ORGANS PARAMETERS AND SERUM BIOCHEMISTRY OF BROILERS GIVEN DRINKING WATER FROM DIFFERENT SOURCES.

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ABSTRACT
A study was carried out to determine the effect of drinking water quality on organs and blood chemistry of broilers using two hundred and forty broilers. Starter mash (22.64% CP, 2963Kcal/Kg energy) and finisher mash (20.35% CP, 3002Kcal/Kg energy) were fed to broilers raised on deep litter management system for eight weeks. Drinking water obtained from river (RV), rain (RN), borehole (BH) as well as pipe-borne (PB) were given to groups of 60 birds subdivided to 3 replicates in a completely randomized design. Drinking water had pH range between 4.4 and 6.7, total hardness ranged between 2.0 and 18.80Mg/L, nitrate ranged between 0.8Mg/L and 10.30Mg/L, while chloride ranged between 2.40Mg/L and 10.30Mg/L. Coliform bacteria was detected only in river water. Organ characteristics assessed after eight weeks of production showed that weight of some vital organs including gizzard, heart, liver, kidney, pancreas and bursa did not differ significantly ($p>0.05$) as a percentage of body weight of broilers; but there were significant differences ($p<0.05$) in weight of lungs being 0.53 (RV), 0.41 (RN), 0.44 (BH) and 0.54 (PB) respectively. Moreover, analysis of blood samples from the broilers revealed that aspartate aminotransferase (AST), did not differ significantly ($p>0.05$) being 157.60 (BH), 161.80 (RV), 176.00 (RN) and 208.40 (PB). There were no significant differences ($p>0.05$) also between treatment means of broilers drinking water from different sources with respect to alanine aminotransferase (ALT) in this trial which ranged between 4.10 and 6.40. Results of creatinine, glucose, triglyceride, urea and cholesterol (Mg/dl) did not differ significantly ($p>0.05$) due to different water sources used in this work; neither did the blood proteins (albumin and globulin). Water from these four different sources did not significantly ($p>0.05$) affect blood chemistry of broilers raised to eight weeks in a deep litter system; but had significant effect ($p<0.05$) on weight of lungs and spleen.

KEY WORDS: Drinking water, broilers, blood chemistry, vital organs

INTRODUCTION

Water is essential to all living organisms and is available in great quantity naturally in springs, streams, rivers, oceans, and as precipitation (rain, fog, mist, frost and dew). Large portion of water is also found in form of snow or ice especially in temperate and arctic regions; while another large portion is located underground which can be obtained through well and boreholes. Scott *et al*. (1982) identified water as necessary medium for maintenance of homeostasis in animals, where it participates in physiological reactions controlling pH, osmotic pressure and electrolytes concentrations; while Li (2009) attributed efficient animal production as well as enhanced fertility to good quality water provision in animal farm. Water is important in washing of utensils/ equipment in animal farm and is a medium through which most medications are offered to animals. A survey of different sources and quality of water used in poultry and pig farms in South Eastern Nigeria (Etuk *et al*., 2011) revealed the major sources of drinking water including borehole, rain, pipe-borne and streams or rivers. Further studies on the quality of these sources (Etuk *et al*., 2014) showed that there is variation in quality of water tested at different periods as values for various parameters varied from report of Etuk *et al.* (2011). The fact that water quality impacts on physiology of chickens as well as performance indices were highlighted earlier (Balnave, 1993; Carter & Sneed, 1996; Kozisek, 2004; and Reutor, 2010). Also, Mohammed (2011) reported effect of water quality in Bassra province on physiology of some vital organs of broilers including liver, kidney, heart, pancreas, spleen and gizzard. Here in South Eastern Nigeria, Etuk *et al*. (2015) has documented effects of different quality of drinking water on growth performance and carcass of broilers.

Apart from production parameters, water quality also impacts on health status of poultry birds. For instance, Vodela *et al*. (1997) stated that, at low vitamin levels in diet, increasing levels of arsenic, cadmium, lead, benzene and trichloroethylene resulted in suppression of natural humeral and cell-mediated immune responses. Major vital health organs such as liver, thymus, and spleen were reported to be affected in broilers drinking water with increase in nitrogen content and reduced pH which may affect immune responses too (Grizzle *et al*., 1996). More adverse effects of poor quality drinking water on fertility (Abass *et al*., 2008), health and welfare (Koelkebeck *et al*., 1999 and Manning *et al*., 2007) are reported. Ibitoye *et al*. (2013) observed significant effects of different drinking water sources on white blood cell counts in broilers in North Western Nigeria. In recent studies (Etuk *et al*., 2013 and Etuk *et al*., 2016), packed cell volume, haemoglobin and white blood cells were significantly varied in broilers drinking water from different sources in South Eastern Nigeria. This is suggestive of the fact that there may be health implications of different drinking water quality on health status of these birds. Further researches into the effect of drinking water quality on vital organs as well as blood chemistry of broilers will enhance the knowledge of the impact on health status of broilers in this region using these four most commonly adopted sources of drinking water.

MATERIALS AND METHODS

The farm trial was carried out in the Poultry Unit of the Teaching and Research Farm, Federal University of Technology, Owerri, Imo state, Nigeria. The geography of this study area was reported earlier (Etuk *et al*., 2016) with temperature range of 26.50 – 27.50^0 C, relative humidity of between 70 -80% and average annual rainfall of 2500 mm.

Experimental Materials and Procedures

Two hundred and forty day-old broiler chicks were purchased from a reputable distributor for this experiment. These were randomly distributed into twelve groups of twenty birds each. Four treatments, including rain water (RN), borehole water (BH), pipe-borne water (PB) and river water (RV) were randomly assigned to three groups, giving 3 replicates per treatment and thus, 60 birds per treatment. Each replicate was housed in separate pens where normal brooding and production activities were carried out. Starter broiler and finisher mashes (table 1) were fed appropriately, and proper medication and vaccination routines were adopted.

Table 1: Experimental diets used in the study

Nutrients	Starter mash	Finisher mash
Crude Protein (%)	22.64	20.35
Fat (%)	3.01	3.16
Fibre (%)	3.37	4.92
Phosphorous (%)	0.49	0.48
Calcium (%)	1.10	1.07
Energy (Kcal/Kg)	2963.00	3002.00

Broilers were raised to eight weeks of age on a deep litter management system in a completely randomized design (CRD).

Rain water (RN) was harvested from the roof of farm offices, river water (RV) was obtained from Otamiri river which is within the University, while borehole water (BH) was obtained from one of the boreholes within the Faculty of Agriculture. However, pipe-borne water (PB) was produced on-farm using filtration and chlorination methods as directed by State's Water Board with Isochlor $^{(R)}$ purchased from a credited dealer. Water collection was normally simple as done by farmers. For laboratory analysis, fresh samples from these water sources were obtained in sterile bottles and physico-chemical and biological analysis were carried out within twenty four hours in the Laboratory of Soil Science and Technology Department, Federal University of Technology, Owerri.

Blood Collection and Analysis

After eight weeks, five broilers were selected randomly from each replicate (15 birds per treatment) for blood analysis. Blood samples were obtained from the jugular vein of each bird selected early in the morning before feeding and taken to laboratory for analysis within two hours of collection. Samples were analyzed for total bilirubin (TB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose (GL), triglyceride (TG), cholesterol (CHL), total protein (TP), albumin (ALB), globulin (GLB), uric acid (URA) and creatinine (CR). AST was measured by monitoring the concentration of oxaloacetate hydrazones formed with 2,4-dinitrophenyl hydrazine; while ALT was measured by monitoring the concentration of pyruvate hydrazones formed with 2,4- dinitrophenyl hydrazine. Uric acid in serum was hydrolyzed to ammonia then measured photometrically, bilirubin was measured by calorimetric method, while measurement of serum albumin was based on its quantitative binding to indicator.

Analysis of Organs of Broilers

Five broilers were randomly selected from each replicate for this purpose, giving a total of 15 birds per treatment. Birds were kept off-feed overnight, weighed and killed by neck slitting. They were then eviscerated, dressed and dissected. The following organs were carefully removed and weighed with a sensitive digital scale including bile, bursa, gizzard, heart, kidney, liver, lungs, spleen and thymus.

Data Analysis

Weights of organs obtained were expressed as percentages of the body weight of birds used. Means for each treatment were computed, and the values were then subjected to analysis of variance using IBM SPSS Statistics 20 package.

Data of blood chemistry obtained were similarly treated and analyzed with the same statistical model.

RESULTS AND DISCUSSION

Table 2 shows the result of analysis of water samples obtained from the different sources. All samples were tasteless, appeared clear, without objectionable odour and had pH range between 4.4- 6.7. River water tended to be more acidic than rain water, pipe-borne and borehole water; but the range was similar to previous reports (Etuk *et al*., 2014 and Etuk *et al*., 2016). Reports by Good (1983) and Grizzle *et al*. (1996) showed that broilers drinking water with lower pH tended to have lower body weight than those drinking water with higher pH, although Blake and Hess (2010) stated that poultry birds accept water on the acidic side better than alkaline water. All the samples in this study were on the acidic side. Oludare and Sikiru (2012) observed some *boreholes* with pH of 3.89 which should be very acidic in Ijebuland, South western Nigeria. Ibitoye *et al*. (2013) observed pH of 6.1 in boreholes in Sokoto, North Western Nigeria,

Table 2: PHYSICO- CHEMICAL AND BIOLOGICAL PROPERTIES OF WATER FROM FOUR SOURCES

Parameters	Rain	River	Borehole	Pipe-borne
Appearance	Clear	Clear	Clear	Clear
Odour	Unobjectionable	Unobjectionable	Unobjectionable	Unobjectionable
Taste	Tasteless	Tasteless	Tasteless	Tasteless
pH	6.7	4.4	6.2	6.4
Temperature(^oC)	24.7	24.4	25.6	25.9
Conductivity(µs/cm)	60.0	93'0	18.0	11.0
Dissolved oxygen (Mg/L)	7.8	7.6	7.7	7.1
Total dissolved solids(Mg/L)	19.50	31.20	3.25	3.50
Total hardness (Mg/L)	10.00	18.80	6.10	2.00
Calcium (Mg/L)	8.25	17.10	3.50	3.00
Magnesium (Mg/L)	2.60	7.50	1.20	0.65
Iron (Mg/L)	0.08	0.22	0.05	0.12
Nitrate (Mg/L)	10.30	4.60	0.78	0.80
Sulphate (Mg/L)	4.20	5.80	1.05	0.60
Chloride (Mg/L)	3.30	6.80	2.40	10.30
Copper (Mg/L)	0.00	0.04	0.22	0.15
Zinc (Mg/L)	0.09	0.43	0.02	0.06
Phosphate (Mg/L)	1.90	1.70	0.30	1.15
Ammonia (Mg/L)	1.08	0.40	0.09	0.06
Coli form (cfu/ml)	0.00	2	0.00	0.00
E- coli	0	0	0	0

while Okechukwu *et al*. (2012) reported minimum pH of 5.0 in springs around Nsukka, South Eastern Nigeria. Levels of nitrate in the four sources of drinking water used here were between 0.99Mg/L (PB) and 2.8Mg/L (RV). These levels were below detrimental level in drinking water prescribed by World Health Organization (WHO, 1980). Nitrate can be converted to nitrite which is more harmful to performance of broilers (Good, 1985; Barton *et al*., 1986). Nitrate is soluble and move with percolating or run-off water from fertilized or manured fields (Pfost *et al.*, 2001). This may be a reason for higher level of nitrate in river water. Nitrite binds strongly to haemoglobin and thus reduces oxygen carrying capacity of blood; and chronic nitrite toxicity results in anorexia and poor coordination (Blake and Hess, 2001).

High levels of sulphates can interfere with intestinal absorption of other minerals such as copper, iron, or lead to a laxative effect in the presence of magnesium; while high levels of iron may encourage bacterial growth leading to diarrhea (Blake and Hess, 2001). In this study, levels of copper, iron and sulphates are reported in table 2. Copper level was highest in borehole water (0.22Mg/L), but was not detected in rain water; iron and sulphates were more in river water, probably due to nature of soil (river bed) or percolation. Coliform bacteria were also detected only in river water suspected to be contamination by human and animal activities near the river side. However, no E-coli were found in all the samples tested. Bacterial contamination of drinking water sources in poultry farms may be less dangerous than chemical contamination since most bacteria are killed during cooking and may rarely affect human consuming such meat. It may, however, be of economic significance when associated with diseases in chicken.

Table 3 shows the organ characteristics of broilers that were given drinking water from these four sources. Gizzard weight (as a percentage of body weight) was 2.71 (RV), 2.66 (RN), 2.38 (BH) and 2.21 (PB). Liver of broilers in the different treatments on average were 2.90 (RV), 2.33 (RN), 3.36 (BH) and 2.66 (PB) respectively. There was no significant difference (p>0.05) between treatment means for these two organs; so also was for heart, thymus, pancreas and bursa. Weight of bursa, expressed as percentage of body weight, was about 0.28 in most treatments which was higher than 0.252 observed at low nitrate-nitrogen level (1.90Mg/L) in water (Grizzle *et al*., 1996). Heart of broilers weighed between 0.47 (PB) and 0.74 (RN) percent of the live weight; while pancreas ranged between 0.15% (RV) and 0.24% (BH). There was no difference that could be attributed to treatment effect in the study. However, weight of kidney (as percentage of body weight), differs significantly (p<0.05) being 0.17 (RV), 0.21 (RN), 0.18 (BH) and 0.25 (PB). These values were very low compared with a range of 0.693 to 0.781reported by Mohammed (2011) using different quality of water in Bassra Province of Pakistan.

Results of serum biochemistry of broilers given drinking water from four different sources are presented in table 4. Alanine aminotransferase (ALT) ranged between 4.10µ/L and 6.90µ/L; while aspartate aminotransferase (AST) ranged between 157.60 µ/L and 208.40 µ/L. There were no significant differences (p>0.05) between treatment means for both parameters. Values observed here for ALT were lower than a range of 16.87 – 23.00 µ/L reported for 14 days old to 42 days old broilers (Café *et al*., 2012), as well as 17-24 µ/L (Mohammed, 2011) in Pakistan. AST measured in this work were also lower than 272.94 µ/L reported by Silva *et al*. (2008) in 42 days old broilers; but higher than reported values of 69 - 76 µ/L (Mohammed, 2011). Serum aminotransferases are particularly used for diagnosis and assessment of liver diseases and may be indicator of the overall health or cardiovascular diseases. They are enzymes which catalyze transfer of amino groups to form hepatic metabolite oxaloacetate. Albumin level (g/l) were between 1.70 and 1.80, while globulins ranged between 1.80 and 2.10 giving a ratio ranging between 0.86 – 0.97. No significant difference (p>0.05) between water sources was noticed in these parameters. Albumin measured here were higher than a range of 1.2 – 1.5, while globulin were lower than 2.0 – 2.6 reported in healthy six-week old broiler hybrids (Ross *et al*., 2007). Nworgu *et al*. (2007) observed albumin range of 2.0- 2.4 and globulin level of 1.10- 1.40 in broilers served fluted pumpkin leaves extract supplement. Results of total bilirubin (Mg/dl) showed that broilers given rain water recorded 0.48 Mg/dl, while those given borehole water had 0.70Mg/dl as the lowest and highest levels in this trial. Total bilirubin reported in normal Hybro-PG broilers at 42 days old (Silva *et al*., 2008) was 0.41Mg/dl which was lower than that observed here. Results of cholesterol level showed a range of 109Mg/dl-120Mg/dl; while glucose ranged between 207Mg/dl and 236Mg/dl. However, there was no effect (p>0.05) of treatment (water sources) on these parameters as well as on triglyceride and uric acid measured in these broilers (table 4).

Table 3: Organ characteristics of broilers drinking water from different sources

Parameters (%)	**RV**	**RN**	**BH**	**PB**	**± SEM**
Average body weight (g)	1998.86	2056.02	2071.50	2065.10	45.34
Gizzard	2.71	2.66	2.38	2.21	0.698
Heart	0.62	0.74	0.54	0.47	0.203
Liver	2.90	2.33	3.36	2.66	0.413
Kidney	0.17	0.21	0.18	0.25	0.015
Thymus	0.06	0.05	0.05	0.04	0.001
Pancreas	0.15	0.18	0.24	0.22	0.013
Spleen	0.11^{b}	0.12^{a}	0.10^{b}	0.12^{a}	0.006
Bursa	0.28	0.28	0.27	0.28	0.007
Lungs	0.53^{a}	0.41^{b}	0.44^{b}	0.54^{a}	0.018

^{ab} Means with different superscripts along a row are significantly different (p<0.05}

Table 4: Serum Biochemistry of broilers given drinking from different sources

Parameters	RN	RV	BH	PB	± SEM
Aspartate aminotransferase µ/L	176.00	161.80	157.60	208.4	10.70
Alanine aminotransferase µ/L	5.60	6.40	4.10	4.50	0.54
Albumin g/dl	1.75	1.80	1.80	1.70	0.01
Cholesterol Mg/dl	111.50	102.00	120.00	109.00	3.50
Glucose Mg/dl	211.00	226.00	236.00	207.00	6.50
Uric acid Mg/dl	5.55	6.00	6.40	4.70	0.23
Creatinine Mg/dl	0.40	0.55	0.35	0.65	0.02
Triglyceride Mg/dl	85.00	82.00	95.00	81.00	2.98
Globulin g/dl	1.80	2.00	2.10	1.95	0.03
Total Protein g/dl	3.55	3.80	3.90	3.65	0.02
Albumin: Globulin ratio	0.97	0.90	0.86	0.87	0.01
Total Bilirubin Mg/dl	0.48	0.55	0.70	0.68	0.01

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