African Journal of Biochemistry Research Vol. 5(8), pp. 244-254, August 2011
Available online at http://www.academicjournals.org/AJBR
ISSN 1996-0778 ©2011 Academic Journals

Full Length Research Paper

Effects of weaning formulae and electrolyte quality of water on rats administered with contaminated water sources

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Accepted 30 June, 2011

The study was aimed at correlating the protein quality of weaning formula with the amelioration of heavy metal toxicity. Wistar albino rats were variously administered borehole water samples, Aba River water samples, simulation experiment (deionized water incorporated separately with lead, cadmium and arsenate) water samples (negative control) and deionized water (positive control). Two weaning formulae viz: SGMC (prepared at 20% dietary protein level) and Rd (prepared at 16% dietary protein level) were fed to the experimental animals. Aba River and borehole water samples had abnormal levels (mg/l) of arsenate (0.25±0.01 to 0.3±0.02), cadmium (0.05±0.001 to 0.11±0.005) and lead (0.17±0.02 to 0.24±0.02), significantly higher ($p<0.05$) than the World Health Organization (WHO) and United States Environmental Protection Agency (USEPA) limit values, but were significantly reduced ($p<0.05$) by boiling and filtering processes of water treatment to meet the WHO and USEPA standards. Growth performance (-25±3.5 to 92±1.2 g), PCV (%) (31.2±0.05 to 50.50±0.05), serum albumin (g/dl) (2.31±0.01 to 4.01±0.05) and NPU% (65±0.03 to 95.01±0.02), were recorded of the diet groups of experimental animals. Regression of serum albumin (g/dl) and NPU%, of SGMC diet group, was significant ($p<0.05$), with Pearson's moment correlation coefficient of 0.9875. Toxicity of heavy metals reduced significantly ($p<0.05$), the performance characteristic of weaning formulae. SGMC weaning formula showed significant ($p<0.05$) efficiency, in ameliorating the toxicity of heavy metals more than the Rd weaning formula.

Key words: Heavy metal toxicity, simulation experiments, weaning formulae, performance characteristics.

INTRODUCTION

Toxic heavy metals enter the aquatic environment from both natural and anthropogenic sources for example, volcanic activity and industrial effluents, respectively. Metal contamination of drinking water is a major problem that is faced by many areas in the world. There is need for an inexpensive remediation technology, for removal of selected metals, such as lead, cadmium and arsenic, from drinking water by limestone-based materials from drinking water by limestone-based materials (Davis and Dixon, 2006).

Aba, a city in the south eastern part of Nigeria, with a population of about 839,000 people, is located at 7°35' E and 5° 35' N. The Aba River, flows through Aba metropolis. It is a continuation of the Imo River which runs through the Okpulor Umuobo in the Ngwa heartland and has become a channel/repository for effluents from chemical and allied products industries, waste products from cattle markets (Garki) and abattoirs, as well as domestic waste water. The implication is that the heavy loadings of the river have invariably caused the natural purifying powers of the Aba River to be overwhelmed,

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which is visibly shown by the mass of dead algae and microscopic plants as a consequence of eutrophication (Ubalua and Ezeronye, 2005).

Effluents discharged from a bottling company, into the Aba River, influence the microbiological and physico-chemical properties of the body of water, thus limiting its use for specific purposes (Ibekwe et al., 2006).

In a scientific investigation (Allinor, 2005), the toxic elements, zinc, manganese, nickel, mercury and the molecule, arsenate, were identified in appreciable amounts, 8.012, 0.861, 0.83, 0.2 and 0.82 ppm, respectively, in fresh and frozen fish samples obtained from the Aba River. These heavy metals were also present in appreciable amounts in Aba River water samples

Periodic characterization of the industrial effluents and wastes that are discharged into Aba River, or that could pollute water tables, is an aspect of environmental impact assessment (EIA). The EIA would provide information that would enable the water resource officer to make recommendations on raw water resource management and water treatment procedures.

Rationale for the use of biochemical, haematological, and nutritional assessment indices in the present study, stem from the facts that PCV%, Hb count and erythrocyte count of blood, of female wistar albino rats made to drink lead acetate polluted water, were significantly ($p<0.01$) reduced (Selmin et al., 2004). Lead interferes with heme synthesis by inhibiting δ-aminolevulinic acid dehydratase (δ-ALAD) and ferrochelatase, which causes increased urinary co-porphyrin and δ-ALA excretion and decreased heme synthesis.

Wistar albino were rats administered with Tiete and Capivara Rivers samples, contaminated with toxic heavy metals, manifested significant, hepatic, renal and pancreatic tissue lesions (Silvaa et al., 1999). Serum protein was decreased significantly in cadmium treated rats (Moshtaghie et al., 1991). Diets deficient in protein impair conjugation (methylation) reactions of arsenic, thus increasing arsenic toxicity (Lammon and Hood, 2004). Marked reduction in total serum proteins, body and organ weights and food consumption, characterized experimental rat models to which were administered 0.5 mg/kg arsenic in drinking water (Gopal-Krishman and Rao, 2006).

Tubular necrosis, interstitial fibrosis and glomerular epithelial cell hypertrophy in kidney sections, characterized adult male wistar rats, administered with drinking water incorporated with 60 µg Cd/g wet weight (Aughey et al., 1984). Mice models administered with drinking water incorporated with arsenic (300 ppb), showed severe type of necrosis and degenerative changes in kidneys distal and proximal tubules (Ferzand et al., 2008).

The rationale for formulation of diet is founded on the importance of the cereal-legume-animal supplement mix.

A 20 to 30% addition of animal protein to a 7: 3 (weight to weight) cereal to legume combination improves the nutritive value of foods and induces good and consistent biological responses in experimental animals. The protein advisory group recommends that the protein contents of weaning foods should be at least 20%, on a dry weight basis (FAO/WHO, 1971; WHO, 2001, 2002). The relevance of using albino rats in nutritional studies, while evaluating the nutritional quality of diets is founded on the fact that wistar albino rats have a dietary requirement for the same ten (10) essential amino acids as human infants. The objectives of the study are:

1. To determine the effect of toxic heavy metals (As, Cd and Pb) on the performance characteristics of weaning formulae.
2. To correlate the protein quality of weaning formulae with the amelioration of heavy metal (As, Cd and Pb) toxicity.
3. To compare underground water (borehole water) with surface water (river water) from a polluted environment, while evaluating drinking water quality, using metabolic/biochemical indices of experimental animals fed on weaning formulae.

MATERIALS AND METHODS

Ethical approval (Appendix) was sought and approved by the Department of Biochemistry, Michael Okpara University of Agriculture, Abia State Nigeria. Water samples were drawn from the Aba river, 500 m from an industrial effluent pipe, 300 m from the cattle market, a stone's throw from the abattoir, near the Aba River bridge, located along Ogbor Hill/Ikot Ekpene Road, in Aba metropolis and also from a water borehole in the neighbourhood of the Aba River, located in Omoba Road, near Assemblies of God (Water-side) Ogbor-Hill Aba.

1. Water samples (5 L) drawn from Aba river and water borehole in Aba metropolis, were boiled, separately, in a boiler, for 20 min, allowed to cool and filtered for a period of 2 h in a filteration unit (Doulton, England), by means of silicaceous filter candles (Prestige ®) and stored in clean plastic containers.
2. Water sample (10 L) drawn from water borehole was deionized, using a B114 Deionizer and an ELGA catridge type C114 (Finlab, Nigeria) and stored in a clean water bottle.

The water samples used in this research study were:

1. Water sample sourced from Aba River (untreated): (surface water): RRW,
2. Water sample sourced from Aba River (boiled and filtered): TRW,
3. Water sample sourced from water borehole in Aba metropolis (untreated): RBW (underground water),
4. Water sample sourced from water borehole in Aba metropolis (boiled and filtered): TBW,
5. Deionized borehole water incorporated with 0.2 mg lead/liter (Pb),
6. Deionized borehole water incorporated with 0.2 mg cadmium sulphate/liter (Cd),
7. Deionized borehole water incorporated with 0.2 mg arsenate/liter (As) and

Table 1. Experimental Layout for the two factors, factorial, randomized complete block design (RCBD).

Drinking water quality	Rd (16%) (reference diet)	SGMC (20%)
	Rd A_1*	SGA_1
0.2 mg As/liter of water	RdA_2	SGA_2
	RdA_3	SGA_3
	Rd P_1	SGP_1
0.2 mg Pb/liter of water	RdP_2	SGP_2
	RdP_3	SGP_3
	Rd C_1	SGC_1
0.2 mgCd sulphate /liter of water	RdC_2	SGC_2
	RdC_3	SGC_3
	RdR_1	SGR_1
Aba River water (raw) (surface water)	RdR_2	SGR_2
	RdR_3	SGR_3
	RdB_1	SGB_1
Borehole water (raw) (underground water)	RdB_2	SGB_2
	RdB_3	SGB_3
	$RdRT_1$	$SGRT_1$
Aba River water (treated)	$RdRT_2$	$SGRT_2$
	$RdRT_3$	$SGRT_3$
	$RdBT_1$	$SGBT_1$
Borehole water (treated)	$RdBT_2$	$SGBT_2$
	$RdBT_3$	$SGBT_3$
	RdD_1	SGD_1
Deionized water	RdD_2	SGD_2
	RdD_3	SGD_3*

*Number of observations = 48 responses (RdA_1… SGD_3) per parameter measured using the experimental animals (exclusive of the basal diet group).

8. Deionized water borehole (reference/control water sample) (DW).

The experimental design is a two factor factorial randomized complete block design (RCBD) (Lentner and Bishop, 1993), whose layout is described in Table 1 and linear model, given by;

Yijkl = μ + TAi + TBi + (TATB)ij + Lk + eijkl.

Yijkl = individual observations.

μ = overall mean.

TAi = treatment effect of ith level of dietary protein treatment.

TBj = effect of jth quality of drinking water.

Lk = effect of kth level of block.

(TATB) ij = effect of interaction between ith level of dietary protein and jth quality of drinking water.

eijkl = random error, which is independently, identically and normally distributed, with zero (o) mean, and constant variance.

Treatments A are: A reference weaning formulae (Rd), prepared industrially at 16% protein level (Nutrend) and a high quality 20% weaning formulae (SGMC), prepared using, processed soya bean seeds, groundnut seeds, maize grains and catfish, No. of levels of treatment A = 2.

Treatments B are: Deionized water, incorporated with 0.2 mg lead/liter, deionized water, incorporated with 0.2 mg cadmium sulphate/liter, deionized water, incorporated with 0.2 mg arsenate/liter, Aba (untreated) river water (RRW), and borehole (untreated) water (RBW), Aba River water (TRW) and borehole water (TBW) and deionized water (DW) (+ve control) : no of levels of treatment B = 8.

Number of experimental units = 16.

Number of replicates per experimental units = 3 wistar albino rats.

Number of experimental materials = 48 wistar albino rats.

Number of replicates in basal diet group = 3 wistar albino rats.

Number of observations = 48 responses (RdA1… SGD3) per parameter measured using the experimental animals (exclusive of the basal diet group).

Number of degree of freedom of error = 25.

Lead content of water samples were quantified according to the method described by Di Nezio et al. (2004) which is based on preconcentration of lead ions on chitosan and dithizone–lea

Table 2. Diet formulation.

S/N	Components	Grams/100 g diet (%) Basal	SGMC (20%)	*Rd (16%)
1	Soya bean seed (flour)	-	17.63	
2	Groundnut seed (flour)	-	4.41	
3	Maize seed (flour)	-	51.42	
4	Catfish	-	10.20	
5	Vegetable (fluted pumpkin leaves)	5.00	5.00	
6	Palm oil	8.00	8.00	
7	Vitamins –minerals-amino acid complex	0.25	0.25	
8	Sucrose (sugar)	1.00	1.00	
9	Garri (processed cassava product)	85.75	2.09	

*Reference diet (Rd): Nutrient prepared industrially by Nestle®, of nutritional value- 16% dietary protein, 63.7% carbohydrates, 9% fat, 4% moisture, 2.3% minerals, ≥ grams vitamins, 417.5 kcal/100 g. SGMC: 19.71% dietary protein, 64.2% carbohydrates, 9.2% lipids, 3.1% moisture, 3.1% minerals, vitamins ≤ 0.69 g, 437.1kcal/100 g.

complex formation in aqueous medium (pH 9), followed by spectrophotometric detection. The method provided a linear range between 25 and 250 µg l^{-1}, a detection limit of 5.0 ng ml^{-1} and a sample throughput of 15 h^{-1}.

Cadmium content of water samples were quantified according to the method described by Jankiewicz et al. (2000), which is a spectrophotometric method of determination of trace amounts of cadmium and involves extraction of the complex of cadmium (II) with dithizonate from the basic medium containing cyanides by means of chloroform, in the presence of sodium-potassium tartrate. Absorbance of the resultant pink solution containing cadmium (II) ions was measured at λ = 520 nm.

Arsenic content of water samples were quantified according to the method described by Bednar et al. (2004) in which use was made of anion exchange chromatography to separate the arsenic species and inductively coupled plasma-mass spectrometry as an arsenic-specific detector.

Two hundred (200) grams, each of raw soya bean seeds, raw groundnut seeds and raw maize seeds, were washed and soaked, separately, in a liter of water, for 11 h, and thereafter, boiled in 800 ml of water, for 2 h. Boiled groundnut seeds and soya bean seeds were dehulled. The samples were dried in the oven for 9 h at 105°C, ground and dried for a further 4 h, at 105°C. Fresh catfish samples were dried in the oven for 24 h at 105°C and ground. Fluted pumpkin vegetable leaves were washed in warm water and dried in the oven for 1 h and ground. The schematic for diet formulation is shown in Table 2.

Fifty-one, six-weeks old, male wistar albino rats were divided into 17 experimental units of 3 animals per experimental unit and housed in stainless steel cages under 12 h light and dark cycles, under humid tropical conditions, acclimatized for a period of 3 days and fed *ad lib* on two different types of weaning formulae viz: SGMC and Rd diets for a period of 28 days. Eight experimental units were fed on the SGMC diet and were orally administered, each of the eight different water samples per experimental unit. Eight experimental units were fed on the Rd diet and also were orally administered, each of the eight different water samples per experimental unit. The basal diet group was placed on a hypothetical protein-free diet. Daily faecal deposits of the animals were collected, pooled, oven dried and weighed. The experimental animals were weighed and killed by cervical dislocation, after the animal feeding trials. Blood samples for haematological and biochemical assays were collected in requisite blood sample bottles and stored in a refrigerator at 4°C. The liver organs were weighed. The carcasses were dried for 17h, in an oven drier at 105°C and stored. The faecal nitrogen content and the carcass nitrogen content of the experimental animals were determined.

Quantitative determination of nitrogen content of carcass and faecal deposits of experimental animals by the Kjeldahl method was carried out by a modified method, similar to that described by (AOAC, 1990). Samples were digested in 250 ml Kjeldahl flask using concentrated tetraoxosulphate VI acid, sodium tetraoxosulphate VI and selenium containg catalyst. The dilute digest was distilled using 50% sodium hydroxide solution in a distillation apparatus and collected in an alcoholic boric acid solution and titrated against 0.1 M hydrochloric acid. The titre value (t ml) was obtained at endpoint (pink colour of solution). N (%) = [(N acid) (ml acid) – (ml bk) (N NaOH) – (ml NaOH) (N NaOH)] × 1400.67/mg sample. Where ml NaOH = milliliters of standard base needed to titrate sample; ml acid = mill iliters of standard acid used for that sample; ml bk = milliliters of standard base needed to titrate 1 ml standard acid minus milliliters of standard base needed to titrate reagent blank carried through method and distilled into 1 ml standard acid; N acid = normality of standard acid; N NaOH = normality of standard base.

Crude protein % = N (%) × 6.25.

The Spun microhaematocrit method of Bull and Hay (2001), and Bull et al. (2003) was used for the quantitative *in vitro* determination of packed cell volume (PCV%).

PCV was measured as the ratio of whole blood hemoglobin to packed red cell hemoglobin. Blood samples were collected by syringe and anticoagulated with di-potassium ethylenediaminetetraacetic.

Whole blood (w. bl) hemoglobin concentration was determined by use of hemiglobincyanide (HiCN: methemoglobin cyanide) reagent. The packed red cell (p.r.c) hemoglobin concentration was determined using the blood-anticoagulant mixture. The tubes containing separately, the w.bl, and p.r.c mixtures, were centrifuged at 10,000 g for 5 min in a “microhematocrit”, followed by the removal of the plasma-containing portion, aspiration and mixture with HiCN reagent [HiCN reagent contains 50 mg KCN, 200 mg $K_3Fe(CN)_6$, 140 mg KH_2PO_4 (anhydrous), 0.5 to 1.0 ml nonionic detergent (for example, Nonidet P40, Triton X-100) and clinical laboratory reagent water, Type 1, to 1000 ml]. The absorbance of the filtered solutions in the tubes was measured with a calibrated spectrophotometer at 540 nm in 1.000 cm cuvettes against a HiCN

reagent blank.
ICSH Reference PCV = (A540 w.bl. × 367.7)/ (A540 p.r.c. × 1100.2) = whole blood hemoglobin/packed red cell hemoglobin.
Spun PCV = (0.9736 × ICSH Reference PCV) + 0.0119

Quantitative *in vitro* determination of albumin in serum was carried out using the method described by Qureshi and Qureshi (2001) and Huang and Fraker (2003), serum albumin was determined using human albumin standards and sigma diagnostics albumin reagent (Sigma, St. Louis, MO) containing bromocresol green. The absorbance of the mixture of the reagent and serum albumin was measured at 578 nm against a reagent blank.

Performance characteristics analysis of Net Protein Utilization [NPU (%)] was carried out using the method employed by Pellet and Young (1980). The slope of the regression line of N intake on N retention is related to net protein utilization. The correlation coefficient of the regression is a measure of the net protein utilization [NPU (%)].

Liver sections were prepared for histopathological studies according to the method described by Brozska et al. (2003), slices of the left liver lobe were fixed in 10% formal saline for 24 h. The fixed tissues were dehydrated and de-alcoholated using increasing concentrations of alcohol and xylene, respectively. Infiltration and embedding of the infiltrated tissues were carried out using paraffin wax. Sections (5 to 6 µm) of the liver were obtained using the microtome (MR 2) (Boeckeler Instruments Inc., USA), and routinely stained with haematoxylin and eosin and the stains differentiated, using 1% hydrochloric acid ethanol. The stained sections were examined in a Digital microscope (Motic DMIII) (Motic China Group Co. Ltd). The magnified images of the liver sections taken are the photomicrographs (Plates 1 and 2).

RESULTS

Table 3 shows the values of lead, cadmium and arsenate in each of the water samples, compared with their corresponding WHO values, in order of consecutive decrease as follows: RRW, RBW, WHO, TRW and TBW. Each of the values of lead, cadmium and arsenate in RRW and RBW samples and their corresponding WHO values differed significantly ($p<0.05$). The TRW and TBW samples had significantly reduced values ($p<0.05$) of each of the toxic heavy metal concentrations.

The growth performance is shown in Table 4, The PCV (%) and the serum albumin concentration (g/dl) are shown in shown in Tables 5 and 6, respectively. The NPU (%) values for the distinct experimental animal groups differed significantly ($p<0.05$). These values are demonstrated in Figure 1, in order of consecutive significant decrease, as follows: DW/ TRW/TBW, RBW, RRW, As, and Pb, among the SGMC and Rd diet groups of experimental animals.

All the Rd diet groups of experimental animals had significantly reduced ($p<0.05$) values of growth performance, PCV%, serum albumin and NPU% compared with their corresponding SGMC diet groups of experimental animals, with the exception of the growth performance of the Pb diet groups of experimental animals, which were not significantly different ($p<0.05$). The basal diet group of experimental animals suffered significant reduction ($p<0.05$) of growth performance, PCV (%), serum albumin and NPU%. Rather than gain weight, the basal diet group, lost weight.

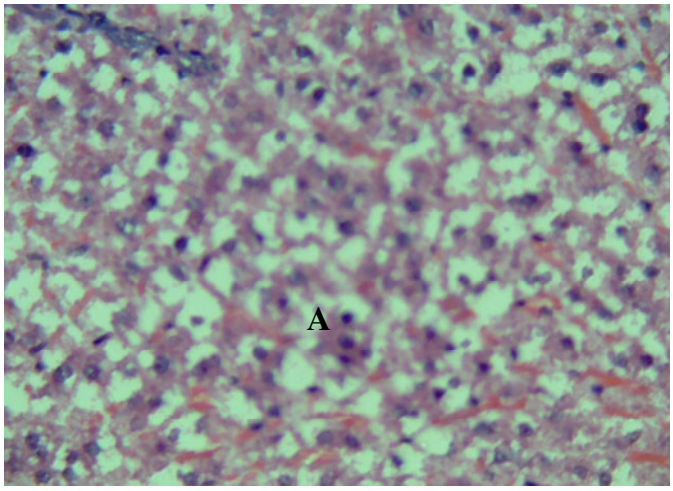

Plate 1. Photomicrograph of lobe section of the liver tissue of a wistar albino rat, administered RRW and SGMC. Plate 1 is the photomicrograph of the liver section of albino rat administered with RRW and SGMC, showing degenerative changes: **A**. Necrotic cells (sparsely distributed black dots).

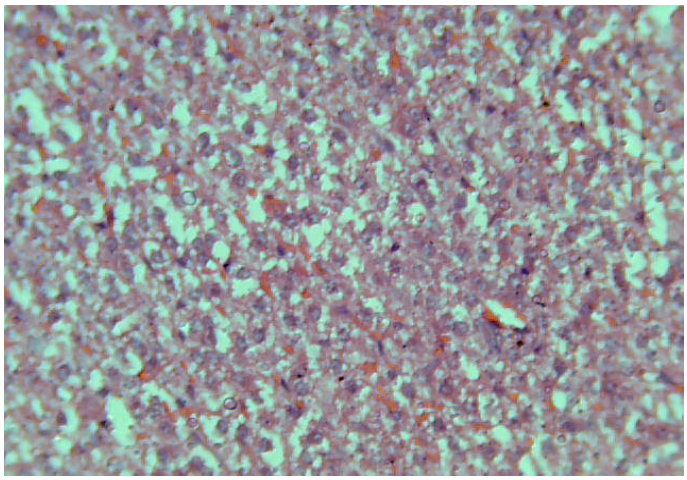

Plate 2. Photomicrograph of lobe section of the liver tissue of a wistar albino rat, administered with TRW and SGMC. Plate 2 is the photomicrograph of the liver section of albino rat administered with TRW and SGMC, showing normal tissue.

Plate 1 is the photomicrograph of lobe section of the liver tissue of a wistar albino rat, administered with Aba raw river water (RRW) and SGMC diet, showing some degenerative changes resulting in the production of necrotic cells. Plate 2 is the photomicrograph of the liver section of albino rat administered with treated Aba River water (TRW) and SGMC diet, showing normal tissue.

Table 3. Chemical characteristics of water sample.

Parameters	Raw river water (RRW)	Treated river water (TRW)	Raw borehole water (RBW)	Treated borehole water (TBW)	WHO/USEPA* Limit Values/ standard
Lead (Pb) mg/l	0.24^{a}± 0.02	0.003^{b}± 0.01	0.17^{c}± 0.02	0.003^{b}± 0.01	0.015± 0.0001
Cadmium (Cd) mg/l	0.11^{a}± 0.005	0.001^{b}±0.0001	0.05^{c}± 0.001	0.001^{b}± 0.00	0.0005^{d}± 0.0001
Arsenate (As) mg/l	0.30^{a}± 0.02	0.001^{b}±0.001	0.25^{c}± 0.01	0.001^{b}± 0.001	0.01^{d} ± 0.001

* Maximum contaminant level/limit values, defined by the World Health Organization (WHO, 1998) and United States Environmental Protection Agency (USEPA, 2010). Results are expressed as means of triplicate ± standard deviation. Means in the same row having same superscripts are not significantly different at 5% level ($p<0.05$).

Table 4. Growth performance/weight gain (grams) of experimental animals.

Drinking water group	Rd (16%) diet group	SGMC (20%) diet group	Basal diet group
As water	31^{a}± 2.25	36^{b} ± 1.5	
Pb water	27.3^{c} ± 1.2	31.0^{c} ± 2.25	
Cd water	36^{b}± 1.5	42^{a} ± 1.2	
Raw river water (RRW)	38^{b}± 2.25	44^{a} ± 0.5	
Treated river water (TRW)	74^{e}± 1.20	90^{f} ± 0.5	
Raw borehole water (RBW)	55^{d} ± 2.0	73^{e} ± 2.5	
Treated borehole water (TBW)	73^{e}± 3.5	90^{f} ± 1.5	
Deionized water (DW)	75^{e}± 0.5	92^{f} ± 1.2	-25^{a} ± 3.5

Values are means ± standard deviation for 3 animals per experimental unit (n =3). Means in the same column having same superscripts are not significantly different at 5% level ($p<0.05$). Means in the same row having same superscripts are not significantly different at 5% level ($p<0.05$).

Table 5. PCV (%) of experimental animals.

Drinking water group	Rd (16%) diet group	SGMC (20%) diet group	Basal diet group
As water	33.1^{a}± 0.01	34.42^{b} ± 0.02	
Pb water	31.2^{b}± 0.05	31.31^{c} ± 0.01	
Cd water	35.3^{c}± 0.02	40.28^{d} ± 0.01	
Raw river water (RRW)	35.3^{c}± 0.03	40.3^{d} ± 0.02	
Treated river water (TRW)	45.5^{g}± 0.03	50.41^{f} ± 0.11	
Raw borehole water (RBW)	36.45^{e}± 0.00	42.5^{a} ± 0.04	
Treated borehole water (TBW)	45.51^{g}± 0.10	50.40^{f} ± 0.11	
Deionized water (DW)	45.5^{g}± 0.11	50.50^{f} ± 0.05	28.02^{a} ± 0.04

Values are means ± standard deviation for 3 animals per experimental unit (n = 3). Means in the same column having same superscripts are not significantly different at 5% level ($p<0.05$). Means in the same row having same superscripts are not significantly different at 5% level ($p<0.05$).

DISCUSSION

The results of the qualitative and quantitative analyses of water samples, showed that values of the concentration of the toxic heavy metals obtained of the Aba River samples, exceeded significantly ($p<0.05$), the maximum contaminant level/limit values, defined by the World Health Organization (WHO, 1998), and United States Environmental Protection Agency (USEPA, 2010), indicating that untreated water from Aba River is not fit for human consumption.

The results of the haematological and biochemical parameters of experimental animals administered with Aba raw river water and simulation drinking water experiments, in the present study, show significant reduction ($p<0.05$) of packed cell volume (PCV%), haemoglobin concentration and serum albumin. These results corroborate the findings of Al-Fartosi (2008), who observed significant reduction ($p<0.05$) of red blood cells, white blood cells and haemoglobin count of male mice, administered with 0.5 mg/kg lead oxide and Wang et al. (2006), who observed significant reduction ($p<0.05$) of

Table 6. Serum Albumin (g/dl) of experimental animals.

Drinking water group	Rd (16%) diet group	SGMC (20%) diet group	Basal diet group
As water	$2.36^{d} \pm 0.01$	$2.68^{a} \pm 0.01$	
Pb water	$2.31^{e} \pm 0.01$	$2.60^{f} \pm 0.03$	
Cd water	$2.42^{a} \pm 0.02$	$2.78^{b} \pm 0.04$	
Raw river water (RRW)	$2.45^{a} \pm 0.03$	$2.8^{b} \pm 0.05$	
Treated river water (TRW)	$3.68^{b} \pm 0.00$	$4.0^{c} \pm 0.03$	
Raw borehole water (RBW)	$2.93^{c} \pm 0.01$	$3.08^{d} \pm 0.05$	
Treated borehole water (TBW)	$3.65^{b} \pm 0.03$	$3.98^{c} \pm 0.03$	
Deionized water (DW)	$3.69^{b} \pm 0.01$	$4.01^{c} \pm 0.05$	$1.78^{a} \pm 0.01$

Values are means ± standard deviation for 3 animals per experimental unit (n = 3). Means in the same column having same superscripts are not significantly different at 5% level ($p<0.05$). Means in the same row having same superscripts are not significantly different at 5% level ($p<0.05$).

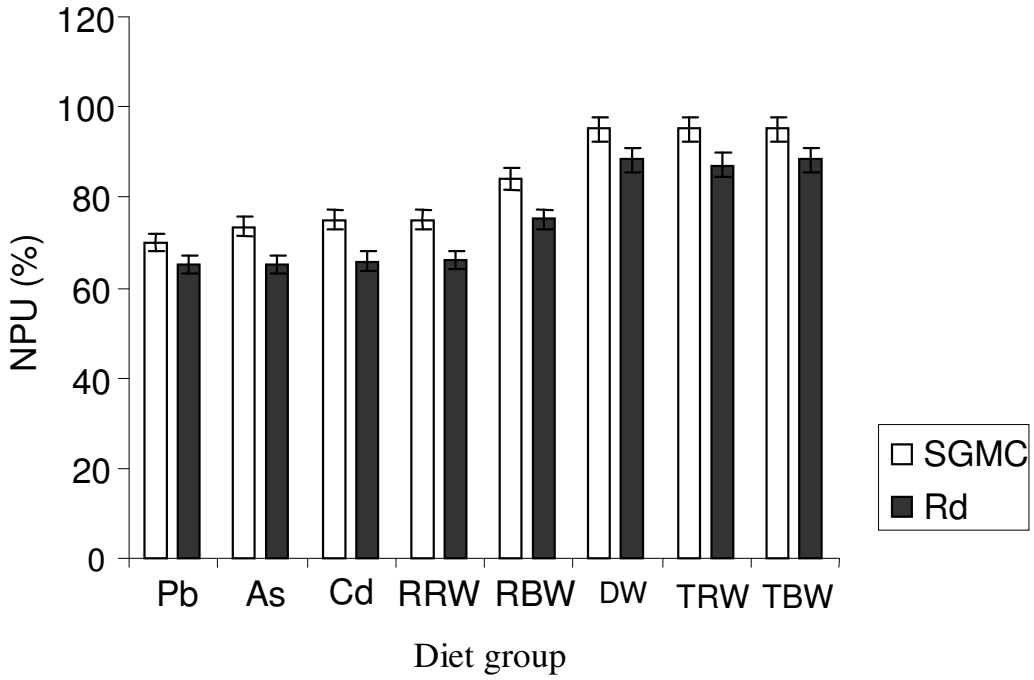

Figure 1. Net protein utilization (npu) % of the weaning formulae.

total serum proteins of experimental animals administered with arsenic.

The SGMC weaning formula comparatively ameliorated the toxicity of lead, more efficiently than the Rd weaning formula. However, these ameliorative effects occur up to a certain high of lead concentration, beyond which the detoxifying effects of the weaning formulae cease to be a function of their comparative dietary protein level. At this point which actually a range (0.23 to 0.24 mg/l), the PCV (%) value of the Rd diet group is significantly equal ($p<0.05$) to the corresponding PCV (%) value of the SGMC diet group of experimental animals, as shown by the convergence of the SGMC and Rd curves in Figure 2. The administration of the Aba raw river water led to degenerative changes associated with necrosis of the liver cells of the experimental wistar albino rats. A finding consistent with the simulation experimental model of Brzoska et al. (2003) and in keeping with the degenerative changes observed of liver tissues of Sprague-Dawley rats, concurrently administered with lead, cadmium and arsenic (Mahaffey and Fowler, 1977).

The correlation between serum albumin (g/dl) and NPU

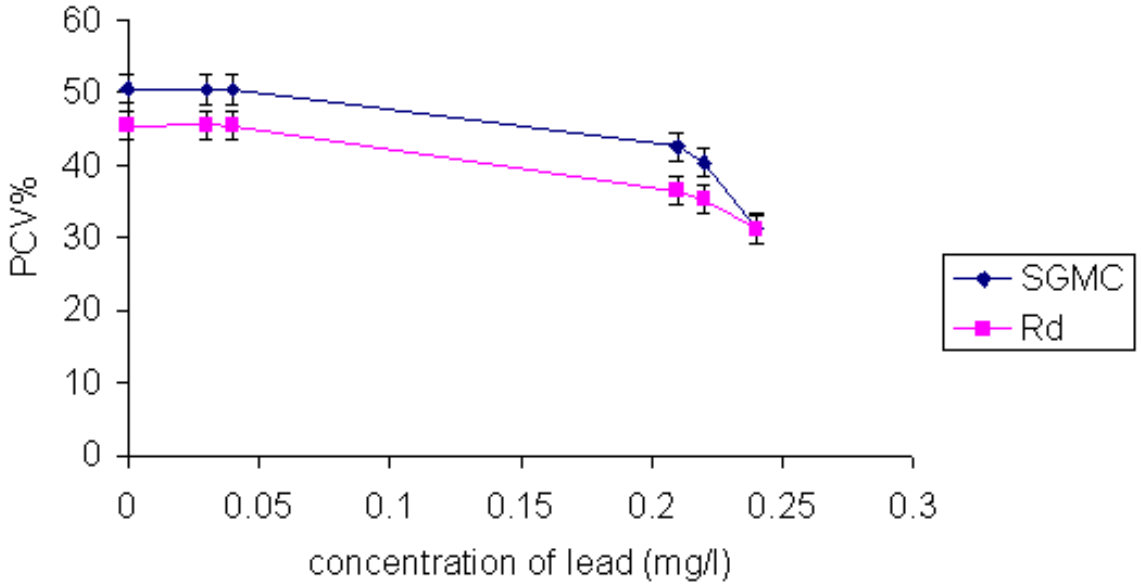

Figure 2. Effect of lead (pb) on the pcv (%) of the experimental groups of animals.

(%) was significant at 5% level, for both the SGMC and Rd diet group of experimental animals. The Pearson's product moment correlation coefficients of the SGMC and Rd diet groups were 0.9875 and 0.8210, respectively. This observation served to indicate that the value of NPU (%) of SGMC weaning formula could be predicted with more accuracy and efficiency than the value of NPU (%) of Rd weaning formula, by means of extrapolation from the regression curves shown in Figure 3.

The performance characteristic value of the SGMC weaning formula fed to the treated (boiled and filtered, deionized) water diet groups, indicate good growth-promoting quality of food proteins and elicited excellent biological responses from experimental animals, in keeping with the findings of Mosha and Bennink (2004).

SGMC weaning formula, is significantly ($p<0.05$), more efficient, in reducing and ameliorating the toxic effect of heavy metals, in the experimental animals, than the Rd weaning formula. The suggested possible mechanism for the amelioration of the toxicity of heavy metals in drinking water by high level protein diets fed to experimental animals is founded on the fact that the ionic metal lattices of the toxic heavy metals form strong electrostatic bonds with the negatively charged R groups (for example, γ-carboxylic group of aspartic acid residue) and terminal α-carboxylic groups of the amino acid residues of the polypeptide chains of dietary proteins. Thus, dietary proteins chelate with toxic heavy metals in the gastro-intestinal tract, making the toxic heavy metals, insoluble and some of the dietary proteins, unavailable for digestion and absorption.

Water treatment (boiling, filtration and deionization), as well as high level dietary protein weaning formulae, improved and optimized the metabolic/biochemical quality of the Aba raw river and borehole drinking water samples. The measured values of growth performance, PCV (%), serum albumin, histopathological indices and NPU (%) of the dietary groups, administered with treated water varieties, fell within the normal range of respective values. Though, these values of the SGMC diet groups were significantly higher ($p<0.05$) than the corresponding values of the Rd diet groups. Thus confirming the excellent electrolyte quality of the treated water varieties which are fit for human consumption/use as portable drinking water.

The silicaceous ceramic candle filters (Prestige®), posses the desirable capacity to reduce, the toxic heavy metal content of water, reminiscent of the capacity of limestone, to reduce, with an efficiency of 90%, the Pb, Cd, and As content of drinking water (Davis and Dixon, 2006).

Conclusion

The Aba untreated river water is not fit for human consumption. Water treatment (boiling and filtration), as well as the high level dietary protein weaning formula (SGMC), improved and optimized the Aba River and borehole drinkable water, metabolic/biochemical qualities, thus ameliorating the toxicity of the heavy metals in polluted waters. The treated water varieties, are fit for human consumption/use as portable drinking water.

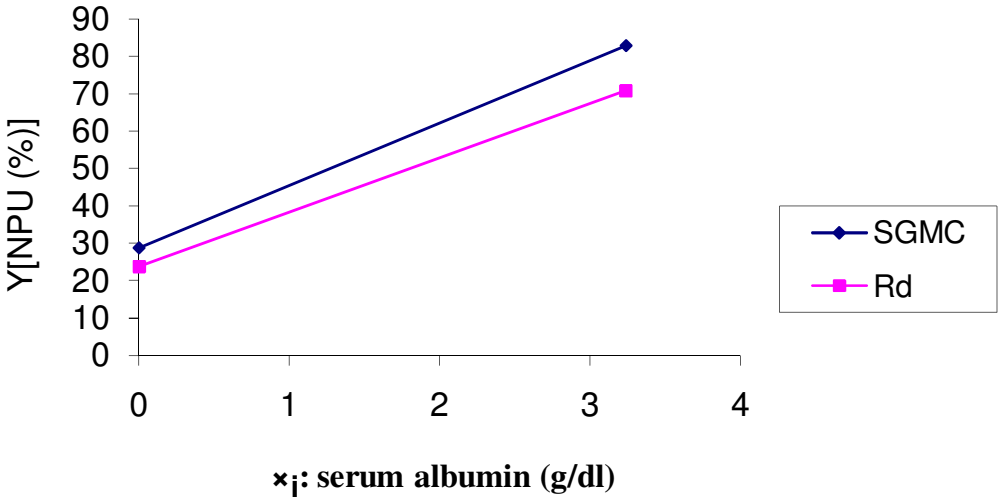

Figure 3. Regression curve of serum albumin (g/dl) and npu%: (SGMC): Y (%) = 28.73 (%) + 16.69 (%* dl/g)x_i g/dl, (Rd): Y (%) = 23.73 (%) + 14.53(%* dl/g) x_i g/dl.

A water treatment plant should be installed/constructed as part of the Aba City water supply rehabilitation scheme.

ACKNOWLEDGEMENTS

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We thank Jesus Christ of Nazareth, the Food of life and the Living Water. We acknowledge with gratitude the resource contributions of Engr. Donald Anyalebechi Obimbas and Engr. and Mrs. Enyichukwu Abraham Obimba, Lois Ogechi Nene Obimba and the technical support of Prof. Johnson Lin.

Abbreviations: SGMC, Weaning formulae prepared at 20% dietary protein, with processed soya bean seeds, groundnut seeds, maize seeds and catfish, diet group; **Rd,** Nutrend-Nestle®, weaning formulae industrially prepared at 16% dietary protein, diet group; **Bd,** basal diet (hypothetical protein-free diet), diet group; **RRW,** water sample sourced from Aba River [untreated (surface) river water], diet group; **TRW,** water sample sourced from Aba River (boiled and filtered-tap river water), diet group; **RBW,** water sample sourced from water borehole in Aba metropolis (untreated borehole water), diet group; **TBW,** water sample sourced from water borehole in Aba metropolis (boiled and filtered-tap borehole water), diet group; **Pb,** deionized water incorporated with 0.2 mg lead/liter, diet group; **Cd,** deionized water incorporated with 0.2 mg cadmium sulphate/liter, diet group; **As,** deionized water incorporated with 0.2 mg arsenate/liter, diet group; **DW,** deionized water (reference/control water sample), diet group; **PCV,** packed cell volume; **NPU,** net protein utilization.

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Appendix

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Vice Chancellor:
Prof. H.O. Edeoga.

Ag. Head of Department
Dr.Okafor P.N.
B.Sc, M.Sc. PhD (Ibadan)
Date: 23-06-2011

TO WHOM IT MAY CONCERN : ETHICAL STANDARDS FOR ANIMAL HANDLING/RIGHT.

This is to certify that the research work carried out by Obimba, Kelechukwu Clarence, titled : **Ameliorative Effect Of Weaning Formulae on Wistar Albino Rats Administered with Heavy Metals contaminated Waters,** was conducted according to Ethical Standards for animal handling/rights of Nigeria.

This was ensured by the supervisory Committee of the Department of Biochemistry, College of Applied and Natural Sciences, Michael Okpara University of Agriculture Umudike. Nigeria.

Yours faithfully,
Polycarp Nnacheta Okafor. (Ph.D).

HEAD DEPARTMENT BIOCHEMISTRY
23/06/11
DATE

Appendix. Ethical approval.