Detection of Heavy Metals and Antibiotic Residues in Poultry Feeds in Selected Areas of Bangladesh

Md. Tarikul Islam, Fatema Akter, Md. Faisal Ferdous, Kamrun Nahar Koly, Sabbya Sachi, Parijat Biswas, Md. Mehedi Hasan, Ummai Wara Khan Chowdhury, Mohammad Abdus Sattar Bhuiyan, Quazi Forhad Quadir and Kazi Rafiq

ABSTRACT

Presence of heavy metals and antibiotics in the poultry feed even in low concentrations is considered as potentially toxic and may cause hazardous effects on animal leading to accumulate in food chain. This study was performed to assess the status of heavy metals and antibiotics residue in different poultry feed samples collected from local markets of Mymensingh and Kishoreganj sadar upazila of Bangladesh. A total of 40 poultry feed samples were analyzed to detect the concentration of three heavy metals- chromium (Cr), cadmium (Cd) and lead (Pb) using atomic absorption spectrophotometry and antibiotic residues using thin layer chromatography. A survey was also conducted among 30 feed sellers in the study areas whose knowledge regarding presence of heavy metals and antibiotics residues in poultry feed was evaluated. Survey data showed that about 80% feed sellers were literate whereas 20% were illiterate. Around 23% feed sellers had knowledge about the presence of heavy metals and 90% had about antibiotic residues in poultry feeds and its detrimental public health effects. The average Cr, Cd and Pb contents in poultry feed collected from Mymensingh sadar upazila were 0.402, 1.630 and 18.314 mgkg\(^{-1}\), respectively. The average content of Cr and Cd in feed collected from Kishoreganj sadar upazila were 7.884 and 0.006 mgkg\(^{-1}\), respectively, whereas Pb content was below the detection level in this area. The results from this study showed that Cd and Pb in Mymensingh sadar upazila, Cr in Kishoreganj sadar upazila, ciprofloxacin, doxycycline and oxytetracycline in both upazilas were present at alarming levels in most of the feed samples which could be harmful for poultry as well as human health. Therefore, it is suggested to take proper steps for monitoring and regular detection of heavy metals and antibiotic residues in poultry feeds with sources identification as we as community-based awareness among the stakeholder.

Keywords: Antibiotic residues, heavy metal residues, poultry feeds, survey.
I. INTRODUCTION

Bangladesh is a developing country where poultry industry is a rising sector. Being an integrated part of the livestock sector, poultry farming plays an important role in the agro-based economy of Bangladesh [1]. Demand for poultry meat especially chicken meat has increased in every region of the world, particularly in developing and Asian countries. An increase in demand has put the owner of farm or farmer under continuous pressure to produce poultry in shortest period of time with maximum output. In this regard, owners of farm, farmers as well as feed manufacturer add various types of antibiotics in poultry feed as growth promoter. For proper development and growth of broilers and layers, commercially produced feeds are used in both small scale and commercial poultry farms due to availability of required nutrients. Many commercial poultry feed producers in Bangladesh mix antibiotics and tannery waste products containing heavy metals in poultry feed as a low-cost protein source which are potentially toxic and if added at excessively high concentration can cause serious health issues in animals and humans.

Originating either from natural or anthropogenic sources, heavy metal toxicity is potentially dangerous because of their bioaccumulation in the body through the food chain, and this can cause hazardous effects on livestock and human health [2]. The most commonly found heavy metals in animal feeds include cadmium (Cd), chromium (Cr), lead (Pb), copper (Cu), arsenic (As), nickel (Ni), and zinc (Zn), among which Cr, Cd and Pb are more harmful for poultry when cross the maximum tolerance level [3]. These toxic metals present in feeds bioaccumulate in primary and secondary consumers body and since these metals are not easily digested, they cause serious effects not only to the tissues but also to the bones and other parts of the body [4].

In the poultry industry, antibiotics are generally used either for therapeutic or prophylactic purposes or as growth promoters. The use of antimicrobial agents for growth promotion purposes in farm animals was prescribed in the mid-1950s [5]. However, recent sporadic use of antibiotics as growth promoter in poultry feeds over maximal residual limit is very alarming for human health. In addition to reducing the sustainability of feeds, the use of antibiotics and synthetic growth promoters lead to presence of residues in animal origin food products (meat, milk, and eggs), as well as in the environment (water and soil pollution) [6]. Even though Bangladesh Animal feed Act 2010 bans use of antibiotic growth promoter in feeds, various researches revealed the existence of antibiotics residue in poultry meat and eggs, consumption of which leads to serious public health hazards [7], [8] and [9]. Furthermore, irrational use of antibiotics in poultry feed results in the development of antimicrobial resistance [10].

Irrespective of their awareness, poultry farmers generally do not have enough facility to analyze and monitor the quality of commercial poultry feeds. Often, they themselves formulate the ration for their own birds based on some random information, where unknowingly they may add various antibiotics growth promoter or contaminate the feed with different toxic heavy metals by using tannery waste as a low-cost protein. The presence of heavy metals and antibiotics residues in poultry feeds which were generally unnoticed by the farm owners causing a devastating effect not only on safe feed production but also in public health. However, there is invariably insignificant data in literature performed on detecting the presence of heavy metals and antibiotics residues in poultry feed available in Mymensingh and Kishoreganj sadar upazila of Bangladesh. Hence, this study is important to meet a void in research.

II. MATERIALS AND METHODS

A. Study Area

This study was designed to assess contamination of heavy metals and antibiotics residues in poultry feed samples collected from local markets at three unions of Mymensingh sadar upazila (Akua union, Baera union, Mymensingh Paurashava) and three unions of Kishoreganj sadar upazila (Jasodal union, Latibabad union, Kishoreganj Paurashava) of Bangladesh during the period from July to December 2018. A survey was performed with pre-tested questionnaire on 30 poultry feed sellers from the study areas on their educational status and knowledge regarding heavy metals and antibiotics residues in poultry feed.

B. Sample Collection

A total of 40 different types of poultry feed samples were collected from the study area and presence of heavy metals and antibiotics residues were detected by atomic absorption spectrophotometry (AAS) and thin layer chromatography (TLC) method, respectively at the Department of Pharmacology and Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh. Samples were collected in properly labeled air tight zip lock plastic bags (250 gm for each sample). Collected samples were kept in room temperature until processing.

C. Sample Preparation for Heavy Metal Detection

Feed samples were oven dried at 60 °C for 48 hours until a constant weight was obtained. After homogenization, all ground samples were kept in polythene zipper bags labeled with specific code number until subsequent analysis. Exactly 1 g of each feed samples was taken into a conical flask and 10 ml of di-acid mixture (HNO3:HClO4 = 2:1) was added into it [11]. Then the samples were digested at 150 °C until the white fumes appear. About 2 mL of H2O2 was added and heated until the digest became colorless. Then the samples were cooled down and filtered through Whatman No. 42 filter paper. After filtering the feed samples, the digests were made up to 50 ml volume by deionized water and kept in labeled air tight polyvinyl bottles. Then the samples were stored in the refrigerator until further analysis [12].

D. Analysis of Heavy Metals using AAS

Determination of different heavy metals (Cr, Cd, and Pb) in poultry feed samples were done by a Flame AAS (Model no: SHIMADZU, AA-7000, Japan). Mono element hollow cathode lamp was employed for the determination of each metal of interest. At first, the AAS was calibrated following the manufacturer’s recommendation. Then filtered samples were run directly for the determination of heavy metals. Finally, the concentration of the metals in poultry feed samples was recorded directly by AAS as described...
previously [12].

E. Detection of Antibiotics Residues in Feed Samples

HPLC grade chemicals and reagents were used with at least 99% purity. The standard drugs ciprofloxacin, enrofloxacin, doxycycline, and oxytetracycline were collected from Sigma-Aldrich via Renata Limited, Bangladesh. The antibiotic standard solutions were prepared by dissolving 0.1 g of powder in 4 ml methanol. Finally, 50 ml of methanol and 50 ml of acetonitrile were mixed to form mobile phase [8].

F. Sample Preparation for Antibiotic Residue Analysis

Four grams of each sample was grinded and blended first and then 10 ml phosphate buffer saline (pH-7.2) was added to the samples. After vortexing, 2 ml 30% Trichloroacetic acid was added and centrifuged (Hettich D-78532, Germany) @ 6000 rpm for 20 min. Supernatant was collected and filtered by Whatman filter paper and funnel. Filtrated fluid was collected in another falcon tube and same amount of diethyl ether was added and kept in room temperature for 10 min. The bottom layer was collected and pooled carefully into screw cap vial and kept into refrigerator for future analysis [13].

G. Detection of Antibiotic Residue by Thin Layer Chromatography

TLC was performed according to [8] and [14] with some modifications. After cutting TLC plate (MN-Germany) into appropriate size (4x5 cm), two straight lines were drawn across the plate approximately 2cm from the bottom and 1 cm from the upper edge with a pencil. On the bottom-line desired spots were marked where analyses were dropped. Then using thin capillary glass pipettes 10 μl of sample was used for spotting. Plate was placed in TLC tank (mobile phase), covered by lid and left until the mobile phase reached the upper line. Spots were visualized in UV detection box (UV light: F18W-Germany) at 256 nm. Finally, retention factor (Rf) was calculated which is the ratio of the distance travelled by the individual sample spots [8] and the distance travelled by the solvent (b), i.e Rf=a/b. Same Rf value of standard and sample is considered as similar compound [8].

H. Data Compilation and Processing

After the laboratory analysis of heavy metals and antibiotic residues, all recorded data were collected and processed using Microsoft Excel software, 2007.

III. RESULTS AND DISCUSSION

A. Questionnaire Survey among Poultry Feed Sellers

The survey was conducted on 30 feed sellers from six geographical areas of Mymensingh and Kishoreganj sadar upazilas about their educational status and knowledge on heavy metals and antibiotic residues in poultry feed. Among the sellers, 20% were illiterate whereas 50%, 20%, and 10% participants have earned their primary, secondary and higher secondary education, respectively (Fig. 1). When considered their knowledge on the presence of heavy metals on feed and its consequences, only 23% sellers have knowledge, and 77% don’t have any idea about it (Fig. 2A). Interestingly, 90% of the sellers have understanding about antibiotic use in feed (Fig. 2B).

B. Heavy Metals in Poultry Feed

In the present study, 40 poultry feed samples were analyzed to detect the presence of heavy metals like Cr, Cd, and Pb. Surprisingly, all samples contained these heavy metals in different concentrations. The maximum residual limit (MRL) of Cd in poultry feed is 0.5 mgkg⁻¹ [15]. The mean Cd content of collected feed from Mymensingh and Kishoreganj sadar upazila were 1.630 mgkg⁻¹ and 0.006 mgkg⁻¹, where the content in Mymensingh sadar was almost three times higher than the MRL (Table I). The Cd content in poultry feed of Mymensingh sadar is comparable to 0.53-3.19 mgkg⁻¹ reported by [16], but much lower than 3.8-33.6 mgkg⁻¹ detected by [17]. On the other hand, Cd content of feed from Kishoreganj sadar is in line with other findings like 0.004-0.249 mgkg⁻¹ [18], 0.038-0.463 mgkg⁻¹ [19] and 0.1852 - 0.0232 mgkg⁻¹ [20].

Mean Pb content of collected poultry feed from Mymensingh sadar upazila was 18.314 mgkg⁻¹ which exceeded the MRL (5 mgkg⁻¹) set by EU [15], while in Kishoreganj sadar upazila it was below the detection level (Table I). Pb content 14.36 mgkg⁻¹ was found in tannery waste contaminated poultry feed in Bangladesh [21]. However, feed from other studies contained much lower Pb content-0.10-3.21 mgkg⁻¹ [18] and 1.10-7.85 mgkg⁻¹ [19]. Nevertheless, [17] determine the Pb concentration ranged 23.2-32.6 mgkg⁻¹ which was higher than that of feeds collected from Mymensingh sadar. There was no recommended limit about Cr in feed by EU [15]. The MRL for Cr concentrations in human food, however, ranges from 0.1 to 0.5 mgkg⁻¹ [18]. The mean Cr content in feed collected from Mymensingh and Kishoreganj sadar upazila were 0.402 mgkg⁻¹ and 7.884 mgkg⁻¹, respectively (Table I). Other studies also detected similar Cr content to Mymensingh sadar e.g 0.38-0.47 mgkg⁻¹ [16] and 0.00-3.02 mgkg⁻¹ [22], but much lower than that of Kishoreganj sadar. However, [23] found Cr content in poultry feed ranged between 17.68-78.39 mgkg⁻¹ which is more or less similar to poultry feed of Kishoreganj sadar. The presence of Cr in poultry feed indicate the presence of solid tannery waste and its wastewater. In this regards, previous study reported that the primary source of Cr in the samples of poultry feed may be from tannery solid waste and its effluent [24]. Arsenic, cadmium, chromium, lead, and mercury are among the priority heavy metals of public health concern due to their high degree of toxicity. These are the systemic toxins that can damage various organs even at lower exposure levels.

Presence of these heavy metals to poultry feed results in serious public health concern. Chronic exposure to low level Cd is associated with renal failure, neurotoxicity, respiratory disorders, bone damage and affects reproductive biology [25], [26]. There are also some evidence of Cd causing renal cancer [27] and prostate cancer [28]. In Bangladesh, significant levels of harmful metals like Cr, Cd and Pb are found in the solid waste produced by the tannery industry, which is then processed into low cost protein concentrate in poultry feed and such contaminated feed are used in poultry industries [29]. Inhalation of Cr results in severe respiratory problems including bronchogenic carcinoma whereas dermal contact causes dermatitis, skin allergies and corrosion [30]. In addition, chronic high level Pb exposure causes peripheral motor neuropathy, hypertension, cardiovascular diseases,
were found in all samples of the two locations due to the high variations in the concentration of heavy metals especially for Cd and Pd. Mymensingh and Kishoreganj sadar upazilas. High variations and antibiotic residues in poultry feed collected from each district, to identify the presence of these antibiotic residues. Results from the present study showed that, in Mymensingh sadar upazila, 95% of feed samples were positive with antibiotic residues where 70% contains oxytetracycline, 35% doxycycline, and 20% ciprofloxacin (Table II). On the other hand, antibiotic residues were detected in 85% feed samples in Kishoreganj sadar upazila where 50% comprises oxytetracycline, 35% doxycycline, and 30% ciprofloxacin. No sample was affirmative for enrofloxacin. Similar study on poultry feed samples in Bangladesh showed the presence of antibiotic residues in 18.89% samples [33]. The results of present study indicate that in recent years, indiscriminate use of antibiotics in poultry feed has drastically increased which is a matter of concern. Although, presence of antibiotic residues namely enrofloxacin (46.67%), ciprofloxacin (30.00%) and amoxicillin (23.33%) in poultry feed was also detected by other researchers [34].

Antibiotics are added to poultry feed for rapid growth and disease prevention. The debate regarding the benefits and harmful effects of antibiotic growth promoters (AGPs) has been in focus for a long period. Antibiotics used as growth promoters tend to be given in feed at sub-therapeutic dose over extended period of time to entire flocks, and are available for purchase over the counter by feed manufacturers and farmers. The concept relies on maintaining poultry fewer days on feed with growth promoters before reaching slaughter weight to make the production system profitable [35]. However, this has serious consequences resulting health hazards with emergence of antibiotic resistance leading to treatment failure in both humans and animals [36]. Another severe concern is that, antibiotic resistance genes can be transferred from human to animal pathogens or vice versa and there is also horizontal transmission within species [37]. Therefore, community-based awareness regarding abuse of antibiotics in poultry feed as growth promoter is crucial. The weakness of this study is use of TLC technique to qualitative detection of antibiotic residues which give misleading results. Therefore, quantitative analysis of the poultry feed with wider range is required to make a definitive conclusion. Another limitation is small sample size with limited area.

This study determined the present scenario of heavy metals and antibiotic residues in poultry feed collected from Mymensingh and Kishoreganj sadar upazilas. High variations in the concentration of heavy metals especially for Cd and Pd were found in all samples of the two locations due to the variation in point of sources of collection. Additionally, majority samples contained oxytetracycline, doxycycline and ciprofloxacin whereas only a minimal number of feeds were free from antibiotic residues. This is very alarming to consider since it has serious public health concern and increased risk of antimicrobial resistance. Moreover, the method described in this study is simple, easy and inexpensive which can be readily adopted by any laboratory for the detection of the concentration of heavy metals and antibiotics residues in poultry feed. Further investigation is required for quantitative analysis of heavy metals and antibiotics residues in poultry feed. Therefore, future research on sources of antibiotics and heavy metals in poultry feed should be intensified to help produce pollution-free environment and safe poultry production in Bangladesh.

**C. Antibiotics Residue in Poultry Feed**

The qualitative analysis of antibiotic residues was done for ciprofloxacin, doxycycline, enrofloxacin and oxytetracycline. A total of 40 feed samples were tested (20 from each district), to identify the presence of these antibiotic residues. Results from the present study showed that, in Mymensingh sadar upazila, 95% of feed samples were positive with antibiotic residues where 70% contains oxytetracycline, 35% doxycycline, and 20% ciprofloxacin (Table II). On the other hand, antibiotic residues were detected in 85% feed samples in Kishoreganj sadar upazila where 50% comprises oxytetracycline, 35% doxycycline, and 30% ciprofloxacin. No sample was affirmative for enrofloxacin. Similar study on poultry feed samples in Bangladesh showed the presence of antibiotic residues in 18.89% samples [33]. The results of present study indicate that in recent years, indiscriminate use of antibiotics in poultry feed has drastically increased which is a matter of concern. Although, presence of antibiotic residues namely enrofloxacin (46.67%), ciprofloxacin (30.00%) and amoxicillin (23.33%) in poultry feed was also detected by other researchers [34].

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**Fig. 1. Educational qualification of feed sellers in Mymensingh and Kishoreganj sadar upazilas (n=30).**

**Fig. 2. Knowledge of feed sellers about (A) heavy metals and (B) antibiotics residues in poultry feeds.**

**TABLE I: AVERAGE CHROMIUM, CADMIUM AND LEAD CONTENTS IN DIFFERENT FEED SAMPLES COLLECTED FROM MYMENSINGH AND KISHOREGANJ SADAR UPAZILA WITH STANDARD REFERENCE VALUES FIXED BY EU**

<table>
<thead>
<tr>
<th>Location</th>
<th>Cr (mg kg⁻¹)</th>
<th>Cd (mg kg⁻¹)</th>
<th>Pb (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mymensingh</td>
<td>0.402</td>
<td>1.271</td>
<td>1.630</td>
</tr>
<tr>
<td>Kishoreganj</td>
<td>7.884</td>
<td>24.728</td>
<td>18.314</td>
</tr>
<tr>
<td>EU (2013)</td>
<td>-</td>
<td>0.5</td>
<td>5</td>
</tr>
<tr>
<td>Reference values:</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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TABLE II: PRESENCE OF ANTIBIOTIC RESIDUES IN DIFFERENT POUlRY FEED SAMPLES COLLECTED FROM MYMENSINGH AND KISHOREGANJ SADAR UPAZILAS

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Mymensingh Detected (n=20) Percentage (%)</th>
<th>Kishoreganj Detected (n=20) Percentage (%)</th>
<th>Overall percentage (%) (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>4</td>
<td>6</td>
<td>30 25</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>14</td>
<td>10</td>
<td>70 50</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>7</td>
<td>7</td>
<td>35 35</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>3</td>
<td>5 15</td>
</tr>
</tbody>
</table>

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**AUTHORS CONTRIBUTION**

MTI and KR contributed equally to this work. MTI, SS, MFF, KNK, MMH: sample collection, analysis. FA, PB, MASB: manuscript writing and revision; KR, QFQ, FA: planning, conceptualization, supervise and revised the manuscript; QFQ, KNK, UWKC, MFF: Sample analysis and data compilation. All authors have read and approved the final manuscript.

**ETHICS APPROVAL**

All experimental procedures in this original project were performed according to the guidelines for the care and use of animals as described by Animal Welfare and Experimentation Ethics Committee (AWEEC), Bangladesh Agricultural University (BAU), Mymensingh-2202 [Approval number: AWEEC/BAU/2018(11)]. However, this part is an observational study in poultry ready feed. The AWEEC, BAU has confirmed that no ethical approval is required.

**DATA AVAILABILITY**

Due to important public health and general public issues, the datasets created during and/or analyzed during the current investigation are not publicly available, however, they are available from the corresponding author upon justifiable request.

**CONFLICT OF INTEREST**

The authors declare no relevant financial or non-financial conflict of interest to disclose.

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