



Technical Note

A proton induced X-ray emission (PIXE) analysis of concentration of major/trace and toxic elements in broiler gizzard and flesh of Tehsil Gujar Khan area in Pakistan



Khawar Nadeem ^a, Javaid Hussain ^b, Noaman Ul Haq ^c, A. Ul Haq ^{a,*}, Waheed Akram ^b, Ishaq Ahmad ^b

^a Department of Basic Sciences, Riphah International University Islamabad, Pakistan

^b NPU-NCP Joint International Research Center on Advanced Nanomaterial's and Defects Engineering, National Center for Physics, Islamabad, Pakistan

^c Comsats University Islamabad, Lahore Campus, Pakistan

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ABSTRACT

Ten gizzard and three flesh samples of the broiler were collected from different locations in Tehsil Gujar Khan District Rawalpindi, Pakistan. The samples were dried, crushed and ground. Pellets were prepared by pressing the powder of the samples and that of the Bovine liver 1577c reference material obtained from NIST, USA. Proton induced X-ray emission (PIXE) installed at National Center for Physics, Islamabad, Pakistan has been used as a reliable and improved technique to determine concentration of various major/trace and toxic elements e.g. S, Cl, K, Ca, Fe, Cu, Mn, Co, Zn, Ti, Cd, Ga, Cr, V and Ni, in the Gizzard and Flesh samples of the broiler. The concentrations of all the detected elements in the samples are statistically significant. The certified and measured values of the elements in the reference material were in agreement with each other within a deviation of 7%. S, Cl, K and Ca are within tolerable limits and are good for human consumption. Ti, V, Cr, Mn, Fe, Co, Ni, Cu and Zn were more than the acceptable limits of World Health Organization, WHO whereas Ga, As, Sn, Sb and Pb are not detected in most of the samples. © 2019 Korean Nuclear Society, Published by Elsevier Korea LLC. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Poultry meat holds an important position in human daily diet due to its unmatched qualities that contains essential amino acids, essential elements and vitamins. Poultry meat can be rapidly cooked and has frequent sensorial and nutritive qualities like poorest in calories and richest in proteins [1] such as high in low fat proteins (than other red meat which support for muscle growth and healthy body weight); high in an amino acid (which increases serotonin level in the brain and act as natural anti-depressant); rich in phosphorus and selenium, (good for teeth, bones, kidney and liver); contains niacin (protects against cancer and genetic (DNA) damage); controls homocysteine levels (which causes cardiovascular diseases); and good for osteoporosis or arthritis in old age.

In 1964, Pakistan International Airlines (PIA) became the first commercial poultry producer and started breeding of layers and broilers whereas poultry feed was produced by lever brothers [2].

According to the dietary reference intake, DRI, one should take 0.8 g of proteins per kg of body weight [3]. 66% of Pakistan population is facing lack or deficiency of protein in their food material [4]. Here the main source of protein in human diet is animal meat. It is worth mentioning that increase in the population has also played a role in the decrease of proteins contents in the daily diet [2]. Chicken meat in Pakistan is easily available at low prices so it is highly preferred and contributes approximately nineteen percent of the overall meat demand and has capacity to produce meat further [2]. The poultry meat industry has made good progress over the years. According to the economic survey of Pakistan (2017–18), poultry meat production in Pakistan [5] was 11.70 million tons in 2015–16 which increased to 13.891 million tons meat during 2017–18. The poultry meat growth has increased by 13.2% in the year 2017–18 as compared to 2016–17.

Poultry feed plays an important role in the growth of poultry birds. For proper growth a poultry birds should be given balanced diet. Poultry feed consists of several ingredients and healthy feed helps farmer to protect the birds from different diseases and help in the weight gain [6,7]. However, some preventive medicines [8] e.g.

* Corresponding author.

E-mail address: anwar.haq@riphah.edu.pk (A. Ul Haq).

Monensin, Kanamycin, Gentamicin, Oxytetracycline, Oxolonic Acid, Norfloxacin, Furazolidone, Flumequine, Erythromycin, Danofloxacin, Trimethoprim, Chloramphenicol, Enrofloxacin etc. are given to the birds to save them from seasonal diseases and achieve good growth rate and weight gain. In case of anemia and weight loss, birds are given high doses of iron and copper salts [9]. It is a common perception that contamination of poultry feed with various environmental pollutants particularly heavy metals, have been used carelessly to avoid business losses. Surtipanti et al. [10], determined the toxic metals contents in 63 samples (from Jakarta local market, Indonesia) of chicken meat, intestine and liver using neutron activation analysis, (NAA) and atomic absorption spectroscopy, AAS. They found that the concentrations of Cu, Cr, Co were higher in chicken liver and intestine and concentrations of Cr, Co, Hg, Cu, Sb, Zn and Se were in the tolerable range in the chicken meat. Kurnaz and Filazi [11] randomly collected 20 samples from 4 different cities including industrial and rural areas of Turkey and determined the concentration of toxic metals Pb, Fe, Cu and Cd in the muscle and liver samples of broiler by AAS. They found that samples from industrial areas possess significantly higher metals levels than from rural areas but were below the standard limits established by Turkish and EU legislation.

Akan et al. [12] reported the concentration of heavy metals in different liver and kidney of Chicken, Cow, Sheep and Goat in the samples from the Kasuwan Shanu market in Maiduguri Metropolis, Bomo State, Nigeria by AAS. They concluded that the concentration of metals is statistically significant. In general concentrations of metals were within tolerable limit except that of Cr and Pb which was higher than the standards of Australia-New Zealand Food Authority, ANZFA (2001). Zhuang et al. [13] reported heavy metals concentrations in five tissues of 48 chickens. One group of chickens was fed with high metal feed while other group of samples was fed with uncontaminated feed. Concentration of Cd, Cu, Zn and Pb were determined by AAS in the kidney, liver, muscle, blood and feathers. They concluded that the concentration of Pb, Cd, Zn and Cu were relatively high in experimental group.

Ismail and Abolghait [14] estimated the concentration of Cd and Pb by AAS in chicken giblets from Ismailia city of Egypt. They randomly collected sixty samples of chicken heart, liver, and gizzards. They reported significantly high concentration of Cd and Pb. Szekeley et al. [15], examined the heavy metals in liver sample of beef, chicken and pork by X-ray fluorescence spectrometry, XRF. The samples were collected from the same location of Budapest (Hungary), over three consecutive weeks. Results of first sampling showed greater amounts of cadmium and lead content with respect to standard value (0.5 mg/kg) (1881/2006 EC Regulation). Result of second sampling is also relevant to the first sampling excluding manganese contents which were five times higher in second sampling. In third sampling the cadmium content is higher than the standard limit and lead content were not detected.

Hussain et al. [16], determined the heavy metals Cd, Pb and Zn in the liver of chickens collected from local market of Al-Basra, Iraq. Five frozen Liver samples of chicken each from nine different brands were analyzed by AAS for concentrations of heavy metals. The amount of Cd and Pb concentration was high in the local chicken livers. The results showed the lead concentration in samples was lower than the tolerable limit. Muhammad et al. [17] studied concentrations of the heavy metals (Fe, Cr, Pb, Cd, Fe and Cr) in the breast, gizzard, liver and kidney of two species i.e. Hacco and Isa Brown of adult layers chickens. Twelve samples from each group were collected in 8 weeks. Inductively Coupled Plasma-Optical Emission Spectrometry, ICP-OED was used. They conclude that the odd layer strains of poultry are within safe limit for dietary intake and safe for consumption.

Rohma Imran et al. [18] estimated the metal concentration in

the chicken heart (11 samples), kidney (6 samples) and 12 samples each of leg tissue, leg bone and wing bone taken from Kasur area by ICP. They concluded that the concentration in all samples were within the tolerable limits except Mn which exceeded the permissible limit set by WHO, only in wing bone and back bone while nickel was high in all chicken parts. Rehman et al. [4] reported heavy elements: Zn, Ag, Mn, Pb, Cu, Fe, Cd and Ni concentrations determined by AAS in different parts i.e. kidney, liver, gizzard, heart, and muscle of broiler chickens from district Bannu, Khyber Pakhtunkhwa, Pakistan area. Higher concentration of Cu, Fe and Zn were detected in domestic chickens as compared to the broiler. They concluded that the meat of broiler chicken contains investigated elements within the tolerable limits set for human diet. Mariam et al. [19] reported the concentration of some trace/macro minerals e.g. lead, arsenic, copper, cadmium, zinc, mercury sodium, potassium and calcium in liver, kidney and lean meat of beef, mutton and chicken meat local markets in Lahore by AAS. Arsenic, lead and mercury concentration in the samples were above tolerable limits which showed that the toxic metals pollution in the Lahore region. Rehman et al. [20] determined the heavy metals in tissue, litter and poultry feed of broiler and household layers by PIXE from district Faisalabad, Pakistan. The results showed high contamination of heavy metals in feed samples that reveal bio-accumulation in different tissues of broiler and also emission in litter which were harmful for human consumption. Khan et al. [21] collected thirty samples each from liver, breast and thigh muscle of broiler from different sales outlets of districts of Khyber Pakhtunkhwa (KPK), Pakistan and analyzed by using AAS. They concluded that level of Cd, Cr, Zn, Fe, Mn and Pb was high as compared to the permissible limits. Jaweria et al. [22] reported that concentrations of Al, Cd, Cr, Co, Fe, Ni and Zn by AAS in various tissues of chicken. They concluded that concentrations of these elements are lower than the permissible limits. Yasir [23] determined the major/minor minerals and toxic metals in 33 samples of the broiler liver, using Proton Induced X-ray Emission, PIXE technique. Concentration of all investigated elements was in the tolerable limits and concluded that liver of a broiler is good to eat. Demirezen and Urcin [24] reported that concentration of heavy metals in feed products for poultry birds is a severe health hazard for the human being as it is reflected in the bird's meat. Subhan et al. [25] determined the changes in biochemical parameters of blood and accumulation of cadmium in various tissue caused by various levels of dietary cadmium chloride (CdCl₂) in broiler chicks by AAS. They found that Cd concentration values were high in kidney rather than in liver and breast samples.

From above mentioned literature review it is established that any toxic material taken by birds in feed, medicine or environment may pollute their meat. The aim of this study was to determine the concentration of major/trace minerals and level of toxic elements in the flesh and gizzards of the broiler samples. Therefore, this study carried out to determine the levels of seventeen major or trace and toxic elements in the boiler gizzard and meat samples collected from Gujar Khan (Rawalpindi District), Pakistan by PIXE.

2. Materials and method

The broiler chicks were selected according to their ages between 34 and 39 days. All samples were collected randomly from different locations of Tehsil Gujar Khan, District Rawalpindi. This area is geographical located at the latitude: 33.2508° N; longitude: 73.2966° E and its altitude is 505 m. Sample collection (Table 1) was divided into three groups. Each group composed of four locations. From each location either gizzard or flesh sample was collected. The three gizzard samples and one flesh sample was collected in each group on dates specified in Table 1. The bird feed, in each group was

Table 1
Tehsil Gujar Khan, Rawalpindi locations from where samples were collected.

S.No	Group	Sample code	Collection area	Feed use	Broiler age	Collection date
1	1	JRG	Jabbar	Islamabad Feed	35	25-02-2017
2		GNG	Gujar Khan	Islamabad Feed	37	12-02-2017
3		MLG	Missa Kaswal	Islamabad Feed	34	03-03-2017
4		KNF	Kalyam Awan	Islamabad Feed	35	22-03-2017
5	2	DAG	Doutala	High Tech Feed	36	25-02-2017
6		SOG	Sukho	High Tech Feed	35	07-03-2017
7		KAG	Kountrila	High Tech Feed	36	29-01-2017
8		KNF	Kallar Syedan	High Tech Feed	39	27-02-2017
9	3	SAG	Sohawa	Kashmir Feed	37	19-03-2017
10		MAG	Mandra	Kashmir Feed	38	25-01-2017
11		NIG	Narali	Kashmir Feed	37	29-03-2017
12		QNF	Qazian	Kashmir Feed	38	16-03-2017

given from same feed manufacturer.

All the samples were thoroughly cleaned with distilled and de-ionized water and dried at 15–20 °C discontinuously in air for ten days. The inner protective layer of gizzard was not removed in gizzard samples. The samples were sliced into small pieces and dried in an oven at 110 °C for six hours until the constant weight was obtained. The samples were ground fine and sifted with a stainless steel drum sieve. Two pellets of diameter 0.8 cm and thickness 0.25 cm were made.

The state-of-the-art 5 MV Pelletron Tandem Accelerator Facility (National Electrostatic Corporation, USA) of National Center for Physics, Islamabad, Pakistan, was used for PIXE analysis. A 2.5 MeV proton beam with 2.0 mm² collimator, was irradiated on the samples placed in the vacuum chamber at 10⁻⁷ torr. The samples were placed at an angle of 90° with respect to the incident beam. Five samples can be mounted at the same time on the holders. The beam current was adjusted at about 2–3 nA and the integrated charge of the beam were monitored up to 2 μC. The characteristic X-rays emitted from the samples were detected by Sirius SD detector positioned at 45° with respect to the incident beam. A 40 μm thick Mylar absorber foil was placed in front of the detector in order to decrease the intensity of low energy X-rays originating from the matrix elements. The energy resolution of the detector was 129 eV at 5.9 keV. The solid angle of the detector was limited to

1531 × 10⁻⁴ stradian. The PIXE data of Standard SRM 1577c as shown in Fig. 1 was collected for different elements to make the H-value file. The H-value or energy dependent X-rays data is given in Table 2. The acquired experimental data were analyzed using the GUPIXWIN software [26].

The recommended dietary allowances are basically defined as average daily intake level that is sufficient to meet the nutrient requirement of about 97–98% healthy persons, whereas, adequate intake is a recommended daily intake value based on experimentally determined approximations or observed nutrient intake by a groups of healthy people that are assumed to be adequate. Levels of Recommended Daily Allowance, RDA/Adequate Intake, AI for major/minor and toxic elements [8], respectively (in each group) are shown in Tables 3–5.

3. Results and discussion

Four major minerals (sulphur, chlorine, potassium and calcium) are measured and presented in Table 3. The range of sulphur measured concentration in broiler gizzard samples is 61 ± 1.0 to 367 ± 0.3 mg/kg and in boiler flesh samples range is 32 ± 2.0 to 87 ± 0.6 mg/kg. The highest concentration level in broiler gizzard and flesh samples were observed at Gujar khan city and Kalyam Awan respectively. Similarly the lowest concentration level in

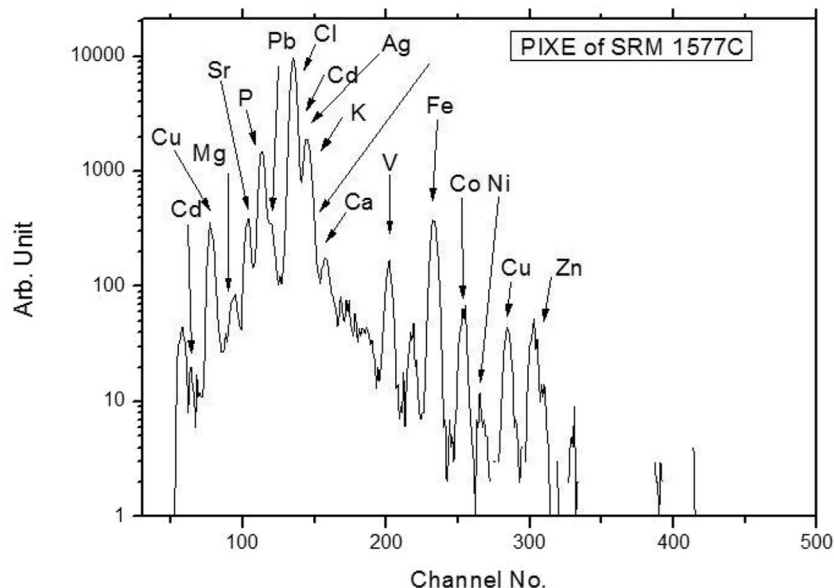


Fig. 1. PIXE analysis of SRM 1577c.

Table 2
H-value for NIST, SRM 1577c ($\mu\text{g/g}$).

Element	Standard value (given)	This work (measured)	% Deviation	H-Value
Ca	131 \pm 10	127 \pm 8	3	3.6
Cd	0.097 \pm 0.001	0.092 \pm 0.011	5.1	0.001531
Cl	2870 \pm 130	2904 \pm 260	1	0.023
Cr	0.053 \pm 0.014	0.055 \pm 0.015	3.7	2.5
Cu	275.2 \pm 4.6	261 \pm 44	5	0.11
Fe	197.94 \pm 0.65	187 \pm 10	5.5	0.021
K	10230 \pm 640	10740 \pm 882	4.9	0.0142
Mg	10.46 \pm 0.47	9.64 \pm 2.45	7	0.0058
Ni	0.045 \pm 0.009	0.049 \pm 0.037	8	0.11

Table 3
Major elements (Minerals; mg/kg) detected in the Broiler Gizzard and Flesh.

S.No	Area	Area code	Group 1: Islamabad Feed			
			S	Cl	K	Ca
1	Jabbar	JRG	187.2 \pm 3.9	614.3 \pm 1.4	3381.9 \pm 0.4	704.9 \pm 4.6
2	Gujar Khan	GNG	367.0 \pm 2.7	1172.1 \pm 1.0	5945.7 \pm 0.3	1414.0 \pm 3.5
3	MissaKaswal	MLG	127.0 \pm 4.6	382.1 \pm 1.7	2353.2 \pm 0.5	658.2 \pm 4.2
4	Kalyam Awan	KNF	87.6 \pm 6.2	117.4 \pm 3.6	5029.1 \pm 0.3	621.4 \pm 9.9
RDA*/AI + limit (mg/person/day)			900	3500	4700	2500
Group 2: High Tech Feed						
1	Doutala	DAG	263.9 \pm 3.2	898.2 \pm 1.1	5135.2 \pm 0.4	984.0 \pm 4.6
2	Sukho	SOG	222.7 \pm 3.6	830.3 \pm 1.1	5870.3 \pm 0.3	855.9 \pm 6.0
3	Kountrila	KAG	208.6 \pm 3.7	503.1 \pm 1.5	5155.9 \pm 0.3	1490.7 \pm 3.4
4	Kallar Syedan	KSF	32.2 \pm 20.3	69.0 \pm 5.8	7145.9 \pm 0.2	678.8 \pm 12.7
RDA*/AI + limit (mg/person/day)			900	3500	4700	2500
Group 3: Kashmir Feed						
1	Sohawa	SAG	323.0 \pm 3.0	1075.0 \pm 1.0	5788.3 \pm 0.4	109 N.D \pm 4.4
2	Mandra	MAG	356.2 \pm 2.9	1213.6 \pm 0.9	7187.0 \pm 0.3	1452.6 \pm 4.0
3	Narali	NIG	47.0 \pm 13.2	92.6 \pm 4.3	6495.7 \pm 0.2	602.7 \pm 12.8
4	Qazian	QNF	87.6 \pm 6.2	94.3 \pm 3.6	6731 \pm 0.3	648 \pm 9.9
RDA*/AI + limit (mg/person/day)			900	3500	4700	2500

*RDA: Recommended Dietary Allowance.

+AI: Adequate Intake.

Table 4
Minor elements, (Minerals; mg/kg) detected in the Broiler Gizzard and Flesh.

S.No	Area	Area Code	Group 1: Islamabad Feed			
			Ti	V	Cr	Mn
1	Jabbar	JRG	10.3 \pm 11.4	12.5 \pm 37.1	124.7 \pm 3.8	11.0 \pm 31.9
2	Gujar Khan	GNG	9.2 \pm 15.0	6.1 \pm 94.6	88.5 \pm 5.4	9.2 \pm 43.3
3	MissaKaswal	MLG	14.7 \pm 9.3	-ND-	26.8 \pm 11.1	7.1 \pm 32.9
4	Kalyam Awan	KNF	1 \pm 13.6	2.5 \pm 251.5	48.9 \pm 10.2	-ND-
RDA*/AI + limit (mg/person/day)				1.8	35	11
Group 2: High Tech Feed						
1	Doutala	DAG	6.6 \pm 13.5	5.4 \pm 96.6	51.4 \pm 7.9	5.2 \pm 62.7
2	Sukho	SOG	20.5 \pm 12.2	-ND-	49.6 \pm 7.9	12.9 \pm 24.5
3	Kountrila	KAG	19.6 \pm 12.5	3.6 \pm 136.9	48.4 \pm 8.3	16.7 \pm 20.2
4	Kallar Syedan	KSF	N.D.	1.7 \pm 55	91.8 \pm 9.0	18.4 \pm 44.2
RDA*/AI + limit (mg/person/day)				1.8	35	11
Group 3: Kashmir Feed						
1	Sohawa	SAG	2.0 \pm 14.0	6.4 \pm 86.3	117.7 \pm 4.4	14.4 \pm 26.6
2	Mandra	MAG	29.3 \pm 15.7	9.0 \pm 67.2	105.8 \pm 5.1	14.7 \pm 27.6
3	Narali	NIG	N.D.	3.8 \pm 163.7	100.5 \pm 5.8	4.3 \pm 114.9
4	Qazian	QNF	N.D.	6.0 \pm 251.5	101 \pm 10.2	16.8 \pm 51.5
RDA*/AI + limit (mg/person/day)				1.8	35	11

*RDA: Recommended Dietary Allowance.

+AI: Adequate Intake.

broiler gizzard and flesh samples was observed at Qazian and Kallar Syedan respectively. The RDA/AI limit for sulphur is 900mg/person/day. Thus concentration of sulphur is within tolerable limit for all

locations in both gizzard and flesh samples. Being part of four amino acids (cytosine, methionine, taurine and cysteine), sulphur performs a number of important functions, such as solidifying the

Table 5
Toxic elements (Minerals; mg/kg) detected in the Broiler Gizzard and Flesh.

S/N	Area	Area Code	Group 1: Islamabad Feed									
			Fe	Co	Ni	Cu	Zn	Ga	As	Sn	Sb	Pb
1	Jabbar	JRG	387.5 ± 6.2	2.7 ± 9.8	9.5 ± 2.2	64.6 ± 3.5	61.5 ± 6.5	1.9 ± 2.8	N.D	N.D	N.D	N.D
2	Gujar Khan	GNG	423.4 ± 6.4	5.6 ± 9.9	10.9 ± 2.8	92.3 ± 3.1	92.4 ± 9.1	N.D	N.D	N.D	N.D	N.D
3	MissaKaswal	MLG	154.0 ± 4.4	2.4 ± 6.3	2.2 ± 2.1	13.6 ± 2.2	40.3 ± 6.4	N.D	N.D	N.D	N.D	N.D
4	KalyamAwan	KNF	176.2 ± 6.1	2.0 ± 5.3	8.1 ± 2.5	5.2 ± 1.9	23.1 ± 4.0	N.D	N.D	N.D	N.D	N.D
RDA*/AI + limit(mg/person/day)			45	0.08	2.5	1.7	40	35	11	10	0.08	1
Group 2: High Tech Feed												
1	Daultala	DAG	292.7 ± 4.1	N.D	11.7 ± 2.7	23.1 ± 2.8	58.3 ± 7.7	N.D	N.D	N.D	N.D	N.D
2	Sukho	SOG	599.7 ± 6.1	N.D	40.6 ± 2.8	60.1 ± 2.7	78.4 ± 7.4	N.D	N.D	N.D	N.D	N.D
3	Kountrila	KAG	300.4 ± 5.5	2.4 ± 8.5	11.2 ± 3.1	11.9 ± 3.1	96.2 ± 3.5	N.D	N.D	N.D	N.D	N.D
4	KallarSyedan	KNF	261.1 ± 13.7	ND	15.6 ± 13	6.1 ± 15	22.6 ± 14.9	2.8 ± 16	N.D	N.D	N.D	N.D
RDA*/AI + limit(mg/person/day)			45	0.08	2.5	1.7	40	35	11	10	0.08	1
Group 3: Kashmir Feed												
1	Sohawa	SAG	422.3 ± 6.1	2.1 ± 10	17.2 ± 1.6	108 ± 2.7	78.4 ± 7.8	N.D	N.D	N.D	N.D	N.D
2	Mandra	MAG	706.4 ± 6.6	6.5 ± 13	17.3 ± 2.9	48.4 ± 3.7	133.4 ± 8.4	N.D	N.D	N.D	N.D	N.D
3	Qazian	QNG	345.4 ± 6.2	N.D	23.4 ± 5.3	32.5 ± 5.6	30.9 ± 6.4	N.D	N.D	N.D	N.D	N.D
4	NARALI	NIF	279.7 ± 5.9	N.D	19.0 ± 6.0	66 ± 4.1	42.9 ± 5.6	1.7 ± 7.1	N.D	N.D	N.D	N.D
RDA*/AI + limit(mg/person/day)			45	0.08	2.5	1.7	40	35	11	10	0.08	1

*RDA: Recommended Dietary Allowance.

+AI: Adequate Intake.

protein structure of skin, hair, nails and joint [8]. Fig. 2 Fig. 2 shows PIXE spectrum of one represented sample of (Fig. 2a)-Broiler flesh, collected from Kalyan Awan and (Fig. 2b)-Broiler gizzard sample, collected from Gujar Khan for different elements.

Chlorine belongs to Group 7 A of the Periodic Table. The elements of this group are called halogens or salt forming elements. In the earth's crust, natural abundance of Cl is 0.017%. The range of chlorine measured concentration in broiler gizzard samples is 94 ± 0.4 to 1213 ± 0.09 mg/kg and in boiler flesh samples range is 69 ± 0.6 to 117 ± 0.3 mg/kg. The highest concentration level in broiler gizzard and flesh samples were observed at Gujar Khan city and Kalyam Awan respectively. Similarly the lowest concentration level in broiler gizzard and flesh samples was observed at Qazian and Kallar Syedan respectively. The RDA/AI limit for chlorine is 3500 mg/person/day. The concentration of chlorine is within tolerable limit in both samples of all locations.

The range of potassium measured concentration in broiler gizzard samples is 2353 ± 0.05 to 7187 ± 0.03 mg/kg and in boiler flesh samples range is 5029 ± 0.3 to 7146 ± 0.02 mg/kg. The highest concentration level in broiler gizzard and flesh samples were observed at Mandra and Kallar Syedan, respectively. Similarly the lowest concentration level in broiler gizzard and flesh samples was observed at Missa and Kalyam Awan, respectively. The RDA/AI limit for potassium is 4700 mg/person/day. The concentration of potassium is within tolerable limit in both samples only at Jabbar and Missa Kaswal whereas in all other locations it is high.

Calcium is found in Group 2 A of the Periodic Table and elements of this group are called alkaline earth metals. In the earth's crust natural abundance of Ca is 3.64%. It is the 5th most bountiful constituent in the human body. The range of calcium measured concentration in broiler gizzard samples is 648 ± 1.2 to 1490 ± 0.3 mg/kg and in boiler flesh samples range is 603 ± 1.3 to 679 ± 1.2 mg/kg. The highest concentration level in broiler gizzard and flesh samples were observed at Kountrila and Kallar Syedan respectively. Similarly the lowest concentration level in broiler gizzard and flesh samples was observed at Qazian and Narali respectively. The RDA/AI limit for calcium is 2500mg/person/day. The concentration of calcium is within tolerable limit in both samples at all locations. Calcium is the most abundant element in the body with more than 99% of the calcium being present in the bones. It builds and

maintains teeth and bones. Calcium deficiency may results in osteomalacia i.e. softening of the bones [8]. Thus it is concluded that concentrations of Sulphur, Chlorine and calcium in the investigated samples is within tolerable limits in all 3 feed groups.

Four Trace minerals i.e. Titanium, Vanadium, Chromium and Manganese are measured and presented in Table 4. The range of titanium measured concentration in broiler gizzard samples is 6 ± 1.3 to 29 ± 2.9 mg/kg and in boiler flesh samples titanium is not detected at all locations. The highest concentration level in broiler gizzard samples was observed at Mandra. The lowest concentration level in broiler gizzard samples was observed at Daultala. Titanium is biocompatible.

The range of vanadium measured concentration in broiler gizzard samples is 3 ± 13.7 to 12.5 ± 3.7 mg/kg and in boiler flesh samples range is 1.7 ± 55.0 to 4 ± 16.3 mg/kg. The highest concentration level in broiler gizzard and flesh samples were observed at Jabbar and Narali respectively. Similarly the lowest concentration level in broiler gizzard and flesh samples was observed at Kountrila and Kallar Syedan respectively. The RDA/AI limit for vanadium is 1.8 mg/person/day. This result showed that the concentration of vanadium is high in all samples. Vanadium is not detected in sample of location Sukho.

Chromium primarily occurs into two forms trivalent and hexavalent. Trivalent Chromium Cr^{+3} are biological active and found in food stuff while on other hand hexavalent Chromium a toxic form Cr^{+6} is the result of industrial pollution. The range of chromium measured concentration in broiler gizzard samples is 27 ± 1.1 to 124 ± 0.4 mg/kg and in boiler flesh samples range is 49 ± 1.0 to 100 ± 0.6 mg/kg. The highest concentration level in broiler gizzard and flesh samples were observed at Jabbar and Narali respectively. Similarly the lowest concentration level in broiler gizzard and flesh samples was observed at Missa and Kalyam Awan respectively. Chromium is an essential element which helps the body to use sugar, protein and fat at the same time it possesses carcinogenic properties as well. Excess amount of Cr may cause adverse health effects [24]. The RDA/AI limit for chromium is 3.5 mg/person/day. This result showed that the concentration of chromium is high in samples of almost all locations except at Missa Kaswal.

The range of manganese measured concentration in broiler gizzard samples is 5 ± 6.2 to 17 ± 2.0 mg/kg and in boiler flesh

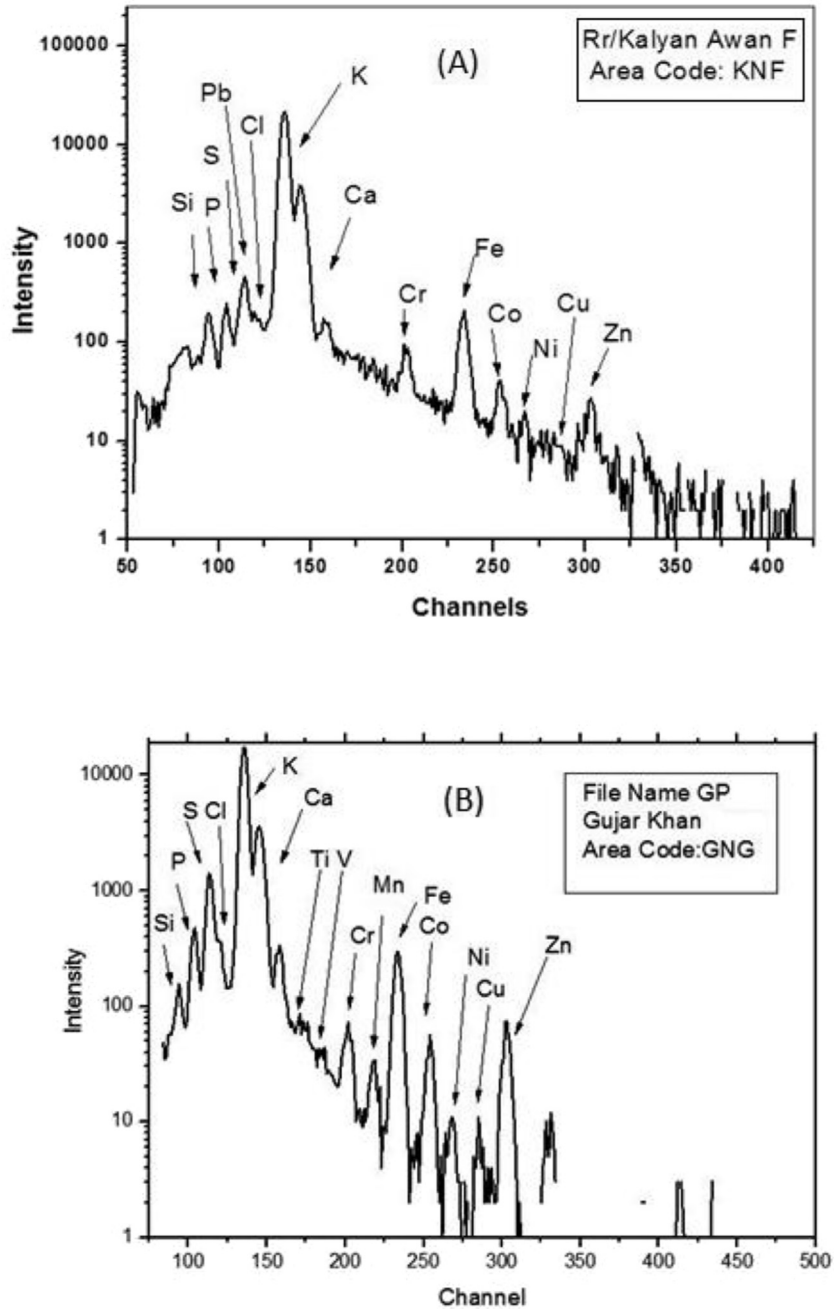


Fig. 2. PIXE spectrum of (a)- Kalyan Awan (KNF) – flesh sample and (b)- Gugar Khan (GNG) – gizzard sample.

samples range is 4 ± 11.5 to 18 ± 4.4 mg/kg. The highest concentration level in broiler gizzard and flesh samples were observed at Kountrila and Kallar Syedan respectively. Similarly the lowest concentration level in broiler gizzard and flesh samples was observed at Daultala and Narali respectively. The RDA/AI limit for manganese is 11mg/person/day. This result showed that the concentration of manganese is low in group I samples whereas high in rest of the samples. Thus it is concluded that concentrations titanium is within tolerable limits whereas high in vanadium, chromium and manganese all three feed groups.

It has been observed that minor (trace) elements have high error values. This may be due to inhomogeneous distribution of these elements, effect of matrix and efficiency of the measuring instruments. This may be improved if latest values of stopping power

are used but Gupix Software does not permit this. Another way to improve is to improve sample preparation techniques.

Ten Toxic elements i.e. Iron; Cobalt, Nickel, Copper, Zinc, Gallium, Arsenic, Tin, Antimony and Lead are measured and presented in Table 5. The concentration of Arsenic, Tin, Antimony and Lead are not detected in any sample or any location. The concentration of Gallium is detected only in 3 out of 12 samples.

The range of iron measured concentration in broiler gizzard samples is 154 ± 4.0 to 706 ± 2.0 mg/kg and in boiler flesh samples range is 176 ± 4.0 to 279 ± 3.0 mg/kg. The highest concentration level in broiler gizzard and flesh samples were observed at Mandra and Narali respectively. Similarly the lowest concentration level in broiler gizzard and flesh samples was observed at Missa and Kalyam Awan respectively. Rahman et al. [22] find very high

concentration of iron in muscle (2030.0 ± 30.0 mg/kg). Iron deficiency is the most common nutrient deficiency. This may be due to an inadequate dietary intake, increased iron requirement, diminished iron absorption or utilization, blood loss, or a combination of these factors. Iron deficiency can lead to anaemia (last stage of iron deficiency). The RDA/AI limit for iron is 45 mg/person/day. The concentration of iron is high in both samples of all locations.

The range of cobalt measured concentration in broiler gizzard samples is 2.0 ± 171.0 to 6.0 ± 101.0 mg/kg and concentration of cobalt is detected only in one boiler flesh samples i.e. 2.0 ± 176 mg/kg in Kalyam Awan location. The highest concentration level in broiler gizzard was observed at Mandra. Similarly the lowest concentration level in broiler gizzard was observed at Kountrila and Kallar Syedan respectively. The RDA/AI limit for cobalt is 0.08 mg/person/day. The concentration of cobalt is high in both samples of all locations except at five locations where it is not detected.

The range of nickel measured concentration in broiler gizzard samples is 2.0 ± 15.7 to 23.0 ± 38.0 mg/kg and in boiler flesh samples range is 8.0 ± 39.0 to 19.0 ± 20.0 mg/kg. The highest concentration level in broiler gizzard and flesh samples were observed at Qazian and Narali respectively. Similarly the lowest concentration level in broiler gizzard and flesh samples was observed at Missa Kaswal and Kalyam Awan respectively. The RDA/AI limit for nickel is 2.5 mg/person/day. The concentration of Nickel is high in both samples of all locations.

The range of copper measured concentration in broiler gizzard samples is 6.0 ± 34.0 to 108.0 ± 5.0 mg/kg and in boiler flesh samples range is 5.0 ± 57.0 to 6.0 ± 80.0 mg/kg. The highest concentration level in broiler gizzard and flesh samples were observed at Sohawa and Narali respectively. Similarly the lowest concentration level in broiler gizzard and flesh samples was observed at Sukho and Kalyam awan respectively. Copper deficiency is characterized by anemia, fatigue, elevated cholesterol levels and poor wound healing. Excessive copper levels have been linked to schizophrenia, learning disabilities, premenstrual syndrome, and anxiety. The permissible limit for copper concentration is 20 mg/100 g (ANZFA) [21]. The RDA/AI limit for copper is 1.7 mg/person/day. The concentration of copper is high in both samples of all locations.

The range of zinc measured concentration in broiler gizzard samples is 31.0 ± 33.1 to 133.0 ± 6.0 mg/kg and in boiler flesh samples range is 22.0 ± 37.0 to 43.0 ± 12.0 mg/kg. The highest concentration level in broiler gizzard and flesh samples were observed at Mandra and Narali respectively. Similarly the lowest concentration level in broiler gizzard and flesh samples was observed at Qazian and Kallar Syedan respectively. The RDA/AI limit for zinc is 40 mg/person/day. Zinc being an essential element is involved in protein synthesis and also a part of many metallo enzymes. The concentration of zinc is high in both samples of all locations.

4. Conclusion

PIXE was used to determine concentration of major/trace and toxic elements viz S (32.2 ± 20.3 to 367.0 ± 2.7), K (69.0 ± 5.8 to 1213.6 ± 0.9), Ca (2353.2 ± 0.5 to 7187.0 ± 0.3), Cl (602.7 ± 12.8 to 1490.7 ± 3.4), Ti (max. 20.0 ± 14.0), V (max. 12.5 ± 37.1), Cr (26.8 ± 11.1 to 124.7 ± 3.8), Mn (max. 18.4 ± 44.2), Fe (154.0 ± 4.4 to 706.4 ± 6.6), Co (max. 6.6 ± 13.0), Ni (2.2 ± 2.1 to 40.6 ± 2.8), Cu (5.2 ± 1.9 to 92.3 ± 3.1), Zn (22.6 ± 14.6 to 133.4 ± 8.4), Ga (max. 2.8 ± 16). The elements such as As, Sn, Sb, and Pb were not detected in the broiler gizzard and flesh samples collected from various locations from District Gujjar Khan, District Rawalpindi, Pakistan. The standard sample of bovine liver No. 1577c, obtained from NIST, USA was used. The certified and measured values of the elements in the

reference material were in agreement with each other within a deviation of 7%. The concentrations of all the elements in all samples were statistically significant. S, Cl, K and Ca were within tolerable limits and good for human consumption. Ti, V, Cr, Mn, Fe, Co, Ni, Cu and Zn were more than the acceptable limits of WHO whereas Ga, As, Sn, Sb and Pb are not detected in most of the samples.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.net.2019.06.005>.

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