Potential Human Health Risks of Tannery Waste-contaminated Poultry Feed

Mohammad Latiful Bari, Hasina Akhter Simol, Nusrat Khandoker, Rokeya Begum, Ummay Nasrin Sultana

Center for Advanced Research in Sciences, University of Dhaka, Bangladesh

Background. For over a decade, solid tannery waste has been converted into protein concentrate and used as a feed ingredient because of its cheap availability. However, as chromium sulfate is commonly used in the tanning process, the chromium (Cr) content of tanned skin-cut wastes (SCW) may enter the edible parts of poultry through feed. Therefore, there is a chance that Cr and other heavy metals may be present in the edible portion of poultry and consequently transfer to humans upon poultry consumption.

Objectives. In this study, skin-cut wastes (SCW)-based poultry feed and the edible parts of chicken fed with this feed were analyzed to understand the potential health risks of their use as poultry feed.

Methods. In the present study, the presence of heavy metal content in SCW, poultry feed, and edible portions of different kinds of chicken was determined using atomic absorption spectrophotometer methods and the associated health risk estimation was calculated by comparing the target hazard quotient (THQ) value and reference daily intake value.

Results. The results revealed the presence of Cr content ranging from 0.12-3.11 mg/kg and lead (Pb) content ranging from 8.06-22.0 mg/kg in SCW. In addition, Cr and Pb were present in the range of 0.27-0.98 mg/kg and 10.27-10.36 mg/kg, respectively, in poultry feed. However, no cadmium (Cd) was found in SCW, but the presence of Cd ranged from 0.03-0.05 mg/kg in feed. When contaminated poultry feed was fed to live poultry, the presence of Cr, Pb, and Cd was observed in the edible portions (i.e., skin, liver, gizzard, and meat). Irrespective of the edible parts and chicken type, Cr values ranged from 0.1-2.440 mg/kg; Pb values ranged from 0.257-1.750 mg/kg; and Cd values ranged from below detection limit (BDL) to 0.037 mg/kg.

Conclusions. The estimated daily intake value, THQ, along with the aggregate hazard index value, indicated a potential risk to consumers through consumption of contaminated chicken. Therefore, the study results clearly demonstrate heavy metals accumulation in chicken due to feeding SCW-based feed. The contaminated chicken further transfers these heavy metals to humans through ingestion. Hence, there is a potential human health risk through consumption of contaminated chicken meat.

Competing Interests. The authors declare no competing financial interests.

Keywords. solid tannery waste, protein concentrate, chromium, poultry, and health risk

complex mixture of both organic and inorganic pollutants which discharge directly into canal or rivers without any appropriate treatment. As a result, heavy metals can build up in the surrounding environment. In addition, use of tannery waste in poultry feed poses additional health risks for consumers, as hazardous waste has the possibility of directly entering the food chain. Chicken is one of the most widely used meats in the world and poultry farms are the main source of chicken. For over a decade, tannery waste, in particular tanned SCW containing a large amount of chromium (Cr), have been used in the manufacture of poultry feed in Bangladesh, which might be the most direct source of Cr contamination in the food chain. The metals of particular concern in relation to their harmful effects to health are Cr, lead (Pb), cadmium (Cd), mercury, tin and arsenic, often referred to as "heavy metals". The toxicity of these metals is in part due to the fact that they bio-accumulate in all living organisms as a result of exposure to metals in food and the environment, including food animals such as chicken, fish and cattle, as well as humans. In this study, heavy metal deposition in different parts of chicken after their consumption of contaminated feed was determined and the resulting human health risk due to consumption of contaminated chicken meats was evaluated.

Methods

Study Area
Our main study area was the Hazaribagh area. Hazaribagh Thana lies in the Dhaka district and has an area of 3.58 square kilometers surrounded by Mohammadpur Thana on the north, Kamrangirchar Thana on the south, Dhanmondi and Lalbagh Thana on the east, and Keraniganj Thana and the Buriganga river to the west (Figure 1). It is situated in the south-west part of the capital of Dhaka. The study area is located between 23°43.85’ and 23°44.05’ N latitude and 90°21.85’ and 90°22.15’ E longitude.

Sampling and Sample Size
For this study, the protein concentrate production processes practiced in the Hazaribagh area were surveyed and local people involved in preparing the protein concentrate were interviewed. This interview and survey helped to determine the amount of solid tannery waste, the frequency of application of skin-cut wastes in feed production, feed producers, feed distribution area, and potential feed sellers, etc. A total 101 samples were collected in pre-sterilized Ziploc bags. Samples were collected from different spots at Hazaribagh and from adjacent areas. These areas were Sonatanagar, Kamrangirchar Fulbaria and Nimitoli feed market, feed mills at Hazaribagh, Hazaribagh bazaar, New-market bazaar and the Nimitoli poultry market (Table 1).

Eighteen chickens were randomly sampled from these areas where local feed producers in Hazaribagh supply the majority of the chicken feed to different poultry sellers at cheap rates. To evaluate the possibility of accumulation of Cr, Cd and Pb in chicken and as well as their possible consumption by humans through consumption of contaminated chicken meat, feed containing Cr=0.98 mg/kg; Pb=10.32 mg/kg, and Cd=0.03 mg/kg was fed to selected chickens for 6 weeks and their meat, skin, liver, gizzard and other parts were analyzed for Cr, Cd and Pb...
Sample Preparation, Digestion and Heavy Metal Analysis
Tannery waste samples were collected from different sources across different groups. These samples were then weighted and put into a Teflon vessel to mix with other chemicals and sealed. The digestion was performed using a CEM microwave digester and the digested samples were serially diluted and the heavy metal content was analyzed with a Perkin-Elmer atomic absorption spectrophotometer (Model A Analyst 200; Illinois, USA). All the samples were analyzed by the same procedure. Dry samples were mechanically ground and weighted to approximately 0.5 g and put into a Teflon vessel; then 6 ml hydrochloric acid (37%, Merck, Germany), 2 mL of concentrated nitric acid (65% Merck, Germany) was added and digested in a microwave oven. The samples were allowed to predigest by standing open for a minimum of 15 minutes before the vessels were sealed and then proceeded to the heating program (CEM system). Microwave digestion was conducted with a 1600W power supply, temperature ranging from 180°C–220°C, and holding time of 15 min.

After digestion, the content in the Teflon vessel was dissolved in deionized water and filtered into a 25-mL volumetric flask quantitatively and brought up to the mark with de-ionized water. The digested sample solutions were subsequently analyzed for the metals Cr, Cd, and Pb by an automatic sampler and analyzed using an air acetylene flame in combination with single element hollow cathode lamps into an atomic absorption spectrophotometer. The correlation coefficient and detection limit for Cr was 0.996 and 0.10 mg/L, 0.997 and 0.20 mg/L for Pb, and 0.996 and 0.01 mg/L for Cd, respectively.

Determination of Estimated Daily Intake (EDI) and Target Hazard Quotient (THQ)
The average estimated daily intake (EDI) of heavy metals by human subjects was calculated using the following equation, recommended by the United States Environmental Protection Agency (USEPA). \(^6\)

**Equation 1**

\[
EDI = C \times IR \times EF \times ED/BW \times AT
\]

where EDI is the average daily intake or dose through ingestion (mg/kg bw/day); C is the heavy metal concentration in the exposure medium (mg/L or mg/kg); IR is the ingestion rate (L/day, or kg/day); EF
is the exposure frequency (260 days/year for people who eat chicken five times a week); ED is the exposure duration (70 years, equivalent to the average lifespan); and BW is body weight (kg). The average adult body weight was considered to be 60 kg, and AT is the average exposure time for non-carcinogens (365 day/year × ED).

The human health risk posed by heavy metal exposure from consuming contaminated chicken is usually characterized by the target hazard quotient (THQ), the ratio of the average estimated daily intake resulting from exposure to contaminated chicken to the oral reference dose obtained by the USEPA, which is an estimation of the maximum permissible risk to a human population through daily exposure. The applied reference dose (RfD) for Cr, Cd, and Pb was 11, 0.005 and 0.005 mg/kg/d, respectively. The THQ based on non-cancer toxic risk is determined by

\[
THQ = \frac{EDI}{RfD}
\]

If the value of THQ is less than 1, the risk of non-carcinogenic toxic effects is assumed to be low. When it exceeds 1, there may be concerns for potential health risks associated with overexposure. To assess the overall potential risk of adverse health effects posed by exposure to more than one metal, the THQs can be summed across contaminants to generate a
The hazard index (HI) to estimate the risk of a mixture of contaminants. The HI refers to the sum of more than one THQ for multiple substances and/or multiple exposure pathways. In the present study, the HI was used as a screening value to identify whether there was significant risk to human health caused by heavy metals through contaminated chicken consumption.

Hazard Index (HI)
The HI was developed to evaluate the potential risk to human health by more than one heavy metal.\cite{Bari et al (2015)} The hazard index is the sum of the hazard quotients as described in the following equation:

\[
HI = \sum HQ = HQ_{Pb} + HQ_{Cd} + HQ_{Cr}
\]

Where \(\sum HQ\) is the summation of hazard quotients of metals and HQ Pb, HQ Cd, and HQ Cr are the hazard quotients for lead, cadmium, and chromium, respectively. It is assumed that the magnitude of the adverse effects will be proportional to the sum of multiple metal exposures. The hazard indexes for the toxic elements Pb, Cd, and Cr were calculated in the present study. When the hazard index exceeds 1.0, there is concern for potential health effects.\cite{Bari et al (2015)}

### Statistical Analysis

All trials were replicated three times. Reported data represented the mean values obtained from three individual trials. Data were subjected to analyses of variance using the Microsoft Excel program (Redmond, Washington, USA). Significant differences in each experiment were established by the least-significant difference at the 5% level of significance.

### Results

Higher Cr concentrations in SCW were recorded in tanned raw skin (3.89 mg/kg), shaving dust (3.44 mg/kg) and unprocessed leather (3.54 mg/kg). All 30 SCW samples contained higher Cr than the maximum residue level (MRL). Pb was found in 5 kinds of SCW samples. Shaving dust contained high (0.42 mg/kg) Pb compared to other forms.
of SCW, and no Cd was found in any of the SCW samples (Table 2). No significant changes in Cr levels were observed after boiling and drying treatments in the SCW samples collected from different sampling spots. Therefore, sampling spot did not have much influence on the Cr content in SCW in the Hazaribagh area. In addition, no change in Cr level except for oxidation would be expected after boiling with water. This finding is in agreement with that of a previous study.

On the other hand, only a small amount of Cd was found in the tested feed sample and no Cd was recorded in the tested protein concentrate samples. A moderate amount of Pb was found in the protein concentrate and feed samples (Table 3).

In the present study, the accumulation of heavy metals differed in the organs depending on type of chicken. In broiler chicken, the maximum Cr accumulation (1.26 mg/kg) was found in the liver and the minimum Cr accumulation (0.474 mg/kg) was found in the skin. Regarding Cd accumulation, the highest Cd content (0.044 mg/kg) was recorded in skin and the lowest Cd content (0.016 mg/kg) in meat. On the other hand, the highest Pb deposition (0.931 mg/kg) was found in meat and the lowest Pb deposition (0.515 mg/kg) was recorded in liver (Table 3). For the Deshi chicken sample (native breed of chicken in the study area), maximum Cr, Cd and Pb accumulations of 0.723 mg/kg, 0.016 mg/kg and 0.723 mg/kg, respectively, were observed in chicken skin, non-detectable levels of Cr were observed in chicken meat, and the lowest Cd accumulation was recorded in Deshi chicken gizzard. For free

<table>
<thead>
<tr>
<th>Sample Group</th>
<th>Cr Content (mg/kg)</th>
<th>Cd Content (mg/kg)</th>
<th>Pb Content (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
<td>Minimum</td>
</tr>
<tr>
<td>Broiler Chicken</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0.162</td>
<td>1.260</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Gizzard</td>
<td>0.216</td>
<td>0.666</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Meat</td>
<td>0.242</td>
<td>0.721</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Skin</td>
<td>0.186</td>
<td>0.474</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mean</td>
<td>0.202</td>
<td>0.780</td>
<td>0.01</td>
</tr>
<tr>
<td>Deshi Chicken</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0.100</td>
<td>0.712</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Gizzard</td>
<td>0.252</td>
<td>0.663</td>
<td>&lt;0.01</td>
</tr>
<tr>
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<td>&lt;0.100</td>
<td>0.283</td>
<td>&lt;0.01</td>
</tr>
<tr>
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<td>&lt;0.100</td>
<td>0.10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mean</td>
<td>0.138</td>
<td>0.440</td>
<td>0.01</td>
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<tr>
<td>Free-ranging Chicken</td>
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<tr>
<td>Liver</td>
<td>0.100</td>
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<td>&lt;0.010</td>
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<tr>
<td>Gizzard</td>
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<td>0.021</td>
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<tr>
<td>Meat</td>
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<td>&lt;0.010</td>
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<tr>
<td>Skin</td>
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<td>0.018</td>
</tr>
<tr>
<td>Mean</td>
<td>0.111</td>
<td>1.400</td>
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</tr>
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</table>

Table 3 — Ranges of Estimated Minimum and Maximum Cr, Pb and Cd Concentrations in Liver, Gizzard, Meat and Skin of Three Types of Chicken (mg/kg) Fed with Contaminated Feed (Cr=0.98 mg/kg; Pb=10.32 mg/kg and Cd=0.03 mg/kg) for 6 Weeks
ranging chickens in the tannery area, the highest Cr and Cd accumulations observed in skin were 2.44 mg/kg, and 0.032 mg/kg respectively. In contrast, the highest level of Pb (1.750 mg/kg) accumulation was observed in liver and the lowest Pb content (0.992 mg/kg) was observed in skin. In addition, the highest Cd accumulation (0.032 mg/kg) was observed in skin and the lowest accumulation (0.015 mg/kg) was observed in liver (Table 3).

The estimated daily exposures for heavy metals due to the consumption of chicken in the study area are given in Table 4. To assess the toxicological significance of various metals, the estimated intakes from diet in this study were compared with those in the recommendations of the Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO), which established a reference value for daily tolerable intake of metals [Cr (11 mg/kg), Cd (0.005 mg/kg and Pb (0.005mg/kg)]. The present study found that the daily exposures of Cd and Pb were higher than the permitted levels (Table 4).

The human health risk from ingestion of contaminated chicken meat was calculated according to the USEPA. To estimate the human health risk from consuming Cr, Cd and Pb-contaminated chicken, reference doses of Cr (11 mg/kg/day), Cd

<table>
<thead>
<tr>
<th>Metal</th>
<th>Exposure rate (minimum avg) mg/kg/day</th>
<th>Exposure rate (maximum avg) mg/kg/day</th>
<th>Exposure rate (minimum avg) mg/kg/day</th>
<th>Exposure rate (maximum avg) mg/kg/day</th>
<th>Exposure rate (maximum avg) mg/kg/day</th>
<th>*Permitted Maximum Tolerable Daily Intake by FAO/WHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr</td>
<td>0.006</td>
<td>0.023</td>
<td>0.004</td>
<td>0.012</td>
<td>0.003</td>
<td>0.041</td>
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<tr>
<td>Cd</td>
<td>0.0003</td>
<td>0.0009</td>
<td>0.0003</td>
<td>0.0005</td>
<td>0.0004</td>
<td>0.0007</td>
</tr>
<tr>
<td>Pb</td>
<td>0.015</td>
<td>0.022</td>
<td>0.012</td>
<td>0.021</td>
<td>0.037</td>
<td>0.042</td>
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</table>


<table>
<thead>
<tr>
<th>Metal</th>
<th>THQ for minimum exposure</th>
<th>THQ for maximum exposure</th>
<th>THQ for minimum exposure</th>
<th>THQ for maximum exposure</th>
<th>THQ for minimum exposure</th>
<th>THQ for maximum exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr</td>
<td>0.0005</td>
<td>0.002</td>
<td>0.0003</td>
<td>0.001</td>
<td>0.0002</td>
<td>0.0037</td>
</tr>
<tr>
<td>Cd</td>
<td>0.06</td>
<td>0.18</td>
<td>0.18</td>
<td>0.10</td>
<td>0.08</td>
<td>0.14</td>
</tr>
<tr>
<td>Pb</td>
<td>3.0</td>
<td>4.4</td>
<td>2.4</td>
<td>4.2</td>
<td>7.4</td>
<td>8.4</td>
</tr>
</tbody>
</table>

Hazard Index (HI) 3.06 4.58 7.16 4.38 7.48 8.54

Table 4 — Minimum and Maximum Average Daily Exposure Value per kg/day Due to Ingestion of Contaminated Poultry

Table 5 — Health Risk Estimation for Cr, Cd and Pb Ingestion from Different Kinds of Chicken
showed higher heavy metal content compared to the other two types of poultry studied. This might be due to the prior accumulation of heavy metals, as they were free ranging and might be consuming raw waste, water, effluent or other discharge from the industries in the tannery area. The highest Cr accumulation was found in chicken skin, followed by liver, gizzard and meat, and these findings are in general agreement with those in other studies. It is well known that the liver is the organ where detoxification takes place. Thus, the accumulation of a toxic substance like Cr in the liver is easily explained. The gizzard is the organ where food is initially processed and may therefore contain Cr. Flesh is predominately protein and contains a lot of moisture. Hexavalent chromium, being soluble in water, is expected to be present in flesh in some quantities. There is also evidence that supplemental Cr of 20 mg/kg in the diet as chromium chloride (CrCl	extsubscript{3}) increases the rate of glucose utilization by the liver of chicks both in vivo and in vitro. Despite the results of the present study, it is clear that the accumulation of heavy metals in different edible portions of poultry is possible if chicken feed is contaminated with heavy metals.

Ingestion of heavy metals (Pb, Cr, Cd) through the food chain by human populations has been widely reported throughout the world. Due to their non-biodegradable and persistent nature, heavy metals accumulated in vital organs in the human body are associated with numerous serious health disorders. The studied heavy metals are known to be potentially toxic and these toxic elements can be harmful even at low concentrations when ingested over a long period of time. Cd is a cancer- and potential mutation-causing element and has lethal effects at low concentrations. Long term exposure to lower levels of Cd in air, food or water leads to a buildup of Cd in the kidneys. Fraser et al. reported that lead exposure causes neurotoxicity and the nature of effects can be toxic (acute, chronic or sub-chronic), neurotoxic, carcinogenic, mutagenic or teratogenic depending on the dose and exposure duration.

Cr poisoning has been implicated in asthma, chronic bronchitis, chronic irritation, chronic pharyngitis, chronic rhinitis, congestion and other acute diseases. Almost all of the sources of Cr in the earth are in the trivalent state (Cr\textsuperscript{3+}), and naturally occurring Cr compounds in the hexavalent oxidation state are rare. Hexavalent chromium (Cr\textsuperscript{6+}) compounds are thus man-made products and are absorbed primarily in the small intestine. Cr toxicity is primarily associated with exposure to hexavalent chromium compounds. Trivalent and hexavalent chromium compounds behave differently in the body. However, Cr\textsuperscript{6+} is believed to be reduced to Cr\textsuperscript{3+} by extracellular fluids before reaching sites of absorption in the small intestine.

Among the three metal elements in the three different chicken samples, the exposure value for Pb in broiler chicken was 1.2-2.29-times higher than that of Deshi chicken or free ranging chicken. The exposure value for Cr and Cd in free ranging chicken was found to be higher compared to other poultry, but lower than the permissible limit (Table 4). The present results indicate that Pd is a major contributor to the potential health risk, with Cd posing the least amount of risk. However, the contribution of Cr and Cd to human health risk at any accumulated level cannot be underrated, because
of their high toxic potential and chronic human health implications.

Cd and Pb are nonessential nutrients that are of direct concern to human and livestock health and may accumulate in the body, particularly in the kidney, liver, and to a lesser extent, in muscle. Only a limited number of instances have been reported where levels in chicken/cattle tissue exceeded maximum acceptable limits for human consumption, but recent work has suggested that chicken may be more susceptible to the accumulation of Cd and Pb than beef cattle.20,21,17 Although it is unlikely that Cd would accumulate in products intended for human consumption, accumulation has been observed in the ovaries and uteri of dairy cows which may have an impact on reproduction.20 The health effects of Pb exposure can include neurological damage, reduced IQ, anemia, nerve disorders, and a number of other health problems. The effects of lead are most severe in children, and at high concentrations, Pb poisoning can lead to death.

Conclusion

In order to conserve the environment and resources, biological remediation processes for heavy metals need to be adopted. In Bangladesh, substantial environmental degradation occurs due to the crude disposal of tannery waste. Tannery waste is a vital source of protein once dechromed. The dechroming rate can be controlled to produce a final product with a low level of Cr which satisfies the requirements for poultry feed. The chemicals used in this vital process do not affect the final quality of the product. Further research needs to be undertaken into these by-products with the aim of establishing their values across a wide range of animal feed.

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