Hexavalent Chromium in Chicken and Eggs of Bangladesh

Shamima Parvin and M Lutfor Rahman

Abstract -- Chickens of different regions of Bangladesh were analyzed for their Cr(VI) content in the different body parts such as liver, gizzard, flesh and brain. In addition, 4 albumen and 3 yolk samples were also analyzed. The USEPA method 3060A of alkaline digestion, followed by spectrophotometric measurement of the colored complex formed with 1, 5 diphenylcarbazide (EPA method 7196A) has been used for most of the measurements. For comparison, Ion Chromatographic technique (EPA method 7199) has also been employed for some samples. All the liver samples showed the presence of Cr(VI), ranging from 0.24 - 15.0 ppm. All the samples of chicken gizzard, too, contained Cr(VI) in the range, 0.15 - 4.7 ppm. About 75% of the flesh samples had Cr(VI) in them. Most of the brain samples gave negative results. Average amounts of Cr(VI) present in these body parts were in the order: Liver > Gizzard > Flesh > Brain.

Index Terms-- Hexavalent chromium Cr(VI), EPA, Spectrophotometry, Ion-chromatography, Tannery, Poultry feed, Dipenylcarbazide

1 INTRODUCTION

Solid tannery waste from Hazaribag tanning industries of Dhaka, Bangladesh is being used as the principal component of poultry feed, fish feed and fertilizers. Previous authors have found the prevalence of heavy metals like lead, chromium, arsenic, mercury, etc. in the waste, in the protein concentrate and in the poultry feed [1]. They have also determined the amount of total chromium in chickens and eggs [2].

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The presence of chromium in popular food items like chicken and eggs has attracted the media attention as well. The two most widely circulated newspapers of Bangladesh, The Daily Star [3], and the Prothom Alo [4] have run articles on the subject. The concern for chromium toxicity has not been limited to Bangladesh alone. In an article entitled, "Are we eating chrome chicken", Sudha [5] describes the transfer of chromium from leather waste to chicken through poultry feed in the Tamil Nadu state of India, which is home to some 900 tanneries. Different body parts of chicken collected from a Lahore market in Pakistan have also been shown to contain high concentrations of chromium [6]. Toxicity of chromium depends on its valence state. Whereas Cr (III) is useful for health, Cr (VI) is highly toxic and can cause mutation, cancer and cell damage [7]. Instead of measuring the concentration of total chromium, which has been done by most of the previous workers, we have directed our attention to hexavalent chromium only. Using the USEPA method, 3060A of alkaline digestion [8] followed by spectrophotometric measurement of the colored complex that is formed on addition of 1, 5 diphenylcarbazide (EPA method 7196A) [9], Mazumder, Hasan and Rahman [10] have found hexavalent chromium in leather waste, poultry feed, and even in some chicken livers of Dhaka city. As the poultry feed that is manufactured from tannery waste of Dhaka is distributed all throughout the country, it would be interesting to

extend this work to chickens of other parts of the country. In addition to liver, other body parts of chicken such as gizzard, flesh and brain have been brought under investigation. Yolk and albumen of some eggs have also been analyzed for their Cr(VI) content. To check the reliability of the spectrophotometric method (7196A), we have also used a more sophisticated and sensitive technique of Ion Chromatography (EPA method 7199) [11] for the measurement of Cr(VI) in some samples.

2 MATERIALS AND METHODS

2.1 Study Area

For the collection of chickens, the cities and towns have been chosen in such a way that they are scattered all over the country of Bangladesh.

2.2 Sample and Sample Size

Chickens (broiler/layer) were collected from Dhaka, Rajshahi, Pabna, Chittagong, Netrokona, Barishal and Khulna. Altogether, 23 chickens were analyzed. Liver, gizzard, flesh & brain were separated from each chicken and digested with appropriate solutions for the analysis of hexavalent chromium. A limited number of eggs were obtained from a Dhaka market and their albumen and yolk were analyzed using the same procedure.

2.3 Transport and Storage of Sample

The collected samples were brought to the laboratory; they were labeled and stored under proper conditions in a freezer below 4^{o}C, until they were handled further. In addition, the samples were kept cool and in the dark during transportation.

2.4 Apparatus

For absorbance measurements, Hach DR/4000 model single-beam and Shimadzu UV-1700 model double-beam spectrophotometers were used. Adjustment of pH was checked using a pH meter, model: Sension ™ 156 of Hach, USA. Ion chromatograph was from Shimadzu, Japan. Digestion of samples was carried out both manually and by using an automatic digester, model: ETHOSD microwave labstation of Milestone.

2.5 Reagents

Nitric acid, sulfuric acid, sodium carbonate, sodium hydroxide, magnesium chloride, phosphate buffer (K_2HPO_4, KH_2PO_4), potassium dichromate, ammonium hydroxide, ammonium sulfate and 1,5 diphenylcarbazide(DPC) were all of analytical grade, obtained from Merck, Germany and used without further purification. Methanol was of HPLC grade.

2.6 Calibration of the Spectrophotometers

Standard solutions of potassium dichromate were used to calibrate the spectrophotometers. The graphs of absorbance versus concentrations were straight lines, the value of R^2 being greater than 0.999. Sandell's sensitivity and detection limit have been found to be 1.6 ppb cm^{-2} & 2.5 ppb and 1.2 ppb cm^{-2} & 1.9 ppb respectively for single-beam and double-beam spectrophotometers.

2.7 Sample Digestion

USEPA Method 3060A is an alkaline digestion procedure for extracting hexavalent chromium from soluble, absorbed, and precipitated forms of chromium compounds in soils, sludge, sediments, and similar waste materials. As is well known, chromium (VI) can be easily converted to Cr (III) in acid medium and Cr (III) can be oxidized to Cr (VI) in alkaline medium. Thus, to determine chromium (VI) in solid matrices, 3 criteria must be satisfied: (1) the extracting solution must be able to dissolve all forms of Cr (VI), (2) the conditions of the extraction must not reduce native Cr (VI) to Cr (III), and (3) the method must not oxidize native Cr (III) contained in the sample to Cr (VI). Method 3060A is supposed to meet these criteria for most solid matrices. Under the alkaline conditions of the extraction, minimal reduction of Cr(VI) or oxidation of native Cr (III) occurs. The addition of Mg^{2+} in a phosphate buffer to the alkaline solution has been shown to suppress oxidation.

2.8 Sample Analysis

EPA Method 7196A involves measurement of absorbance of a colored complex formed by the addition of 1, 5 DPC to the solution containing Cr (VI) at 540 nm. The details of procedure have been described elsewhere [10].

EPA Method 7199 uses ion chromatography to separate the chromate ion from other species by passing through a chromatographic column. This is then allowed to react with DPC and the absorbance is measured with a spectrophotometer.

2.9 Ion Chromatographic Procedure

The sample was digested in the automatic digester, where the parameters like temperature, pressure and time of digestion were fixed beforehand.

- The Cr (VI) extract obtained after digestion of the sample was adjusted to a pH of 9 - 9.5 with a buffer solution.
- A measured volume was introduced into the ion chromatograph.
- A guard column containing Dionex IonPac NG1 or equivalent removed organics from the sample before the Cr (VI) was separated on an anion exchange separator column.
- A mixture of NH_4OH and $(NH_4)_2SO_4$ was used as the elluant.
- The sample was guided through an analytical column packed with a high capacity anion exchange resin capable of resolving CrO_4^{-2} from other sample constituents (Dionex IonPack AS7)
- Conc. H_2SO_4 was added to make the medium acidic.
- Post-column Cr (VI) was allowed to react with diphenylcarbazide and absorbance of the colored complex was measured.

The experimental set-up was calibrated with respect to standard solutions of $K_2Cr_2O_7$. The graph of concentration versus area was a straight line with R-values being 0.9995 and 0.9991.

Detection limit for the instrument was 0.15 ppb.

3. RESULTS AND DISCUSSION

23 liver, 23 gizzard, 21 flesh and 21 brain samples of chicken, collected from different regions of Bangladesh, have been analyzed for their Cr (VI) content. In addition, 4 albumen and 3 yolk samples have also been analyzed. Ion chromatographic technique has been employed for 1 liver, 1 gizzard, 2 albumen and 1 yolk samples. For spectrophotometric measurements, at least 5 readings were taken for the absorbance of the sample after appropriate treatment. The mean and the standard deviation were calculated using the Excel program. Some samples became colloidal on digestion and as a result, unsuitable for photometric measurements. Therefore, no absorbance data have been recorded against these samples. Consolidated results for liver, gizzard, flesh, brain and eggs have been given in Tables 1, 2 and 3.

3.1 Reproducibility of the Results

Table 1 shows the results of chicken liver and gizzard. An examination of the standard deviations in column 5 reveals that the results are highly reproducible except for the sample no's 10 & 21. The chromium (VI) content in these two samples are rather low (0.36 & 0.24) and errors are expected to be large. The digital spectrophotometers display results in multiples of 0.001 units and the low values such as 0.001, 0.002 and 0.003 for a particular sample would give an error of 50%. Most of the other results have less than 10 % errors.

It is seen from the Tables 1 and 2 that average amount of Cr (VI) present in different body parts of chicken follows the pattern:

Liver > gizzard > flesh > brain and as expected, errors follow the reverse pattern:

Brain > flesh > gizzard > liver.

3.2 Reliability of the Spectrophotometric Results

In order to check the reliability of results obtained through measurement of absorbance of the colored complex, a more sophisticated and reliable experimental technique, namely, Ion Chromatography has been used. The chromium content of the same sample was measured using both the techniques and the results are shown in Table 4 and in Fig. 1 and 2.

TABLE 1
Amount of Cr (VI) in Chicken Livers and Gizzards

Region	Sample Number	Age in Months	Body Weight in gm	Amount of Cr(VI) in Livers (mg/kg)	Amount of Cr(VI) in Gizzards (mg/kg)
Rajshahi	1	18	3200	5.892 ± 0.04	2.253 ± 0.375
Rajshahi	2	12	2400	2.607 ± 0.19	1.962 ± 0.2721
Khulna	3	6	1800	Colloidal	0.932 ± 0.1425
Pabna	4	6	1800	Colloidal	1.309 ± 0.0438
Pabna	5	4	1700	Colloidal	0.744 ± 0.228
Chittagong	6	10	2200	2.56 ± 0.12	4.765 ± 1.725
Chittagong	7	8	1900	2.396 ± 0.1	1.695 ± 0.19
Netrokona	8	5	1400	Colloidal	1.077 ± 0.2115
Barishal	9	15	3000	15.04 ± 0.43	0.751 ± 0.093
Barishal	10	7	1600	0.362 ± 0.26	3.728 ± 0.307
Dhaka	11	8	1800	Colloidal	3.858 ± 1.025
Dhaka	12	5	1600	3.197 ± 0.16	1.372 ± 0.257
Dhaka	13	24	3500	13.29 ± 1.51	3.889 ± 0.607
Dhaka	14	9	2000	5.926 ± 0.49	1.312 ± 0.351
Dhaka	15	9	1900	5.148 ± 0.09	0.688 ± 0.091
Dhaka	16	9	1800	4.72 ± 0.23	1.5 ± 0.313
Dhaka	17	18	3000	7.189 ± o.12	3.88 ± 0.225
Dhaka	18	12	2500	12.81 ± 0.07	0.552 ± 0.043
Dhaka	19	4	1200	7.78 ± 0.24	1.4 ± 0.336
Dhaka	20	5	1200	Colloidal	0.43 ± 0.15
Dhaka ,Using Ion Chromatography	21	8	1700	3.456	0.904
Dhaka , Using Spectrophotometry	21	8	1700	1.008 ± 0.12	0.646 ± 0.049
Native Chicken From Rajshahi	22	12	1000	Colloidal	3.403 ± 0.387
Native Chicken From Rajshahi	23	12	1000	Colloidal	1 ± 0.257

TABLE 2
Amount of Cr (VI) in Chicken Flesh and Brain

Region	Sample Number	Age in Months	Body Weight (gm)	Amount of Cr (VI) in Flesh (mg/kg)	Amount of Cr (VI) in Brain (mg/kg)
Rajshahi	1	18	3200	3.6028 ± 0.4834	0.261 ± 0.18
Rajshahi	2	12	2400	1.661 ± 0.3667	0.942 ± 0.404
Khulna	3	6	1800	ND*	-
Pabna	4	6	1800	0.265	0.025 ± 0.056
Pabna	5	4	1700	1.392 ± 0.088	ND
Chittagong	6	10	2200	3.688 ± 1.248	0.337 ± 0.0861
Chittagong	7	8	1900	1.24 ± 0.816	ND
Netrokona	8	5	1400	ND	ND
Barishal	9	15	3000	ND	1.5914 ± 0.5004
Barishal	10	7	1600	1.427 ± 0.374	ND
Dhaka	11	8	1800	0.746 ± 0.181	Colloidal
Dhaka	12	5	1600	1.395 ± 0.6	ND
Dhaka	13	9	1900	1.792 ± 0.41	4.744 ± 0.894
Dhaka	14	9	1800	ND	ND
Dhaka	15	18	3000	ND	ND
Dhaka	16	12	2500	1.648 ± 0.175	ND
Dhaka	17	4	1200	0.92 ± 0.05	0.213 ± 0.23
Dhaka	18	5	1200	0.848 ± 0.237	ND
Dhaka	19	4	1000	ND	ND
Native Chicken From Rajshahi	20	12	1000	Colloidal	Colloidal
Native Chicken From Rajshahi	21	12	1000	0.703 ± 0.271	ND

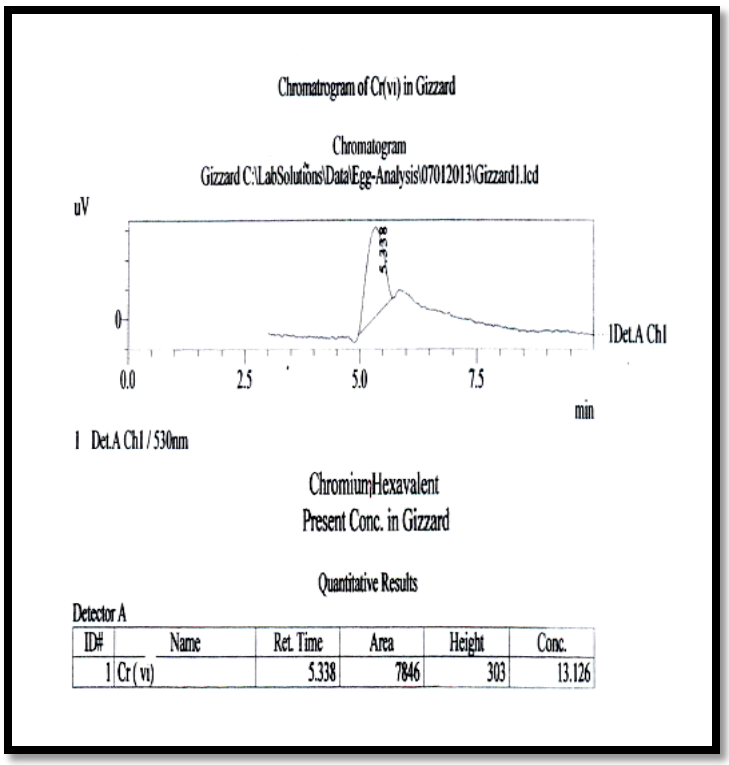
ND*= Not detectable

TABLE 3
Amount of Cr (VI) in Chicken Eggs

Region	Laboratory Technique	Sample Number	Amount of Cr (VI) in Albumen (mg/kg)	Amount of Cr (VI) in Yolk (mg/kg)
Dhaka	Spectrophotometry	1	ND	ND
Dhaka	Spectrophotometry	2	ND	ND
Dhaka	Spectrophotometry	3	0.415 ± 0.306	0.191 ± 0.03
Dhaka	Ion Chromatography	3	0.497	0.36
Dhaka	Ion Chromatography	4	0.938	-
Dhaka	Spectrophotometry	4	1.03 ± 0.327	-

TABLE 4
Comparison of Spectrophotometric and Ion Chromatographic Results

Sample No.	Body Parts/ Eggs	Amount of Cr (VI) (mg/kg) by Photometry	Amount of Cr (VI) (mg/kg) by I. C
1	Liver	1.008 ± 0.12	3.456
2	Gizzard	0.646 ± 0.049	0.904
3	Albumen	0.415 ± 0.306	0.497
4	Albumen	1.03 ± 0.33	0.938
5	Yolk	0.191 ± 0.03	0.36



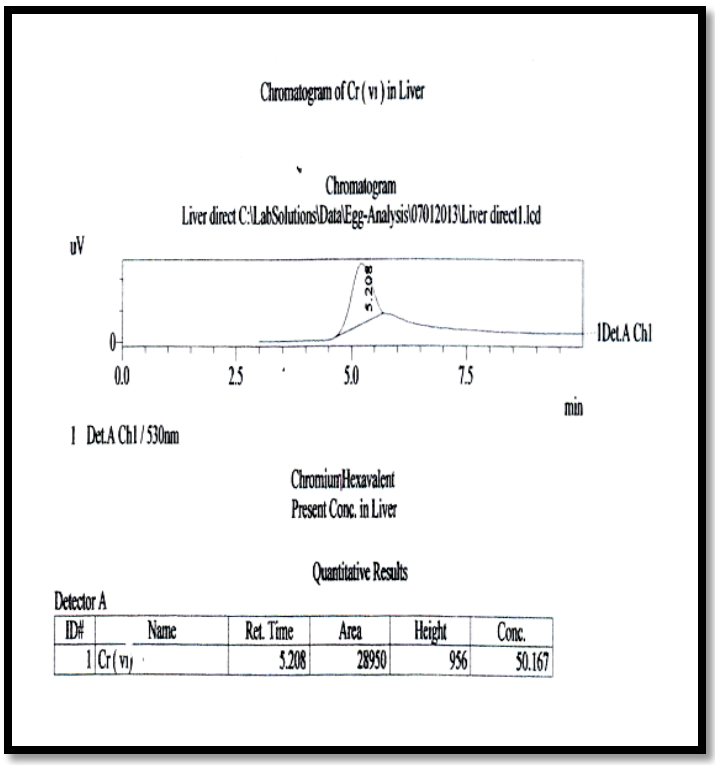
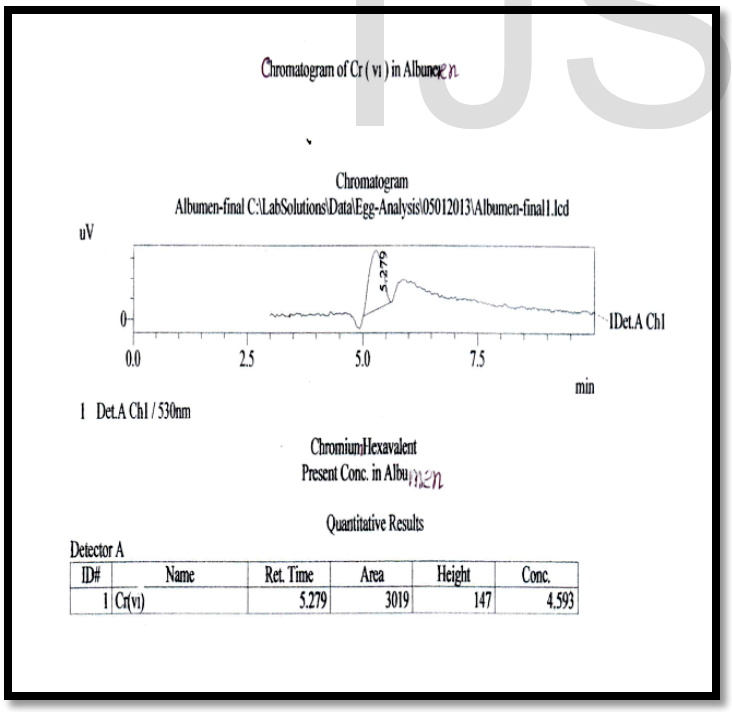


Fig.1. Ion chromatography peaks for gizzard and liver



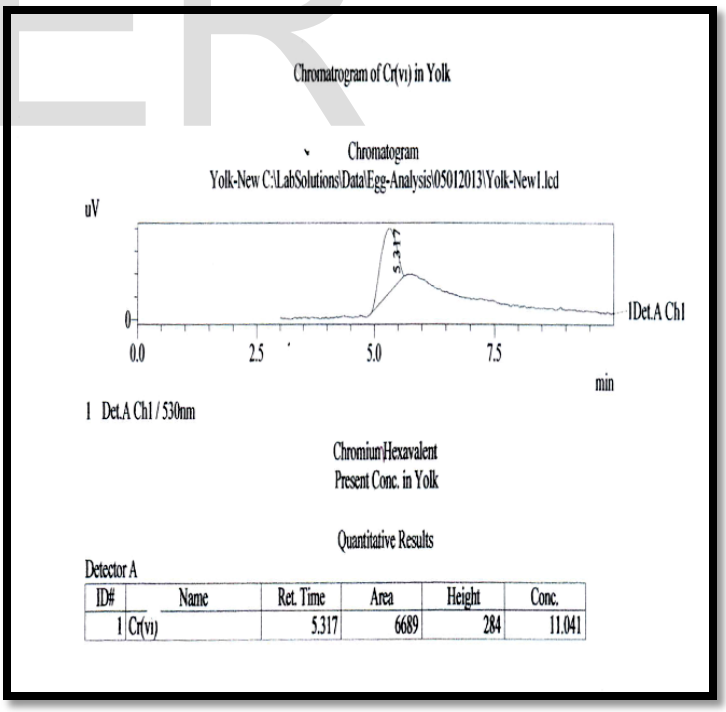


Fig.2. Ion chromatography peaks for albumen and yolk

It would be seen that Ion Chromatography has given higher amounts than the spectrophotometry except for a sample of egg albumen. This is in agreement with literature values. According to the New Jersey Chromium Workgroup [12], higher results are obtained by Method 7199 than by Method 7196A. This is expected in view of the fact that reduction of Cr (VI) occurs when reducing material from the matrix reacts with Cr (VI) during the neutralization process. In the present circumstances, chicken parts/eggs contain a substantial amount of reducing material. The Method 7199 removes some of these reductive species through use of a guard column in the front end of the instrumentation.

3.3 Relation between the Age of the Chicken and the Amount of Cr (VI) in Different Body Parts

Age of the chicken seems to be an important factor in the accumulation of Cr (VI) in different body parts. Most of the high values of Cr (VI) have come from chickens of relatively mature age (1 year and above), but there are exceptions. It must be mentioned, however, that the information about the age of a chicken was obtained from whom the chickens were bought and is likely to be inferred rather than accurate.

3.4. Variation over the Region

As expected, there is random variation of Cr (VI) content with different regions of the country. Barishal has the highest value for liver and the next two amounts come from Dhaka. The highest value for gizzard is from Chittagong; again the next three values come from Dhaka. Chittagong holds the top position for flesh, followed closely by Rajshahi. Dhaka has the highest value for brain.

3.5. Amount of Cr (VI) in Different Body Parts

The highest value for Cr (VI) in the liver as found in the present study is in general agreement with literature values [5]. It is well known that liver is the organ where detoxification takes place. Thus the accumulation of a toxic substance like Cr (VI) in liver is easily explained. Gizzard is the organ where the food is processed at the initial stage and may contain some Cr(VI). Flesh is basically protein and contains a lot of moisture. Hexavalent chromium, being soluble in water, is expected to be present in flesh in some quantities. Brain is small in size and mainly contains lipid. Inorganic substances are not that likely to be present in significant quantities.

Only a limited number of eggs from Dhaka have been analyzed. Both method 7196A and method 7199 have detected small amounts of Cr (VI) in some albumen and yolk. Hossain et al, through a comprehensive study on a large number of eggs, have found the presence of total Cr at an average level of 1.9 ppm [2].

3.6 Health Risk due to the Presence of Cr (VI)

The Office of Environmental Health Hazard Assessment (OEHHA), California has set the maximum allowable dose level (MADL) for chromium (hexavalent compounds) as 8.2µg/day by the oral route of exposure [13]. The MADL for a week would be 57.4 µg. If we assume the average amount of Cr (VI) present in chicken to be 1 ppm, one needs to consume only 57.4 g of chicken per week to reach this limit.

The ion chromatographic value for albumen is around 0.5 ppm. This means that about 115 g per week will exceed this limit. From this calculation, it seems obvious that on an average, most people of Bangladesh are taking great risk in consuming chicken and eggs. Cr (VI) being mutagenic, future generation is also liable to be affected.

Conclusion

Most of the liver and gizzard samples and about 75% of flesh samples have been found to contain Cr (VI). This is an alarming situation. Poultry feed, manufactured from solid tannery waste of Hazaribag, is distributed throughout the country. To prevent Cr (VI) from entering the food chain, either or both of the following steps may be taken:

Manufacturing process of poultry feed should be engineered in such a way that minimum conversion of Cr (III) to Cr (VI) takes place.

Cr (VI) has to be eliminated from the feed through appropriate treatments.

The third option of stopping altogether the production of poultry feed from solid tannery waste may not be economically attractive.

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