

Levels of Toxic Trace Metals and Mineral Elements in Broiler Chicken Raised at Nsukka, Nigeria: An Evaluation of Possible Potential Health Risk

J.N. ASEGBELOYIN^{*}, N.N. UKWUEZE, J.N. IHEDIOHA and U.S. ORUMA

Department of Pure and Industrial Chemistry, University of Nigeria, Nsukka, Nigeria

*Corresponding author: E-mail: niyi.asegbeloyin@unn.edu.ng

Received: 2 January 2015; Accepted: 2 I	March 2015; Published online: 22 June 2015;	AJC-17346
---	---	-----------

The study assessed the concentrations of some trace metals and mineral elements in some broiler chicken from Nsukka, Nigeria and estimated the potential health risk associated with their consumption. Dried meat samples were digested with freshly prepared 1:1 HNO₃/ H_2O_2 solution and metal concentrations were determined spectrophotometrically. The ranges of mean concentrations (mg/kg) were: Mn (0.01-0.09), Cd (0.01-0.7), Cr (1.21-2.94), As (0.01-2.13) Pb (0.50-1.50), Se (0.01-0.03), K (7.11-33.86), Ca (15.22-177.15), Na (12.41-43.81), Mg (60.50-548.03) and P (7.75-88.53). The concentration (mg/kg) of trace metals in some of the animal parts were above some regulatory limits. The estimated intakes of As and Pb were above the Provisional Tolerable Daily Intake (PTDI) while the Target Hazard Quotient (THQ) of As was very high. Possible potential health risk could arise from consumption of the broiler chicken with respect to arsenic.

Keywords: Toxic trace metals, Mineral elements, Broiler chicken, Target hazard Quotient, Risk assessment.

INTRODUCTION

Broilers are birds of the species Gallus domesticus that have been breed specially for rapid growth so that they will on average reach a weight in excess of 2 kg in six weeks of age having consumed less than 2 kg of feed for each kilogram gain in weight¹. Broiler production is one of the quickest ways to bridge the animal protein supply situation in Nigeria. Broilers as simple stomached animals to a large extent depend on grains, legumes and cereals for a great proportion of their ration. These feeding stuffs are grown on soils that have varying degrees of mineral elements and toxic trace metals. Considerable amounts of trace elements get into the environment by human activity². It has been reported that industries, traffics and agricultural technologies have accounted for the largest discharge of trace elements into the soil, water and air³. Trace elements are known to be toxic to all ecosystem components both on the aquatic and terrestrial system⁴. Incorporation of trace elements into the food chain of animals can lead to chronic diseases, reduce fertility and injure the cardiovascular and nervous systems⁵. Meanwhile broilers require mineral elements such as Ca, P, Na, Cl and Mg in relatively large quantities as against Mn, I, Fe, Co, Cu, etc. required in small quantities. Minerals are needed for skeletal tissue development and maintenance⁶. Joint Food and Agriculture Organization (FAO)/WHO Expert Committee on Food Additives (JECFA)

and European Commission (EC) have set the Provisional Tolerable Daily Intake (PTDI) limits and maximum levels (ML) for certain contaminants. These parameters are relevant in assessing toxic element concentrations in both the human body and foodstuffs⁷⁻¹⁰. The PTDI limits are end points used for contaminants such as toxic elements with cumulative properties, representing permissible human daily exposure to those contaminants that are unavoidably associated with the consumption of contaminated foodstuffs. The limits and maximum levels represents the maximum concentration of contaminants in foodstuffs which is acceptable and poses no human health risk¹⁰. In view of the growing public health concern on providing only meat that are safe to the consuming public, the present study was designed to assess the mineral elements and trace elements in broilers meat parts sampled in Nsukka, a town located in South Eastern region of Nigeria and also determine any possible potential health risk due to excess exposure to the toxic metals.

EXPERIMENTAL

Thirty broiler birds were bought from different poultry farms in Nsukka in August, 2014. The birds were killed and dissected to obtain the different parts required for analysis which include the liver, heart, muscle, intestine and gizzard. Samples for analysis were dried to a constant weight at 105 °C. The dry samples were ground using porcelain mortar and pestle

and thereafter stored in desiccators. 10 g of dried samples was digested with 60 mL of freshly prepared 1:1 HNO₃:H₂O₂ solution at 160 °C on a hot plate for about 1 h until the contents reduced to about 5 mL11. The digest was transferred to a standard flask and made up to 25 mL with distilled water. The solution were aspirated into an optima 2000DU Perkin Elmer ICP-OES (USA) for the determination of Se, As, Cr, Mn, P and Cd. Appropriate standards supplied by Perkin Elmer were used. Calcium, Mg and Pb were determined by using an atomic absorption spectrometer (UNICAM, 969, UK) equipped with air-acetylene flame while Na and K were determined with a flame photometer (Gallenkamp Flame analyser UK). The data obtained were subjected to a one-way Analysis Variance (ANOVA) according to the procedure of Steel and Torrie¹². Significantly different means were separated using the methods of Duncan¹³.

Quality assurance procedure: Appropriate quality control measures were carried out. Sample blanks, prepared by taking 60 mL of the digestion mixture through the digestion procedure were analyzed for all the metals. Detection limits were determined by lowest possible dilution and the values (mg/L) were: Pb (0.004), Cd (0.002), Cr (0.02), Mn (0.01), As (0.02) and Se (0.01). The digestion method was validated by carrying out recovery experiment. This was done by determining the concentrations of the metals in triplicate samples of un-spiked and spiked liver samples. Spiking was done by adding the different metal solutions to 10g of ground liver which was later subjected to the digestion procedure.

Recovery (%) =
$$\frac{X-Y}{Z} \times 100$$

where X = concentration of the spiked sample, Y = concentration of the un-spiked sample; Z = concentration of the metal ion added.

Health risk assessment: The health risk associated with consumption of broiler chicken meat was determined by evaluating the target hazard quotient (THQ)¹⁰. The THQ value for the toxic trace metals were determined by using the following equation:

$$THQ = \frac{Efr \times EDtot \times EDI}{RfDo \times BW \times ATn} \times 10^{-3}$$

where THQ is target hazard quotient; EF is exposure frequency (350 days/year); ED is exposure duration, (70 years); Estimated daily intake (EDI) is $\sum_{i=1}^{3}$ MI×MC [MI = meat ingestion (g/ person/day); MC =metal concentration in (mg/kg); subscript i = 1-3 corresponding to muscle, liver and gizzard]; RfD is the oral reference dose (mg/kg/day); BW is the average body weight (60 kg), AT is average time for non-carcinogens (365 days/year × ED). Oral reference doses (RfD) mg/kg/day for the metals are: Cd (0.001), Pb (0.004), As (0.0003), Se (0.005) and Mn (0.14)¹⁴. Information on the daily consumption rate of boiler chicken for an adult (muscle = 64 g/day; gizzard = 68 g/day and liver 37 g/day) were obtained from literature¹⁵.

RESULTS AND DISCUSSION

The mean % recoveries of the metals and % precision (Table-1) showed good recoveries and accuracy, thus validating the method of analysis. Table-2 shows the mean concentration of toxic trace metals in the different broiler meat parts. Results showed that the concentration of Mn was significantly (P <0.05) higher in the intestine and liver. Manganese concentration (mg/kg) of 0.09 and 0.08 was observed in the intestine and liver respectively. These values differ significantly from Mn concentration (mg/kg) of 0.06, 0.03, 0.02 and 0.01 observed for the heart, muscle, gizzard and neck respectively. Similarly, the Cd value of 0.07 mg/kg observed for the intestine was significantly higher (P < 0.05) than values recorded for other parts. As concentration followed the same trend as Mn and Cd concentration. Significantly (P < 0.05) higher values were observed in the intestine, however the concentration of As in the gizzard was also relatively high. Pb concentration was significantly (P < 0.05) higher in the breast muscle and this differed significantly (P < 0.05) from 0.80, 0.60, 0.50, 0.50 mg/kg observed for the parts from the liver, gizzard, neck and intestine respectively. A Se concentration of 0.03 mg/kg

TABLE-1 PERCENT MEAN (n =3) RECOVERY FOR THE METALS IN LIVER SAMPLES					
Elements	Spiked (added) concentration (µg/mL)	Concentration of unspiked sample (µg/mL)	Concentration of spiked sample (µg/mL)	Recovery (%)	Precision (%)
Mn	0.200	0.032	0.230	99 ± 1.15	1.16
Cd	0.100	0.020	0.123	103 ± 4.00	3.88
*Cr	0.250	0.480	0.690	84 ± 2.08	2.48
As	0.100	0.020	0.110	90 ± 1.00	1.11
Pb	0.100	0.032	0.126	94 ± 2.00	2.13
Se	0.100	0.010	0.090	80 ± 2.00	2.50
*N f 1	1				

*Muscle samples were used.

TABLE-2 MEAN CONCENTRATIONS OF TOXIC TRACE METALS (mg/kg DRY WEIGHT)						
Trace metals	Neck	Heart	Gizzard	Intestine	Muscle	Liver
Mn	$0.01^{d} \pm 0.01$	$0.06^{b} \pm 0.02$	$0.02^{d} \pm 0.02$	$0.09^{a} \pm 0.01$	$0.03^{\circ} \pm 0.01$	$0.08^{a} \pm 0.01$
Cd	$0.01^{\circ} \pm 0.01$	ND	$0.04^{b} \pm 0.01$	$0.07^{a} \pm 0.02$	$0.03^{\circ} \pm 0.01$	$0.04^{\rm b} \pm 0.01$
Cr	$1.21^{\circ} \pm 0.10$	ND	$1.70^{\rm b} \pm 0.07$	$2.94^{a} \pm 0.11$	$1.50^{\rm b} \pm 0.06$	ND
As	$0.02^{\circ} \pm 0.01$	$0.02^{\circ} \pm 0.01$	$2.10^{a} \pm 0.44$	$2.13^{a} \pm 0.80$	$1.20^{\rm b} \pm 0.50$	$0.02^{\circ} \pm 0.01$
Pb	$0.50^{\circ} \pm 0.12$	ND	$0.60^{\circ} \pm 0.13$	$0.50^{\circ} \pm 0.50$	$1.00^{a} \pm 0.07$	$0.80^{\rm b} \pm 0.08$
Se	$0.01^{b} \pm 0.01$	ND	$0.01^{b} \pm 0.01$	$0.03^{a} \pm 0.01$	$0.01^{b} \pm 0.01$	$0.01^{b} \pm 0.01$
a^{bcd} Pow means with different superscripts are significantly different at (P < 0.05). ND = not detectable						

^{abcd}Row means with different superscripts are significantly different at (P < 0.05), ND = not detectable.

observed for the intestine was significantly (P < 0.05) higher than the values observed for other parts. Table-3 shows the mean concentration of some mineral elements observed in the cut broiler parts. Potassium concentration was significantly (P < 0.05) higher in the liver. A concentration of 33.86 mg/kg observed for the liver was significantly (P < 0.05) higher than a concentration of 19.41, 17.11, 10.46, 9.81 and 7.11 mg/kg observed for cut parts from the heart, breast muscle, intestine, gizzard and neck respectively. The neck had significantly higher concentration of Ca and P than other cut parts studied, relative high concentration of Ca and P were also observed in the liver. Sodium concentration was significantly higher in the gizzard. The Na concentration of 43.81 mg/kg observed in the gizzard was significantly higher than values observed in other cut parts. Significantly (P < 0.05) higher concentration of Mg was also recorded in the gizzard.

High levels of the toxic trace metals was observed in the intestine and liver of broiler chicken. It has been established that intestinal absorption and pulmonary uptake are the major routes of metal entrance into the body of higher organisms¹⁶. The significantly higher concentration of toxic trace metals in the small intestine and liver observed in this study agrees with this view. This may also be connected with the physiological role of the intestine as the active site of digestion. The concentrations (mg/kg) of Pb recorded in all parts studied were above 0.10 and 0.50 mg/kg stipulated by the European Union for poultry meat and edible offal's respectively¹⁷. This is indicative of high levels of lead in the environment. Lead pollution could arise from automobile exhaust gases, which arise from anti knocking agents added in gasoline. Other sources are untreated waste effluents of industry, which find their way to irrigation channels and hence pollute the fodder through soil. Lead is a cumulative poison and poses the greatest problem of chronic intoxication. Reports have shown that ingested Pb ions bind with the haemoglobin and plasma protein of the blood due to high affinity for proteins, thus inhibiting the synthesis of red blood cells and vital transport of oxygen¹⁸ and when this binding capacity is exceeded, Pb passes into the bone marrow, liver and kidney¹⁸. The concentration of Cd recorded for the intestine exceeded the Codex Committee of Food

Additives and Contaminants draft guideline of 0.05 mg/kg while levels in other tissues were within the guideline value¹⁹. It has been reported that poultry feed sold in South Eastern Nigeria contains high levels of lead (1.10-7.33 mg/kg) and moderate levels of cadmium (0.038-0.463 mg/kg) and manganese $(26.91-76.74 \text{ mg/kg})^{20}$. The range of lead and cadmium in this study were lower than Pb (0.01-4.60 mg/kg) and Cd (0.01-5.68 mg/kg) reported in chicken and turkey meat from southern Nigeria²¹. Also, lead (52.41-61.29 mg/kg) and cadmium (5.53-11.48 mg/kg) earlier reported for broiler exotic meat by some workers²² were higher than results observed in the present study. Arsenic levels in muscle, gizzard and intestine were above the US Food and Drug Administration limit of 0.50 mg/kg recommended for edible tissues of chicken and turkey²³. The principal sources of arsenic compounds are the arsenical pesticides, smelters and coal fired power plants. Smelting of non-ferrous metals and production of energy from fossil fuel are among the industrial sources that lead to arsenic contamination of air, water and soil²⁴. More so, arsenic is used in some veterinary drugs, including those used to treat birds and animals used for commercial food products²³. The arsenic concentration in this study is lower than $44.09 \pm 3.62 \text{ mg/kg}$ reported in poultry from Lahore, Pakistan²⁵. The detected chromium levels reported in this study were above the Brazilian maximum levels (0.10 mg/kg) in meat and fish²⁶. Chromium sources could be from waste effluents discharged from textile, pigment, leather tanning and electroplating industries, incineration of municipal refuse and sewage sludge. Manganese levels in this study were much lower than the WHO/EU maximum limit $(0.20 \text{ mg/kg})^{27}$. The levels of selenium were also low compared to 0.04-0.12 and 0.1-0.81 mg/kg reported in meat and meat products in India²⁸ and United States²⁹ respectively. The mineral elements obtained in the study were moderate. These are essential minerals which are beneficial to man. Potassium affects the trans-membrane potential and also plays its role as the major determination of intracellular ionic strength³⁰. Calcium is essential constituent of the body and is the most important intracellular cation. It acts as a second messenger in various signal transduction cascades²⁵. Sodium is also an important cation in the body and is involved in

TABLE-3 CONCENTRATION OF THE ESSENTIAL MINERALS (mg/kg DRY WEIGHT)						
Mineral Elements	Neck	Heart	Gizzard	Intestine	Muscle	Liver
K	$7.11^{d} \pm 0.20$	$19.41^{b} \pm 0.30$	$9.81^{d} \pm 0.15$	$10.46^{\circ} \pm 0.14$	$17.11^{b} \pm 0.13$	$33.86^{a} \pm 0.18$
Ca	$177.5^{a} \pm 0.40$	$15.22^{d} \pm 0.24$	$17.04^{d} \pm 0.10$	$15.12^{d} \pm 0.09$	$36.77^{\circ} \pm 0.34$	$80.44^{b} \pm 0.17$
Na	$12.41^{d} \pm 0.22$	$17.55^{\circ} \pm 0.15$	$43.81^{a} \pm 0.60$	$30.40^{\rm b} \pm 0.50$	$30.55^{\text{b}} \pm 0.40$	$10.44^{d} \pm 0.18$
Mg	$60.50^{\text{b}} \pm 0.44$	$40.33^{\circ} \pm 0.44$	$70.55^{\text{b}} \pm 0.68$	$54.80^{\text{b}} \pm 0.55$	$20.77^{d} \pm 0.17$	$40.23^{\circ} \pm 0.9$
Р	$88.50^{\circ} \pm 0.78$	$7.75^{d} \pm 0.55$	$8.55^{d} \pm 0.44$	$7.63^{d} \pm 0.23$	$15.55^{\circ} \pm 0.11$	$40.23^{\text{b}} \pm 0.11$
abcd Bow means with different superscripts are significantly different at (P < 0.05)						

^{abcd}Row means with different superscripts are significantly different at (P < 0.05

TABLE-4 ESTIMATED DAILY INTAKE (µg/day FOR A 60 kg ADULT) AND TARGET HAZARD QUOTIENT OF THE TOXIC TRACE METALS Pb Cr Meat parts Cd As Se Mn Muscle 64 1.92 76.8 0.64 96 1.92 Gizzard 40.8 2.72 142.8 0.68 115.6 1.36 EDI 29.6 0.74 0.37 2.96 Liver 1.48 Total 134.4 6.12 220.34 1.69 211.6 6.24 THQ 0.54 0.1 11.73 0.005 0.0007

maintaining trans-membrane potential. It has been reported that the most important mineral constituent of the skeleton is calcium and that bone is a metabolic pool of calcium and phosphorus³¹. The relatively high calcium content of the neck is consistent with reports in literature³². The present result also revealed a Ca:P ratio of approximately 2:1 for all parts of the broiler studied. It has been reported that animals require Ca and P in large quantity for proper body function and that for optimal performance; Ca and P need to be present at required levels. The ideal Ca to P ratio (Ca:P) has been reported to be approximately 1:1 to 2:1³³. The estimated daily intakes (EDI) of some of the metals were estimated by multiplication of the daily consumption of chicken meat with the metal content in chicken meat³⁴. The recent Provisional Tolerable Daily Intake (PTDI) for cadmium set by JECFA is 50 µg/day for a 60 kg adult³⁵. The intake of Cd from broiler chicken is quite low (12 %) of PTDI. However, the intakes of As (183 %) of PTDI (120 $\mu g/day$ for 60 kg adult 17 and Cr (106 %) of Recommended Daily Intake (RDI) of 50-200 µg/day for adults and adolescents³⁶ were very high. The Pb intake (63 %) of PTDI (214 μ g/day) for a 60 kg adult³⁷ is appreciable. The daily intake of selenium and manganese in this study were very low. A maximal daily safe dietary selenium intake of 400 µg has been suggested for adults³⁸. Daily intake of small amounts of manganese is needed for growth and good health in humans otherwise deficiency of manganese can cause nervous system problems³⁹. The health risks from consumption of broiler chicken meat were determined based on the target hazard quotient (THQ). The THQ is a ratio of determined dose of a pollutant to a reference dose level. If the ratio is less than 1, the exposed population is unlikely to experience obvious adverse effects⁴⁰. Target hazard quotient has been recognized as a useful parameter for evaluation of risk associated with the consumption of metal contaminated food⁴¹. The THQ of this study is presented in Table-2. With the exception of As, the THQs of the other metals were less than 1, thus signifying no probable health risk. The Pb and Cd THQs in this study were similar to values reported in cow meat for an urban population in Enugu State, Nigeria⁴². The THQ of arsenic is quite high (11.73), thus indicating a relatively high health risk to the consumers. Low level exposure to arsenic is associated with skin, vascular and nervous system disorders¹⁷. Also the International Agency for Research into Cancer (IARC) has classified arsenic as a human carcinogen¹⁷, as a result of increased incidence of cancer in occupationally exposed people.

Conclusion

Levels of lead and arsenic were appreciable in broiler chicken meat compared to the permissible limit set by some regulatory bodies. The THQs were less than 1 for the trace metals except for As which has a very high value, thus indicating probable health risk. This is of utmost concern for safety of the consumers. Diffuse dispersion and exposure to these metal contaminants by the birds may occur from contaminated agricultural land from which the feed stuff were obtained, runoff from waste dumps and contaminated water, thus increasing the metal burden transferred into the food chain. Therefore, there is need for a drastic reduction in these environmental contaminants in order to safeguard the food chain. This study will contribute to baseline data on the intake values for the safety of the population in Nigeria and for further studies.

ACKNOWLEDGEMENTS

The authors are grateful to N.H. Ugwuonah and N.J. Ogbonna for their help in sampling and laboratory assistance.

REFERENCES

- A.J. Smith, Poultry-The Tropical Agriculturalist, CTA Macmillan, London (2001).
- A. Bessenyi, Study of toxic metals (Cd, Pb, Hg, and Ni) in Rabbits and Broilers Chickens. (2003). Available at: http://phd. Univet.hu/lapak/ erbekezes/Bersenyi-T-E. doc. Accessed 20th October, 2009.
- S. Fekete, A. Bessenyui, I. Kadar, R. Glavits, J. Konez and L. Zoldag, Acta Vet. Hung., 49, 301 (2001).
- A. Bessenyi, S. Fekete, I. Hullar, J. Kadar, M. Szilagyik, R. Glavits, M. Kulesar, M. Mezes and L. Zoldeg, *Acta Vet. Hung.*, 47, 181 (1999).
- A. Bersényi, S.G. Fekete, Z. Szõcs and E. Berta, *Acta Vet. Hung.*, **51**, 297 (2003).
- J.A. Oluyemi and F.A. Roberts, Poultry Production in Warm Wet Climates, Spectrum Books Limited, Ibadan, pp. 49 (2000).
- WHO, Summary and Conclusions, Joint FAO/WHO Expert Committee on Food Additives (JECFA/73/SC), 73rd Meeting, Geneva, 8-17 June 2010, Geneva: WHO; 2010 [cited 2011 Jan 7]. Available from: http:// www.who.int/foodsafety/publications/chem/summary73.pdf.
- WHO, Summary and Conclusions (JECFA/72/SC), Joint FAO/WHO Expert Committee on Food Additives, 72nd Meeting, Rome, 16-25 February 2010, Geneva: WHO; 2010 [cited 2011 Jan 7]. Available from: http://www.who.int/foodsafety/chem/summary72_rev.pdf.
- Commission regulation (EC) No. 1881/2006, Setting Maximum Levels for Certain Contaminants in Foodstuffs, Off. J. Eur Union, L 364/5-L 364/24 (2006).
- The International Program of Chemical Safety (IPCS), JECFA glossary of terms. The International Program of Chemical Safety (IPCS). Geneva: WHO; 2011 [cited 2011 Jan 7]. Available from: http:// www.who.int/foodsafety/chem/jecfa/glossary.pdf.
- Food and Agriculture Organization of the United Nations, Manual of Methods in Aquatic Environmental Research, Part 9; Analysis of Metals and Organochlorines in Fish, FAO Fisheries/Technical paper 12 (1983).
- R.G.D. Steel and J.H. Torrie, Principles and Procedures of Statistics, McGraw Hill Co. Inc., New York (1980).
- 13. D.B. Duncan, *Biometrics*, **11**, 1 (1955).
- US E.P.A. Risk-based Concentration Table, Philadelphia PA. United States Environmental Protection Agency, Washington DC. (2000).
- N.C. Oforka, L.C. Osuji and U. Onwuachu, Arch. Appl. Sci. Res., 4, 675 (2012).
- 16. D. Horky, J. Illek and A. Pechova, Vet. Med., 43, 331 (1998).
- Food Safety Authority of Ireland, Mercury, Lead, Cadmium, Tin and Arsenic in Food, (Toxicology factsheet series issue no. 1; 2009), www.fsai.ie/workarea/downloadassest.aspx?id=841, Accessed 28 July 2013.
- R.P. Belile, in eds.: G.D. Clayton and F.E. Clayton, Patty's Industrial Hygiene and Toxicology, John Wiley & Sons Inc, New York, USA, edn 2, vol 2, Part C, (1994).
- Anonymous, Commission Regulation (EC) NO: 78/2005 of 16 January 2005 amending Regulation EC No: 466/2001 as regards heavy metals. Off J L 16/43. 20/1/2005 pp.43 (2005).
- C.O.B. Okoye, C.N. Ibeto and J.N. Ihedioha, *Adv. Appl. Sci. Res.*, 2, 63 (2011).
- 21. C.M.A. Iwegbue, G.E. Nwajei and E.H. Iyoha, *Bulg. J. Vet. Med.*, **11**, 275 (2008).
- C.O.B. Okoye, A.U. Aneke, C.N. Ibeto and J.N. Ihedioha, *Int. J. Appl. Environ. Sci.*, 6, 49 (2011).
- Agency for Toxic Substance and Disease Registry (ATSDR), Environmental Health and Medicine Education. 2010, http://www.atsdr.cdc.gov/csem/csem.asp?csem=1&po=8. Accessed 13 Aug, 2013.
- D.C. Chilvers and P.J. Peterson, in eds.: T.C. Hutchison and K.M. Meema, Global Cycling of Arsenic, In: Lead, Mercury, Cadmium and Arsenic in the Environment, John Wiley & Sons Inc., Chichester and New York, pp. 279 (1987).

- 25. I. Mariam, S. Iqbal and S.A. Nagra, Int. J. Agric. Biol., 6, 816 (2004).
- 26. A. Ikem and N.O. Egiebor, J. Food Compos. Anal., 18, 771 (2005).
- 27. WHO/EU, WHO and EU Drinking Water Quality Guidelines for Heavy Metals and Threshold Values Leading to Crop Damage (1993).
- T.R. Mahalingam, S. Vijayalakshmi, R.K. Prabhu, A. Thiruvengadasami, A. Wilber, C.K. Mathews and K.R. Shanmugasundaram, *Biol. Trace Elem. Res.*, 57, 223 (1997).
- 29. O.A. Levander, Annu. Rev. Nutr., 7, 227 (1987).
- S.O. Man, in eds.: E.S. Maurice, A.O. James S.L. Moshe and Febiger, (Water, Electrolyte and Acid-Base Balance. In: Modern Nutrition in Health and Disease, edn 8, Part I, pp. 127 (1994).
- D.N. Baron, A Short Textbook of Chemical Pathology, ELBS Edition, (Holder and Stoughton Educational, Britain, pp 138 (1980).
- R. Smith, in eds.: J.S. Garrow and W.P.T. James, Human Nutritional and Dietetics, Harcourt Brace and Co. Ltd., London, edn 9, pp. 162 (1998).
- National Research Council, Nutrition Requirements of Beef Cattle, National Academics Press, Washington DC (1996).
- S. Shimbo, Z.-W. Zhang, T. Watanabe, H. Nakatsuka, N. Matsuda-Inoguchi, K. Higashikawa and M. Ikeda, *Sci. Total Environ.*, 281, 165 (2001).

- Joint FAO/WHO Expert Committee on Food Additives (JECFA), Evaluation of Certain Food Additives and Contaminants: Summary and Conclusion on the Seventy-third Meeting, Geneva (2010).
- National Research Council, Food and Nutrition Board, Recommended Dietary Allowances, National Academy Press, Washington, DC, edn 10 (1989).
- WHO, Evaluation of Certain Foods Additives and Contaminants, Forty-First Report of the Joint FAO/WHO Expert Committee on Foods Additives (JECFA), Geneva: WHO Technical Report Series 837, pp. 53 (1993).
- WHO, Trace Elements in Human Nutrition and Human Health, Geneva (1996).
- 39. O. Demirezen and K. Uruc, Meat Sci., 74, 255 (2006).
- P. Zhuang, M.B. McBride, H. Xia, N. Li and Z. Li, *Sci. Total Environ.*, 407, 1551 (2009).
- L.R. Hough, N. Breward, S.D. Young, N.M.J. Crout, A.M. Tye, A.M. Moir and I. Thornton, *Environ. Health Perspect.*, **112**, 215 (2003).
- 42. J.N. Ihedioha and C.O.B. Okoye, *Ecotoxicol. Environ. Saf.*, **93**, 101 (2013).