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Original Research

Composition of Chemical Elements in Edible Offal and Muscle of Semi- extensively Reared Indigenous Doom Pig Breed of Northeast India and its Correlation with Feed and Environment

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Abstract

With the increase in anthropogenic activities and other long-range transport of pollutants, there is a high probability of accumulation of heavy metals in foods consumed by pigs which in turn may amass in edible offals. Therefore, the present study was undertaken to evaluate sixteen chemical (heavy and non-heavy) elements in edible offal and *Longissimus dorsi* muscle of indigenous Doom pig breed of India. Additionally, the values for highly toxic elements were compared with maximum residue limits (MRLs) stated by the regulatory authorities. The elements namely- Sodium (Na), Magnesium (Mg), Potassium (K), Calcium (Ca), Manganese (Mn), Iron (Fe), Zinc (Zn), Selenium (Se), Copper (Cu), Cobalt (Co), Chromium (Cr), Nickel (Ni), Arsenic (As), Cadmium (Cd), Mercury (Hg) and Lead (Pb) were determined in the tissues as well as in feed, drinking water and soil by Inductively Coupled Plasma-Optical Emission Spectrometry. Among the elements determined, the non-heavy metals (K, Na, Mg and Ca) were found to be the highest both in tissues (edible offal and muscle) and feed, drinking water and soil followed by Fe, Zn and Mn which are essential heavy metals. Ni was found to exceed the European Food Safety Agency allowed limits. Spearman correlation test shows significantly ($p<0.0001$) positive relationships between the element of tissues and feed, drinking water and soil. The work underscores the elemental analysis on hitherto understudied consumable edible offal for value addition to the food industry.

KEYWORDS
Doom pig, Edible offal, ICP-OES, Muscle, Non-heavy, Heavy elements.

INTRODUCTION

In Northeast India, pig holders practice low input pig rearing system (Das *et al*., 2005), where feed is mainly consists of local vegetations, remnants of crop and kitchen waste. This type of rearing and feeding system causes uncertainty about availability of nutrients, especially minerals. Doom pig breed is distributed in western region of Assam. This breed is known to have good meat quality, taste and palatability and its meat and meat by-products have high demand among the consumers. They are sold at higher prices than other pig breeds.

Offal of pig is considered as edible and is recommended by nutritionist because they contain essential amino acids, vitamins, and minerals (Kicinska *et al*., 2019). Even though offal is recommended for human diet, there is a high probability of accumulation of heavy metals in animal organs which has been a great concern of discussion in recent years. Toxic elements in muscles are lower than those found in offal products (Lopez-Alsonso *et al*., 2007). Studies suggest that even in low amount, the toxic elements (Pb, Cd, Hg and Al) are known to cause many disorders to humans (Dlugaszek, 2019). They are also known to accumulate in the food chain (Nikolic *et al*., 2017).

Dlugaszek (2019) pointed out that, chemical elements cannot be synthesized in animal body and can only be obtained through feed and drinking water. Animal feed is regarded as one of the most important factors that influence the accumulation of elements in the tissues (Kicinska *et al*., 2019). Previous studies on the mineral status of feed and in the blood serum of exotic (Hampshire and Large White Yorkshire) breeds and indigenous pigs of Mizoram, India, reported high mineral content of Ca, P and Na in indigenous than exotic pigs (Kumaresan *et al*., 2009).

The present study determines the concentration of sixteen elements in edible offal (heart, kidney, liver, small intestine, large intestine, and spleen) and muscle (*Longissimus dorsi*) of Doom pig breed (accession no. INDIA_PIG_0200_DOOM_09006 of Northeast India, NBAGR, 2008), and its correlation with the feed and environmental samples (drinking water and soil i.e. 0 cm-depth).

MATERIALS AND METHODS

Sample collection

The study was approved by the Institutional Animal Ethics Committee, Bodoland University, Assam, India (Vide No. IAEC/BIOTECH/2019/3). Samples were collected from five semi-extensive indigenous pig farms of Kokrajhar district, Assam, India. The pigs are reared under semi-extensive (local feed i.e. non

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specified diet, free-roaming within the paddocks) system. Local feed mainly included local vegetations, remnants of crop and kitchen waste. Ten months old pigs (n=12) were slaughtered in a commercial abattoir, with live weights of around 48-51 kg. Six edible offal and one muscle samples from each of the 12 pigs were collected namely: heart (41.66±1.45 g), liver (900.33±63.80 g), kidney (201±1.52 g), spleen (98.66±0.88 g), small intestine (562.66±31.52 g), large intestine (1696±54.30 g) and *Longissimus dorsi* muscle from loin region (169.23±9.4 g). The samples were conventionally chilled for 24 h in a chiller at 4˚C and were homogenized using a mechanical grinder. Drinking water and soil were collected from the farm where the pigs were reared. All the samples were stored at -20˚C in polyethylene bags until further analysis.

Digestion of samples and analysis

The samples were digested using wet digestion procedures as reported by ASEAN Manual of Food Analysis (2011). 1.5 g of the oven dried sample was digested in 8 mL of concentrated HNO_3 and 2 mL of $HClO_4$ overnight to predigest the sample. Next day, the samples were kept in oven for 5 hrs at 100˚C. They were cooled down and checked for clear/transparent samples. After analyzing the samples, they were diluted up to 100 mL with deionized water. The solution is filtered with Whatman filter paper No. 541 and analyzed.

The analysis of the sixteen elements (Na, Mg, K, Ca, Mn, Co, Se, Pb, As, Cr, Cu, Fe, Ni, Zn, Hg and Cd) was performed by ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry), Model No. 7600, Thermo Fisher Scientific.

Statistical Analysis

The mentioned data were statistically analyzed using SPSS, Version 26.0. Partial Least squares discriminant analysis (PLS-DA) was applied to differentiate between the investigated samples according to element data. Spearman correlation test was applied to determine the correlation between investigated tissues and feed and environmental samples (soil and drinking water). P-values <0.05 were considered statistically significant.

RESULTS

Non-heavy metals in edible offal

The non-heavy metals analyzed in the study (Na, Mg, K, and Ca) is presented in Table 1. Na (122.843 mg/kg) was found to be the highest in spleen followed by kidney (97.196 mg/kg) while lowest was found in *Longissimus dorsi* (13.640 mg/kg). While that of K was found the highest in *Longissimus dorsi* (210.880 mg/kg), followed by liver (101.458 mg/kg) and the lowest in small intestine (32.150 mg/kg).

Similar to K, Mg was found the highest in *Longissimus dorsi* (14.063 mg/kg), followed by liver (5.230 mg/kg) and the lowest was reported in large intestine (2.424 mg/kg). Ca content was found to be the highest in liver (7.813 mg/kg), followed by small intestine (6.021 mg/kg) whereas that of kidney and large intestine were quite similar (4.140 and 4.980 mg/kg respectively) and the lowest was detected in heart (2.243 mg/kg). It is seen that both K and Mg were the highest in *Longissimus dorsi*.

Heavy metals in edible offal

Among the heavy metals, the non-toxic elements that were analyzed (Mn, Zn, Fe, Se, Cu and Co) are depicted in Table 1. Mn was found the highest in small intestine (15.576 mg/kg) and the lowest was in *Longissimus dorsi* (0.197 mg/kg). Fe and Zn were found the highest in liver (11.706 mg/kg and 1.879 mg/kg respectively) while the lowest was in *Longissimus dorsi* (0.769 mg/kg) and that of Zn was seen in heart (0.326 mg/kg). Se, Cu and Co was the lowest among the analyzed non-toxic elements ranging from 0.608 to 0.005 mg/kg.

The other heavy metals that are analyzed are regarded as potentially toxic elements (Cr, Ni, As, Cd, Hg and Pb) depicted in Table 2. Considering the effects of these toxic elements on human health, it is necessary to monitor the levels of these elements

Table 1. Concentration of non- heavy and heavy metals in edible offal and muscle of Doom pig (mg/kg).

Parameters	Non-heavy metals				Heavy metals (Non-toxic)					
	Na	Mg	K	Ca	Mn	Zn	Fe	Se	Cu	Co
Liver	77.578	5.23	101.458	7.813	0.217	1.879	11.706	0.013	0.157	0.006
Kidney	97.196	3.031	78.564	4.14	0.726	1.701	7.354	0.056	0.608	0.008
Heart	31.205	2.766	52.76	2.243	0.593	0.326	2.551	0.01	0.09	0.084
Small Intestine	29.882	3.716	32.15	6.021	15.576	1.727	2.635	0.012	0.047	0.018
Large Intestine	32.233	2.424	34.209	4.98	2.882	0.722	0.89	0.011	0.009	0.054
Spleen	122.843	3.275	60.203	5.49	0.421	0.737	9.594	0.017	0.036	0.005
Longissimus dorsi	13.64	14.063	210.88	4.722	0.197	1.626	0.769	0.005	0.007	0.004

Table 2. Concentration of potentially toxic heavy metals in edible offal and muscle of Doom pig (mg/kg).

Parameters	Potentially toxic heavy metals					
	Cr	Ni	As	Cd	Hg	Pb
Liver	0.032	0.063	0.012	0.012	0.006	0.14
Kidney	0.006	0.022	0.005	0	0.004	0.107
Heart	0.01	0.027	0	0	0.004	0.114
Small Intestine	0.035	0.048	0	0	0.004	0.029
Large Intestine	0.031	0.055	0	0.015	0.003	0.035
Spleen	0.037	0.039	0.005	0.019	0.004	0.015
Longissimus dorsi	0.007	0.008	0.004	0.004	n.d.	0.06

and analyze with their maximum residue limits (MRL's). The mean values of Cr ranged from 0.037 mg/kg in spleen to 0.006 mg/kg in heart, while that of *Longissimus dorsi* showed 0.004 mg/kg. The obtained results were below the allowed range provided by the WHO of 0.1 mg/kg. Ni concentrations ranged from 0.008 to 0.063 mg/kg. The daily allowed intake for Ni is 0.0028 mg/kg and 0.005 mg/kg body weight for adults according to European Food Safety Agency (2015) and the data from this study in all the samples was found to exceed the allowed range.

The maximum limit proposed for As by the China's National standards (GB2762-2012) is 0.5 mg/kg. The arsenic content determined in the investigated samples (0.012 – 0.004 mg/kg) did not exceed the given limited allowance. The highest Cd levels was found in spleen (0.019 mg/kg), followed by liver (0.012 mg/kg), while that of *Longissimus dorsi* was found to be lowest (0.004 mg/kg).

Hg was found highest in liver (0.006 mg/kg), followed by heart (0.004 mg/kg) and kidney (0.004 mg/kg). According to USDA Foreign Agricultural Service (2006), the maximum limit allowance of Hg is 0.05 mg/kg and it can be concluded that the studied samples did not exceed the limit. The Pb content in all the edible offal ranged from 0.140 to 0.015 mg/kg, while that of *Longissimus dorsi* muscle was found to be 0.060 mg/kg. According to FAO and WHO, the maximum daily allowance intake of Pb is 0.214 mg/kg for an average adult (Gu *et al.*, 2015) and did not exceed the limit allowance.

Partial Least squares discriminant analysis (PLS-DA) was applied to the investigated samples according to element data. The PLS-DA loading plot shown in Fig 1, shows that kidney, liver and spleen were easily discriminated from rest of the edible offal.

Non-heavy and heavy metals in environmental samples

The concentration of non-heavy and heavy elements in environmental samples (drinking water, 0 cm-depth soil) and feed are provided in Table 3. K (23.439 mg/kg) was reported to be the highest in feed, followed by Ca (21.508 mg/kg). In soil Mg content was found the highest with a mean of 15.215 mg/kg. Among essential heavy metals, Fe (60.650 mg/kg) was found to be the highest in soil.

Fig. 2, showing all the investigated elements including both non-heavy and toxic heavy metals (also depicted in Table 4) of the feed, drinking water and 0 cm-depth soil were clearly separated from each other using PLS-DA plots. The Hg was in the middle as it was not found in any of the determined parameters.

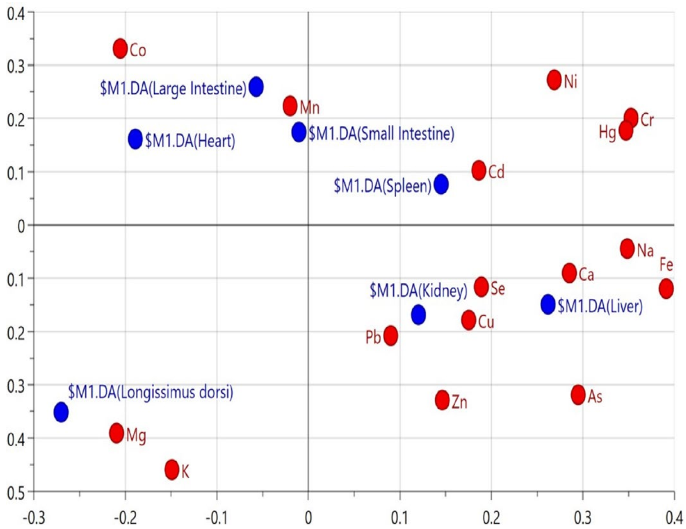

Fig. 1. PLS-DA score (R^2X =1, R^2Y =1 and Q^2 = 1) and loading plots of the sixteen chemical elements of the edible offal and muscle of indigenous Doom pig. Red dots represent the investigated elements and blue dots represent liver, kidney, spleen, large intestine, small intestine, heart and *Longissimus dorsi*.

Correlation between edible offal and muscle with feed and environmental samples

Correlation between the edible offal and environmental samples (drinking water, 0 cm-depth soil) and feed are given in Table 5. The relationship between the sixteen element profiles of tissues (edible offal and muscle) and feed and environmental samples are analyzed based on Spearman's correlation coefficient (r). The correlation seen between the edible offal and environmental and feed was positively significant (p<0.0001).

The liver, kidney, heart, small intestine, large intestine, spleen

Table 3. Concentration of non-heavy and heavy metals in drinking water, feed and 0 cm-depth soil (mg/kg).

Parameters	Non-heavy metals				Heavy metals (non-toxic)					
	Na	Mg	K	Ca	Mn	Zn	Fe	Se	Cu	Co
Drinking water	10.306	0.835	8.895	4.18	2.38	0.012	3.33	0.009	0.032	0.006
Feed	11.177	8.601	23.439	21.508	1.907	0.559	2.001	0	0.014	0.009
0 cm-depth soil	4.773	15.215	18.467	4.717	9.672	0.858	60.65	0.002	0.152	0.812

Table 4. Concentration of potentially toxic heavy metals in drinking water, feed and 0 cm-depth soil (mg/kg).

Parameters	Potentially toxic heavy metals					
	Cr	Ni	As	Cd	Hg	Pb
Drinking water	0.044	0.047	0.002	0.001	n.d.	0.048
Feed	0.005	0.015	0.01	0.006	n.d.	0.056
0 cm-depth soil	0.029	0.129	0.022	0.002	n.d.	0.018

Table 5. Correlation matrix of elemental composition between edible offal and muscle with feed, drinking water and soil.

Variables	Liver	Kidney	Heart	Small Intestine	Large Intestine	Spleen	*Longissimus dorsi*
Feed	0.9109 (<0.0001)	0.8698 (<0.0001)	0.9072 (<0.0001)	0.8851 (<0.0001)	0.8830 (<0.0001)	0.8477 (<0.0001)	0.9197 (<0.0001)
Drinking water	0.9110 (<0.0001)	0.9029 (<0.0001)	0.8904 (<0.0001)	0.9198 (<0.0001)	0.8765 (<0.0001)	0.8735 (<0.0001)	0.8866 (<0.0001)
0 cm-dept soil	0.8262 (<0.0001)	0.8359 (<0.0001)	0.8800 (<0.0001)	0.8697 (<0.0001)	0.8344 (<0.0001)	0.8374 (<0.0001)	0.8010 (<0.0003)

and *Longissimus dorsi* has the highest correlation with the drinking water. Small intestine had the strongest correlations with drinking water. Relatively higher r values were also found between edible offal and feed, ranging from 0.844 to 0.919.

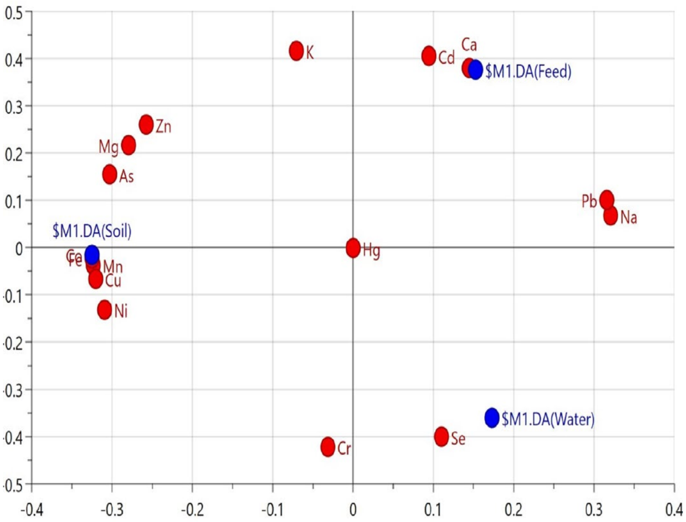

Fig 2. PLS-DA score (R^2X =1, R^2Y =1 and Q^2 = 1) and loading plots of the feed, drinking water and 0 cm depth soil. Red dots represent the investigated elements and blue dots represent the feed, drinking water and 0 cm-depth soil.

DISCUSSION

Mineral content in pork is affected by various factors like, breed of pig, feed type, management practices and environment. Kidney is an organ that helps to determine toxicity level, (Lentini *et al*., 2017). The non-heavy metals (K, Na, Mn, Mg and Ca) analyzed in this study was the highest in the kidney of Doom pig, followed by iron content which is an essential heavy metal. Among all the minerals, K and Na concentration were found to be the highest in *Longissimus dorsi*. Earlier study on muscles of Ghungroo pig too had high content of K and Na (Daimari *et al*., 2022) than other minerals. The present study revealed high content of Na and Fe in spleen. It may be due to the ability of spleen to removes old red blood cells, store blood, reusing element iron, and participating in body's immune system (Zhou *et al*., 2019).

Ca content was found to be the highest in liver, Nikolic (2017) too found high concentrations of Ca in liver. Mn (15.576 mg/kg) was found to be the maximum in small intestine which is more than *Longissimus dorsi* (0.197 mg/kg) and liver (0.217 mg/kg). Nikolic (2017) too showed similar results in liver, kidney, and muscle (*longissimus thoracis et lumborum*).

Fe and Zn are essential heavy metals. They are known to involve in many body functions (Dlugaszek *et al*., 2019). In the present study, both Fe and Zn were found to be the highest in liver, while that of *Longissimus dorsi* was found to be lowest. Earlier studies on cross breed pigs from Serbia revealed high content of Fe and Zn in liver and low in muscle (Nikolic *et al*., 2017). The mean of Se, Cu and Co detected in edible offal and muscle of semi-extensively reared Doom pig were very low in the present study.

Determination of potentially toxic elements (Cr, Ni, As, Cd, Hg and Pb) in edible offal and muscle of Doom pig is important as it affects the human health. Cr is regarded as an essential trace element (Tuzen, 2009). Cr is considered important part of diet as it is involved in insulin function and lipid metabolism (Bratakos *et al*., 2002), also its excess consumption is known to cause gastrointestinal bleeding and necrosis of the kidney (Ihedioha *et al*., 2014). Cr was found to be the highest in spleen and the lowest in kidney. Earlier study by Mi (2020) in liver, kidney and large intestine of Tibetan pigs found high Cr than MRL concentration (more than 0.1 mg/kg provided by WHO), while the obtained findings were below the allowed range.

Ni is considered an essential element for humans (Yipel *et al*., 2017). Ni content was detected in all tissues and was found to be above the allowed range in this study. Again, in the study conducted by Nikolic (2017) Ni was not detected in any of the tissues (muscle, liver, and kidney).

As (arsenic) is known as a carcinogen causing lung, liver, skin, and bladder cancers (Kapaj *et al*., 2006). Similar to results from this study, other studies too presented low concentration of As (Mi *et al*., 2020). Also, it was seen that arsenic content did not exceed the allowed limit. Accumulation of Cd can hamper kidney function, cause skeletal damage, and reproductive deficiencies (Commission of the European Communities, 2001). Cd is reported to be carcinogenic and teratogenic (Simoniello *et al*., 2011). Cd was detected in all offals and *Longissimus dorsi* but did not exceed the maximum tolerance level. The MRL provided by European Commission (2006) for Cd in liver and kidney for human consumption are 0.5 and 1 mg/kg respectively and the samples in the present study did not exceed the limit.

Hg was detected in all offals, but not in *Longissimus dorsi*. However, in none of the samples Hg exceeded the allowed limit. Hg is toxic to the developing fetus and has a high carcinogenic property (Ikem and Egilla, 2008).

The cognitive development and intellectual performances are affected by lead intake and known to increase blood pressure and cause cardiovascular diseases in adults (Commission of the European Communities, 2001). Mi (2020) reported that in liver and kidney of Tibetan pigs, Pb exceeded the maximum allowance limit. In the present study, lead was detected in all tissues but that was within the limit.

Feed is the main source of essential and non-essential elements. Non-essential elements like, Cd, Pb and Cr remain in small concentration in environment (Sager, 2007). Soil too, is known to have heavy metals (Kabata-Pendias, 2001). In semi-intensive production system, soil ingestion has a significant role in soil exposure as pigs are known to show rooting behavior even though there is enough food (Beattie and O'Connell, 2002).

Feed was found to contain the high number of elements, followed by soil and drinking water. Among heavy metals, Fe was found to be the highest in soil which was in accordance with the study of intensive and extensive pigs from Serbia where iron content of soil was the highest compared to concentrated feed and forage (Nikolic *et al*., 2017). Among the non-heavy metals, K was found to be the highest in this study. Concentrations of Se, Cu and Co were quite low in feed, soil and drinking water. Studies done by Nikolic (2017), concentrations of Se and Co were similar with the obtained results except for soil content, but Cu content was higher than finding from the current study.

Arsenic, lead, mercury and cadmium are regarded as the most toxic elements to be found in feed. The maximum tolerance level for arsenic, cadmium, mercury, and lead in animal feeds are 30 mg/kg, 10 and 0.5 mg/kg, 0.2 and 2 mg/kg and 10 and 30 mg/kg respectively (NRC, 2005; AAFCO, 2019). As, Cd, Hg and Pb content in feed were 0.01 mg/kg, 0.006 mg/kg, 0.00 mg/kg and 0.056 mg/kg. Therefore, the feed in this study was considered harmless with respect to the level of tolerance for consumption.

Positive correlation is seen between the tissues (i.e. edible offal and muscle) and environmental samples and feed. Conclusion can be drawn that accumulation of metals in meat and meat by-products are directly associated with environmental status of the living area which is in conformity with Kumaresan (2009). It is found that the tissues (liver, kidney, heart, small intestine, large intestine, spleen, and *Longissimus dorsi*) have the highest correlation with the drinking water. Out of all organs, small intestine had the strongest correlations with drinking water. This might be due to the biological function of the small intestine which can absorb ~90% of nutrients and water from the food (Liao *et al*., 2018).

CONCLUSION

All edible offal and muscle (*Longissimus dorsi*) have levels of Ni, which exceed the maximum tolerance level. The toxic elements in feed, drinking water and soil did not exceed and are within the safe ranges. Moreover, feed, soil, drinking water and pig's rooting behavior have a strong impact on the elemental composition of edible offal and muscle of Doom swine breed. The present study highlights the elemental status of the Doom pig's offal. The findings will be helpful for possible modification for feeding strategies by the rearers, so that good quality and safe products can be served to consumers.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest related.

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