.4	27.8	88.0	182.1	
8	26.6	28.3	85.5	180.2	
Average	26.2	27.9	85.2	183.2	
			16.00 h		
Week	Wet-bulb temp. (°C)	Dry-bulb temp. (°C)	Relative humidity (%)	Temp.-humidity index	
5	28.0	35.6	53.7	142.4	
6	28.2	35.3	56.0	145.0	
7	27.8	33.9	58.4	152.2	
8	30.3	34.1	72.3	153.0	
Average	28.6	34.7	60.1	148.1	

Table 2: Average temperature (°C) of water supplied to the birds during the experimental period

	*ORD				**COLD			
Week	08.00 h	12.00 h	16.00 h	Average	08.00 h	12.00 h	16.00 h	Average
5	27.1	30.3	31.7	29.7	8.1	8.3	7.7	8.0
6	26.9	30.4	31.6	29.6	7.4	7.3	6.3	7.0
7	27.2	29.8	31.4	29.5	8.4	6.1	5.6	6.7
8	27.4	29.7	31.2	29.4	11.3	10.0	8.9	10.4
Average	27.2	30.1	31.5	*29.5*	8.8	7.9	7.1	*8.0*

*ORD = ordinary water **COLD= cold water in this study during hot-dry season was 29.5°C. It is higher than the average water temperature 16°C reported by Glatz (2001) in Australia. This is expected since the ambient temperature in Australia is lower than the temperature in the tropics. Cold water lowered RT when the temperature was high. This is in agreement with the reports of van Kampen (1988) but contrary to the reports of Damron (1991) and Degen *et al.* (1992) for laying hens and broiler breeder hens respectively, it is expected that the birds may respond differently compared to growing broilers. Moreover, response depends on degree of coldness of water, and degree and length of heat exposure of the birds. Drinking water temperature determines the water's ability to act as a heat sink (Glatz, 2001). Efficacy of cold water seems to be ambient temperature dependent. Beker and Teeter (1994) found out that drenching broiler chickens with water of 12.8°C under thermal comfort had no effect on the body temperature 15 minutes post-drenching but the effect was felt when the birds were placed under hot condition. The mean ambient temperature during data collection at 16.00h was 34.7°C (Table 1), which was higher than the thermoneutral zone for broilers.

Lowering of broilers' RT by vitamin C addition agrees with the reports of Kutlu and Forbes (1993) and Sayed and Shoeib (1996) that ascorbic acid reduces skin and rectal temperature of broiler chickens, but is contrary to the report of Miraei-Ashtiani (2004). The latter reported that vitamin C inclusion in broiler feed did not result in any difference in cloacal temperature when compared with chickens receiving diet without vitamin C supplementation. The use of vitamin C is associated with the suppressed stress responses indicated by reduction in plasma corticosterone level (Mahmoud *et al.*, 2004), which is a good indicator of stress in birds. Vitamin C acts at the level of adrenal cortex where it reduces the synthesis and release of corticosterone. The reduced amount of corticosterone in circulation helps to maintain the body temperature.

It was observed that cold water was more effective during the 5th and 6th weeks of age while the effect of vitamin C was pronounced during 7th and 8th weeks. This calls for attention of managers of broiler farms. It means that water of lower temperature can be used in the early part of growing phase in broilers and vitamin C during the later phase.

Cold water reduced the RR in broiler chickens. van Kampen (1988) gave a similar report that respiratory rate was reduced in chickens maintained at high air temperatures and offered cooled drinking water. Chaiyabutr (2004) reported that one of the physiologic responses to heat in birds is increase in respiratory frequency and that panting occurs when the deep body temperature of poultry reaches 41-43°C. birds depend mainly on panting as they have no sweat glands like ruminants(Grieve, 2003) . . The high heat capacity property of water is an advantage in lowering the core body and blood temperature. The temperature of the blood reaching the brain is one of the stimuli that gear up the response to heat. With water of low temperature absorbed from the gastro-intestinal tract into the vascular system, this may lower blood temperature, thereby limiting the response to high ambient temperature.

With the use of vitamin C, a reduction in panting rate occurs with supplemental adrenal ascorbic acid from water ingested. Kutlu and Forbes (1993) had earlier reported that RR was reduced in heat-stressed birds given vitamin C.

Broiler chickens given COLD water had

Table 3: Effects of water temperature and vitamin C supplementation on rectal temperature (°C) of broiler chickens reared during hot-dry season

	Water temperature			Vitamin C		
Week	ORD	COLD	±SEM	-C	+C	±SEM
5	42.13[a]	41.72[b]	0.064	41.98	41.86	0.064
6	42.37[a]	42.12[b]	0.068	42.33	42.16	0.068
7	42.17	42.08	0.062	42.24[a]	42.00[b]	0.062
8	42.22	42.08	0.053	42.26[a]	42.04[b]	0.053
Average	42.22[a]	42.00[b]	0.032	42.20[a]	42.02[b]	0.032

*Water temperature x Vitamin C

Week	ORD-C	ORD+C	COLD-C	COLD+C	±SEM
5	42.21	42.04	41.75	41.69	0.090
6	42.38	42.35	42.27	41.98	0.097
7	42.25	42.09	42.23	41.92	0.087
8	42.30	42.14	42.22	41.93	0.075
Average	42.29	42.15	42.12	41.88	0.045

[a,b] Means in the same row with different superscripts differ significantly ($P<0.05$)
* ORD-C = ordinary water (25-30°C) without vitamin C supplementation
ORD+C = ordinary water (25-30°C) with vitamin C supplementation (0.5g/litre water)
COLD-C = coldwater (5-10°C) without vitamin C supplementation
COLD+C = cold water (5-10°C) with vitamin C supplementation (0.5g/litre water)

Table 4: Effects of water temperature and vitamin C supplementation on respiratory rate (breaths/minute) of broiler chickens reared during hot-dry season

	Water temperature			Vitamin C		
Week	ORD	COLD	±SEM	-C	+C	±SEM
5	59.59[a]	49.59[b]	2.670	56.56	52.63	2.670
6	60.30	56.30	1.946	60.15	56.44	1.946
7	63.52	64.59	1.926	65.37	62.74	1.926
8	61.04	57.56	1.366	62.04[a]	56.56[b]	1.366
Average	61.11[a]	57.01[b]	1.040	61.03[a]	57.09[b]	1.040

*Water temperature x Vitamin C

Week	ORD-C	ORD+C	COLD-C	COLD+C	±SEM
5	62.00	57.19	51.11	48.07	3.776
6	60.52	60.07	59.78	52.82	2.753
7	64.30	62.74	66.44	62.74	2.724
8	65.26	56.82	58.82	56.30	1.931
Average	63.02	59.20	59.04	54.98	1.436

[a,b] Means in the same row with different superscripts differ significantly ($P<0.05$)
* ORD-C = ordinary water (25-300C) without vitamin C supplementation
ORD+C = ordinary water (25-300C) with vitamin C supplementation (0.5g/litre water)
COLD-C = coldwater (5-100C) without vitamin C supplementation
COLD+C = cold water (5-100C) with vitamin C supplementation (0.5g/litre water)

higher packed cell volume, red blood cell count, haemoglobin concentration and white blood cell count (Table 5). There is no clear indication in literatures of the effects of water temperature on most of the haematological parameters in broiler chickens. However, Altan *et al.* (2000) reported that broilers subjected to heat stress had reduced packed cell volume. The PCV value obtained in birds that received COLD water (30.4%) is within the normal range reported for chickens (24.9 - 45.2%) by Mitruka *et al.* (1977), but values obtained for ORD water (23.8%) was below the range. It can then be explained that the effect of high ambient temperature in broilers was a reduction in PCV, which may be corrected by offering of cold water. Mitruka *et al.* (1977) stated that the number of erythrocytes in chicken is influenced by physiological conditions of the animal. Cell morphology is affected by heat stress, red blood cells in broilers become longer and thinner compared to unstressed ones. These changes could be adduced to dehydration of the cells (Maxwell *et al.,* 1992). Cold water improved the number of RBC in broiler chickens during hot-dry season in this study. Haemoglobin concentration was higher in broilers that received COLD water than ORD water. This may be linked to the fact that haemoglobin is carried on red blood cells. Thus the more the RBC, the higher haemoglobin content would be. Various forms of stressors, including heat cause changes in the numbers of leucocytes (Rosales, 1994; Yalcin *et al.*, 2004). Heat stress reduced total white blood cell count in laying hens (Mashaly *et al.*, 2004). Cold water reduced the effect of heat stress on WBC count in this study. The heterophil:lymphocyte ratio has been a reliable index for determining stress status in poultry (Satterlee *et al.*, 1989). Higher H:L ratio is an indication of stress in chickens (El-Lethey *et al.*, 2003). Heat stress has been found to decrease lymphocyte numbers in cockerels. In the present study, COLD water was found to increase heterophil count and H:L ratio over that of ORD birds. Lymphocyte count was similar for the COLD and ORD water treatment. This is contrary to expectation. The reason for this is not however clear.

The reduced heterophil count and increased lymphocyte count obtained in broilers given water with vitamin C indicates its efficacy in reducing the detrimental effects of heat stress (El-Lethey *et al.*, 2003). This was further established by the reduced H:L ratio. Under stress-conditions, there is an increased consumption of ascorbic acid especially in the leukocytes and the liver which is usually lower than the amount biosynthesized in the kidney. Endogenous administration of ascorbic acid increases the concentration in the plasma and the uptake into the adrenal cortex to bridge the gap between synthesis and utilization. This becomes necessary because as long as ascorbic acid is not exhausted in the adrenal cortex, the synthesis and release of corticosterone is regulated and stressed cells remain alive.

Minka and Ayo (2003) gave a similar report of the efficacy of vitamin C in *Shika Brown* pullets transported by road for 6h during the hot-dry season. The workers observed decreases in packed cell volume and haemoglobin values and an increase in the values of heterophil/lymphocyte ratio post-transportation in birds not given vitamin C. However, post-transportation values were not different from those obtained pre-transportation in birds offered vitamin C. Ascorbic acid in ORD was more effective in lowering effects of heat as it reduced heterophil count and H:L ratio and increased lymphocyte count.

In homeothermic animals, thyroid

Table 5: Effects of water temperature and vitamin C supplementation on haematological parameters and hormonal responses of broiler chickens reared during hot-dry season

	Water temperature			Vitamin C		
Parameter	**ORD**	**COLD**	**±SEM**	**-C**	**+C**	**±SEM**
PCV (%)	23.8[b]	30.4[a]	1.07	27.4	26.7	1.07
RBC($x10^{12}$/L)	1.29[b]	1.66[a]	0.059	1.49	1.47	0.059
Hb concentration (g/dL)	7.9[b]	10.2[a]	0.36	9.1	8.9	0.36
MCHC (%)	33.1	33.4	0.13	33.1	33.4	0.13
MCH (μμg)	60.7	61.2	0.60	60.9	61.0	0.60
MCV (μ^3)	183.6	183.4	1.86	184.2	182.8	1.86
WBC ($x10^9$/L)	11.8[b]	18.6[a]	1.24	16.1	14.3	1.24
Heterophil (%)	63.9[b]	65.8[a]	0.48	65.6[a]	64.2[b]	0.48
Lymphocyte (%)	33.1	31.9	0.46	31.8[b]	33.2[a]	0.46
Eosinophil (%)	0.88	0.67	0.125	0.83	0.72	0.125
Monocyte (%)	2.12[a]	1.56[b]	0.149	1.73	1.94	0.149
H:L ratio	1.95[b]	2.08[a]	0.042	2.07[a]	1.95[b]	0.042
Plasma K^+ (mmol/L)	3.27	3.36	0.062	3.28	3.35	0.062
Plasma Na^{++} (mmol/L)	133.9	133.6	1.44	133.9	133.5	1.44
Plasma glucose (mg/dL)	164.1	175.7	5.45	176.3	163.4	5.45
Plasma protein (g/dL)	38.8	39.2	0.49	38.7	39.3	0.49
T_3 (ng/mL)	1.79	1.95	0.062	1.91	1.83	0.062

***Water temperature x Vitamin C**

Parameter	**ORD-C**	**ORD+C**	**COLD-C**	**COLD+C**	**±SEM**
PCV (%)	23.9	23.7	31.0	29.8	1.52
RBC($x10^{12}$/L)	1.30	1.29	1.68	1.64	0.083
Hb concentration (g/dL)	7.9	7.9	10.3	10.0	0.51
MCHC (%)	32.9	33.3	33.3	33.5	0.18
MCH (μμg)	60.2	61.2	61.6	60.9	0.85
MCV (μ^3)	183.3	183.9	185.1	181.7	2.64
WBC ($x10^9$/L)	12.7	10.9	19.6	17.6	1.75
Heterophil (%)	65.7[a]	62.2[b]	65.6[a]	66.1[a]	0.68
Lymphocyte (%)	31.4[b]	34.7[a]	32.2[ab]	31.7[ab]	0.65
Eosinophil (%)	0.88	0.89	0.78	0.56	0.177
Monocyte (%)	2.01	2.22	1.44	1.67	0.210
H:L ratio	2.09[a]	1.80[b]	2.05[ab]	2.10[a]	0.060
Plasma K^+ (mmol/L)	3.19	3.34	3.37	3.36	0.088
Plasma Na^{++} (mmol/L)	136.6	131.2	131.3	135.8	2.04
Plasma glucose (mg/dL)	168.4	159.7	184.2	167.1	7.70
Plasma protein (g/dL)	38.8	38.7	38.5	39.8	0.69
T_3 (ng/mL)	1.74	1.84	2.08	1.82	0.088

[a,b]Means in the same row with different superscripts differ significantly ($P<0.05$)
* ORD-C = ordinary water (25-30°C) without vitamin C supplementation
ORD+C = ordinary water (25-30°C) with vitamin C supplementation (0.5g/litre water)
COLD-C = coldwater (5-10°C) without vitamin C supplementation
COLD+C = cold water (5-10°C) without vitamin C supplementation (0.5g/litre water)

hormones regulate basal metabolic rate and are essential for the maintenance of high and constant body temperature (Darras *et al.*, 2000). The major hormone of thyroid gland, thyroxine (T_4), is considered to be a prohormone of the more biologically active 3,5,3'-triiodothyronine (T_3). The T_3 is converted from 5'-monodeiodination (the outer ring) of T_4 by type I iodothyronine deiodinase in the liver and kidney, or by type II deiodinase in the brain, pituitary gland and brown adipose tissues (He *et al.*, 2000). The T_3 and T_4 play important roles in regulating metabolism and thermogenesis of chickens. Previous reports have shown heat-induced decreases in thyroid hormone of poultry (Williamson *et al.*, 1985; Geraert *et al.*, 1996). However, the plasma T_3 in broiler chicken in this study was no affected by both water temperature and vitamin C supplementation. Williamson *et al.* (1985) had stated that reduction in T_3 values obtained in heat-stressed chickens or feed-restricted birds is a consequence of reduced feed intake. This was corroborated by Darras *et al.* (2000). Abioja *et al.* (2011) has earlier reported no difference in feed intake of broilers offered cold water compared with those offered ordinary water. No difference in feed intake may explains the reason for no effect of treatment on T_3.

Conclusion

Cold water lowered broilers' RT, RR and monocyte count but increased PCV, RBC, Hb concentration, WBC, heterophil count and H:L ratio compared with ordinary water at ambient temperature during hot-dry season. Lowering drinking water temperature during hot-dry season may be beneficial in helping broilers in coping with heat stress. In the same vein, addition of vitamin C to drinking water helped in lowering body temperature, respiratory rate and improving the broilers' well-being during hot season.

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