The Distribution of Thiocyanate in the Serum, Sections of the Digestive Tract and some Organs of Domestic Chicken (Gallus domestic) Given Different Concentration of Cyanide Directly and in their Feed

Kadiri Helen Ejiro Asagba Samuel Ogheneovo
Department of Biochemistry Delta State University Abraka Nigeria

Abstract
The distribution pattern of thiocyanate a metabolic product of cyanide detoxification was examined in the oesophagus, crop, proventriculus, ileum, duodenum, large intestine, gizzard, caeca and cloaca (sections of the digestive tract) as well as in the serum, liver, kidney heart and brain of the domestic chicken (Gallus domesticus) given different doses (1, 2 and 3 mg kg\(^{-1}\) body weight of cyanide) directly (by gavage) and in the diet for different time period (4, 8 and 12 weeks) was investigated in this study. A total of eighty-four-day-old birds were used for this experiment. The chicks were divided into seven groups of twelve birds each: Group I - normal control, Group II, III and IV - received 1, 2 and 3 mg CN kg\(^{-1}\) b.w. as Sodium cyanide (NaCN) directly respectively, while Group V, VI and VII received 1, 2 and 3 mg CN kg\(^{-1}\) b.w. as NaCN in their feed respectively. Thiocyanate concentration in the tissues was determined by Essers method. The study revealed that the accumulation and distribution of CN\(^{-}\) and Thiocyanate in the organs and sections of the digestive tract was influenced by time and mode of exposure. Irrespective of the duration of exposure, the duodenum had the highest concentration of cyanide, in birds offered cyanide in their diet, conversely after 12 weeks the ileum had the highest concentration in birds treated with CN\(^{-}\) directly. Irrespective of the mode or duration of exposure also, the cloaca had the highest concentration of Thiocyanate as compared to other sections of the digestive tract of the bird. In addition at each concentration of exposure, the level of cyanide in the organs decreased after 12 weeks of exposure and irrespective of the mode or dose of exposure, the kidney had the highest CN\(^{-}\) level, while the liver had the highest Thiocyanate level. Thiocyanate concentration was also found to be significantly higher in the serum, sections of the digestive tract and organs of those given cyanide directly compared with those given cyanide contaminated feed.

Keywords: Cyanide, Thiocyanate, domestic chicken, digestive tract

Introduction
Cyanide is well known among the public as a very poisonous substance. However man and animals are regularly ingesting variable levels of cyanide in certain natural diets (Gee, 2010; Nwaichi, 2013). Indeed estimates suggest that the amount taken in some of such diets are sometimes considerable (Orjiekwe et al., 2013; Guede et al., 2013)

The major sources of cyanide in the diet are cyanogenic glycosides and an example of a cyanogenic plant is cassava (Manihot esculenta crantz), which contains linamarin and lotaustrali (Cho et al 2013; Orjiekwe et al., 2013). This food plant is a favoured crop for farmers in Africa because it is tolerant to poor soil conditions and adverse growing conditions(Cooke and Coursey, 1981). Others include common foods such as sorghum, linseed, maize, millet, sweet potatoes and bamboo shoots(Cho et al., 2013; Cressey, 2013). The maximum yield of cyanide from some of these sources could be as high as 100-300mg/100g of tissue (Nwaichi, 2013; Nunn et al., 2010). The high demand for cereals due to increasing human population and their use by millers for compounding livestock feeds coupled with the need for livestock products have led to the use of unconventional feeds for animal production (Tewe, 1994). These unconventional feed materials include sorghum, spent grains and wheat offals (by-product of sorghum and wheat malting respectively) as well as cassava (Adeyemo et al., 2014; Ukwuru and Egbonu, 2013; Okafor and Nwabuko, 2003). As a result of the increasing use of cassava in animal feeding there is greater exposure to dietary toxins from cyanogenic glycosides. It is generally accepted that the toxicity of cyanogenic glycosides is due entirely to the release of free cyanide(Adekanye et al., 2013; Davis, 1981). The use of cassava in animal feeds presents two major problems: the presence of cyanogenic glycosides in the tuber, and the remarkably low protein levels in fresh and dried cassava(Aro et al., 2010; Oppong- Anane, 2010). The goitrogenic effects of cassava are well documented (Aro et al., 2010; Davis, 1981) and the release of cyanide could contribute to the detrimental effects which can occur when intake of this food is not held at a low level(Davis, 1981). Many studies have reported the death of birds from cyanide poisoning through several routes, including exposure to cyanide salts or ingestion of cyanogenic plants(Henny et al 1994; Ma and Pristos, 1997).

The enzyme rhodanese (E.C. 2.8.1.1., Thiosulphate:cyanide sulphurtransferase) which is a ubiquitous enzyme has been known to be responsible for the biotransformation of cyanide to thiocyanate(Okonji et al.,
2010; Preston et al., 2013) and the liver has been considered to be the major source of rhodanese and is also believed to be the major site of cyanide detoxification, however some researchers have shown that considerable variations exist in rhodanese distribution in different parts of the digestive parts of the chicken at different ages (Al-Qarawi et al., 2001; Aminlari et al., 1997). There have also been studies on the distribution of cyanide radical in the tissues of animals (NTP, 1993; Borrowitz et al., 1994; Idonijea et al., 2013) and it has also been shown that the thiocyanate formed from cyanide in the mammalian tissue is largely secreted into the stomach contents of rats and rabbits, but reabsorbed in the gut to be partly excreted in the urine and partly reabsorbed into the stomach contents (Okoh and Pitt, 1982). However, with respect to birds, there have been limited studies on the distribution of thiocyanate in the digestive tract of the bird.

Animal Procurement
A total of eighty four one-day old birds purchased from Zartech farms Sapele, Delta state, Nigeria, were used for the study. The birds used in this study were maintained in accordance with the guidelines approved by the animal ethical committee, Delta state university Abraka, Delta State Nigeria. The chicks were kept in a standard wooden cage made up of wire gauze net and solid woods. The chicks were fed with starters mash for three weeks and thereafter, they were fed with growers mash, both mash were purchased from Top feeds, PLC, Sapele, Delta state, Nigeria. The chicks were also given tap water ad libitum

Experimental Design
The chicks used for the experiment were divided into seven groups of twelve birds each, the groups were given the following treatments. The chicks were brooded on deep litter using 100 watt bulbs, flat plastic feeders and shallow drinkers for the first two (2) weeks of the experiment. The birds were fed starter mash experimental diets for four (4) weeks. Feed and water were provided ad-libitum. The birds were vaccinated against gumboro disease at the second and fourth weeks of age as first and second doses respectively. The experimental birds (Group II – IV) were intoxicated with cyanide every morning using gavage but they are fed with normal mash and tap water. Groups V-VII experimental birds were fed with different concentrations of cyanide in their feed every morning and normal tap water. The weights of the chicken were taken before administering the cyanide every morning. A third of the birds in each group was given this treatment for four weeks, while another third was for eight weeks. The final third in each group was treated for twelve weeks. Thus each group had four birds for each of the duration of exposure. The treatments are as shown below

Group I- normal control.
Group II - received 1 mg CN/kg body weight directly
Group III - received 2 mg CN/kg body weight directly
Group IV – received 3 mg CN/kg body weight directly
Group V – exposed to 1 mg CN/kg feed
Group VI – exposed 2 mg CN/kg feed
Group VII – exposed 3 mg CN/kg feed

Collection of Samples
After completing the duration specified for each sub group chicks were weighed and sacrificed under anaesthesia and sections of the digestive tracts and organs were collected.

The tissues of the digestive tract of the chicken were weighed and 20% homogenates were prepared using 10% sucrose solution. The homogenates were centrifuged and the supernatants obtained were used for biochemical analysis.

Estimation of Thiocyanate
Thiocyanate was measured in the oesophagus, is oesophagus, crop, proventriculus, ileum, duodenum, large intestine, gizzard, caeca and cloaca by spectrophotometric (Sorbo 1953)

Results
The distribution of thiocyanate in the different sections of the digestive tracts of birds is presented in Table 1. Thiocyanate was present in all sections of the digestive tract of the birds in all groups including the control birds at 4, 8 and 12 weeks of exposure. Significant increases (p<0.05) in thiocyanate levels in the different sections of the digestive tract were observed in all the treated groups except group V relative to control group value. The thiocyanate levels in the different sections of the digestive tract of birds given 3mg/CNkg body weight were found to be significantly higher (p<0.05) than those of birds given lower doses, when analysed at the end of each exposure period. Similarly the thiocyanate levels in most sections of the digestive tract of birds exposed 3mg CN tainted food were significantly higher (p<0.05) than those of birds fed lower cyanide in food for the different periods of exposure. In addition, the thiocyanate levels in the different sections of the digestive tract of birds fed cyanide in their diet were lower than that of birds given corresponding levels of cyanide directly.
The pattern of distribution of thiocyanate in the sections of the digestive tract after 4 weeks of direct exposure is cloaca > proventriculus > ileum > duodenum > large intestine > gizzard > crop > caeca > gizzard > oesophagus for 1mg/kg body weight (group II); cloaca > proventriculus > ileum > large intestine > duodenum > crop > caeca > gizzard > oesophagus for 2mg/kg body weight (group III); cloaca > proventriculus > ileum > large intestine > duodenum > crop > caeca > oesophagus for 3mg/kg body weight (group IV). Similarly the pattern of thiocyanate distribution in the digestive tract of birds exposed to cyanide for 4 weeks is cloaca > proventriculus > ileum > duodenum > large intestine > caeca > gizzard > crop > oesophagus for 1mg CN tainted diet (group V); cloaca > proventriculus > ileum > duodenum > crop > large intestine > caeca > gizzard > oesophagus for 2mg CN tainted diet (group VI) and cloaca > proventriculus > ileum > duodenum > crop > large intestine > caeca > gizzard > oesophagus for 3mg CN tainted diet (group VII).

Birds exposed directly to cyanide for 8 weeks had the following pattern of distribution of thiocyanate: cloaca > proventriculus > ileum > caeca > crop > large intestine > gizzard > duodenum > oesophagus for 1mg/kg body weight (group II); cloaca > proventriculus > ileum > caeca > crop > large intestine > duodenum > gizzard > oesophagus for 2mg/kg body weight (group III) and cloaca > proventriculus > ileum > caeca > crop > large intestine > duodenum > gizzard > oesophagus for 3mg/kg body weight (group IV). Similarly the pattern of distribution of thiocyanate in the organs of birds given cyanide contaminated feed for 8 weeks is cloaca > proventriculus > ileum > crop > large intestine > gizzard > duodenum > oesophagus for 1mg CN tainted feed (group V); cloaca > proventriculus > ileum > crop > caeca > large intestine > gizzard > oesophagus > duodenum for 2mg/kg body weight (group VI); cloaca > proventriculus > ileum > crop > large intestine > caeca > gizzard > oesophagus > duodenum for 3mg CN tainted diet (group VII). While birds exposed to cyanide for 12 weeks directly had the following pattern of distribution of thiocyanate in their organs: cloaca > proventriculus > ileum > large intestine > crop > caeca > gizzard > duodenum > oesophagus for 1mg/kg body weight (group II); cloaca > proventriculus > ileum > crop > large intestine > gizzard > caeca > oesophagus > duodenum for 2mg/kg body weight (group VI); cloaca > proventriculus > ileum > crop > large intestine > caeca > gizzard > oesophagus > duodenum for 3mg/kg body weight (group VII). Similarly the pattern of distribution of thiocyanate in birds given cyanide contaminated diet for 12 weeks is cloaca > proventriculus > ileum > large intestine > crop > caeca > gizzard > oesophagus for 1mg CN tainted feed (group V); cloaca > proventriculus > ileum > large intestine > crop > caeca > duodenum > gizzard > oesophagus for 2mg/kg body weight and cloaca > proventriculus > ileum > large intestine > crop > caeca > duodenum > gizzard > oesophagus for 3mg/kg body weight and cloaca > proventriculus > ileum > large intestine > crop > caeca > duodenum > gizzard > oesophagus for 3mg CN tainted feed (group VII). These results show that irrespective of the mode or duration of exposure the cloaca had the highest concentration of thiocyanate as compared to other sections of the digestive tract of the bird. The study also shows less thiocyanate in all sections of the digestive tract of birds exposed to cyanide via feed compared to those exposed to the toxicant directly.

The distribution of thiocyanate in the sera and organs of birds is shown in Table 4.3. Significant increase (p<0.05) in serum thiocyanate at the end of 4 weeks of exposure were observed in all the treated groups relative to control. At the end of 8 weeks of exposure, significant increases in serum thiocyanate were observed in all the treated groups except groups II and V. At the end of 12 weeks of exposure significant increases (p<0.05) in thiocyanate was observed in the sera of all the treated groups when compared with the control. The serum thiocyanate level in birds administered 3mg/ CN kg body weight was significantly higher (p<0.05) than those of birds given lower doses of cyanide directly at the end of all exposure intervals. Similarly the level of serum thiocyanate in birds exposed 3mg CN tainted food was significantly higher (p<0.05) than those of birds exposed to lower cyanide in their diet. However the serum thiocyanate levels of birds fed cyanide in food were lower than birds given corresponding levels of cyanide directly when thiocyanide was analysed at the end of each exposure interval.

Thiocyanate was also present in all the organs of the birds in all the groups including the control birds at the end of exposure all exposure intervals. However there was a significantly higher (p<0.05) thiocyanate concentration in the organs of all treated birds when compared with the control at the end of all exposure interval except in group V (liver, pancreas and brain at the end of week 4 and liver, pancreas, kidney and heart at the end of week 8 and 12). Like in the serum, the thiocyanate contents of the organ of birds given 3mg/kg body weight directly and via food were higher than that of those given lower doses of the toxicant. Moreover the thiocyanate contents of organs of bird given cyanide containing feed were lower than those treated with corresponding level of cyanide directly during the exposure periods.
The distribution of thiocyanate in different sections of the digestive tract.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>DIRECT EXPOSURE</th>
<th>EXPOSURE VIA FOOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organ</td>
<td>(+CN)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Group I</td>
<td>Group II</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>(+CN)</td>
</tr>
<tr>
<td>Oesophagus</td>
<td>0.35±0.0^a</td>
<td>0.55±0.0^a^b</td>
</tr>
<tr>
<td>Crop</td>
<td>0.80±0.0^a</td>
<td>1.08±0.0^a</td>
</tr>
<tr>
<td>Gizzard</td>
<td>0.73±0.0^a</td>
<td>1.15±0.0^a</td>
</tr>
<tr>
<td>Proventriculus</td>
<td>1.68±0.0^e</td>
<td>2.13±0.0^e</td>
</tr>
<tr>
<td>Ileum</td>
<td>1.51±0.0^a</td>
<td>1.98±0.0^a</td>
</tr>
<tr>
<td>Duodenum</td>
<td>1.18±0.0^a</td>
<td>1.75±0.0^a</td>
</tr>
<tr>
<td>Large Intestine</td>
<td>1.19±0.0^a</td>
<td>1.39±0.0^a</td>
</tr>
<tr>
<td>Caeca</td>
<td>0.87±0.0^a</td>
<td>1.04±0.0^a</td>
</tr>
<tr>
<td>Cloaca</td>
<td>1.82±0.0^a</td>
<td>2.22±0.0^a</td>
</tr>
</tbody>
</table>

The results are expressed as mean± Standard Deviation (n=4) for organs.

Thiocyanate concentrations are expressed in µg/g tissue for the organs.

Values not sharing a common superscript on the same horizontal row differ significantly (P<0.05).

The distribution pattern of thiocyanate in the organs of birds given cyanide directly for 4 weeks of exposure is as follows: liver > heart > pancreas > brain > kidney for 1mg/kg body weight (group II); liver > heart > pancreas > kidney > brain for 2mg/kg body weight (group III); liver > heart > pancreas > brain > kidney for 3mg/kg body weight (group IV). While the pattern of thiocyanate distribution in the organs of birds offered cyanide in vivo for 8 weeks is liver > pancreas > heart > brain > kidney for 1mg/kg body weight (group II); liver > heart > pancreas > brain > kidney for 2mg/kg body weight (group III); liver > heart > pancreas > brain > kidney for 3mg/kg body weight (group IV). Thiocyanate distribution pattern in birds given cyanide directly for 12 weeks was the same as that of birds given cyanide directly for 4 weeks except in group II birds the distribution pattern is as follows: liver > heart > pancreas > brain > kidney. Also, the pattern of distribution of thiocyanate in the organs of birds offered cyanide in their food for 4 weeks of exposure is liver > pancreas > heart > brain > kidney for 1mg/kg body weight (group V); liver > pancreas > heart > brain > kidney for 2mg/kg body weight (group VI) and pancreas > heart > liver > brain > kidney for 3mg/kg body weight (group VII). Similarly the pattern of thiocyanate distribution in organs of birds given cyanide in their diet for 8 weeks is liver > pancreas > heart > brain > kidney for 1mg/kg body weight (group V); liver > pancreas > heart > brain > kidney for 2mg/kg body weight (group VI) and liver > pancreas > heart > brain > kidney (group VII). While the pattern of distribution of thiocyanate in the organs of birds given cyanide in their food for 12 weeks is liver > pancreas > heart > brain > kidney for 1mg/kg body weight (group V); liver > pancreas > heart > brain > kidney for 2mg/kg body weight (group VI) and liver >
heart > pancreas > brain > kidney for 3mg/kg body weight (group VII). Thus this study reveals that regardless of the mode or duration of exposure the liver accumulated the highest level of thiocyanate as compared to other organs. Again the thiocyanate concentration in the liver and other organs of birds exposed to cyanide treated diet is less than that of the birds given corresponding concentration of cyanide directly.

Table 2: The distribution of thiocyanate in the serum and organs of birds exposed to cyanide directly and in their feed.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>DIRECT EXPOSURE</th>
<th>EXPOSURE VIA FOOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organ</td>
<td>Group I (+CN)</td>
<td>Group II (+CN)</td>
</tr>
<tr>
<td>4weeks exposure</td>
<td>Serum</td>
<td>0.80±0.05a</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>0.85±0.06a</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>0.47±0.04a</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>0.71±0.03a</td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td>0.80±0.35a</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>1.27±0.40a</td>
</tr>
<tr>
<td>8weeks exposure</td>
<td>Serum</td>
<td>0.70±0.57a</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>0.71±0.05a</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>0.48±0.08a</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>0.20±0.02a</td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td>0.59±0.08a</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>0.91±0.05a</td>
</tr>
<tr>
<td>12weeks exposure</td>
<td>Serum</td>
<td>0.60±0.08a</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>0.70±0.09a</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>0.55±0.07a</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>0.40±0.04a</td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td>0.30±0.09a</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>0.53±0.07a</td>
</tr>
</tbody>
</table>

The results are expressed as mean± Standard Deviation (n=4)
Thiocyanate concentration is expressed in mg/g tissue for the organs and mg/ml for serum
Value not sharing a common superscript on the same row differ significantly (P<0.05)

Discussion
The higher level of thiocyanate in the serum of cyanide treated groups (Table 2) when compared with the control is an indication of the attempt of the birds to detoxify ingested cyanide. Thiocyanate is the main detoxification product of cyanide in animals(Weimeyer et al., 1986; Okoh and Pitt, 1981). It is also known to be the most reliable biomarker for cyanide exposure being a stable metabolite of this radical(Okafor et al., 2006; Haque and Bradbury, 1999; Rosling, 1994). The plasma half-life of thiocyanate has been reported to be 3 days(Rosling, 1994) and levels therefore reflect the mean daily load.

In the present study thiocyanate was found to be high in the liver (Table 2) and this is consistent with other studies that the liver is the main site of metabolism and detoxification in animals(Rosling, 1994) Like in the case of cyanide, the route-specific difference may be related to first-pass metabolism in the liver, following oral dosing, and initial deposition at the portal of entry, following exposure. High thiocyanate concentration was also observed in the heart and this might reflect the high demands of this organ for energy that is provided mostly by the aerobic pathways. (Oh et al., 1977; Ogata and Volini, 1990).

The significantly high thiocyanate level (Table 1) observed in the cloaca is probably because this is where there is a mixing of the digestive wastes together with wastes from the urinary system (urates).Previous studies by several researchers particularly in other vertebrates has shown a high level of thiocyanate in the urine of animals. The high level of thiocyanate also observed in the proventriculus is also not unexpected as this organ is the first section of the digestive tract in which feed is digested. In the proventriculus food is digested through
the secretion of pepsin and hydrochloric acid (Duke, 1986). The acidic condition in this section might result in spontaneous hydrolysis of cyanogenic glycoside and liberation of cyanide which is easily absorbed (Conn, 1978). Presence of high thiocyanate level in the proventriculus of poultry, particularly in sub-mucosal layers (Aminlari et al., 1997), probably ensures cyanide detoxification by rhodanese before it reaches general circulation and this is consistent with other studies (Oh et al., 1977; Aminlari and Shahbazi, 1994).

The result also showed that irrespective of the age of the bird the distribution pattern of thiocyanate in the digestive tract of the bird was consistent unlike the distribution pattern of rhodanese enzyme that was reported to vary with age (Al-Qarawi et al., 2001; Aminlari et al., 1997).

**Conclusion**

Thiocyanate (SCN) was found to be present in the serum, sections of the digestive tract and organs of both treated birds and control. However, accumulation and distribution of cyanide and thiocyanate in the serum, digestive tract and organs of the domestic chicken exposed to different concentrations of cyanide directly and in their feed was influenced by time and mode of exposure. Highest level of cyanide level was indicated in the kidney after 12 weeks of exposure among the organs while in the digestive tract, the duodenum had the highest concentration in those given cyanide in their diet and the ileum in those given cyanide directly irrespective of the mode of exposure. Thiocyanate concentration was found to be highest in the liver amongst the organs investigated and in the cloaca in the sections of the digestive tract. The study reveals also reveals that the mode of metabolism of cyanide in birds is quite similar to that of other vertebrates and that the degree of toxicity of cyanide in birds is a function of its mode of administration.

**Acknowledgements**

I wish to appreciate Tetfund for their sponsorship

**References**


Toxicol. Cyanides. 16: 209-216


The IISTE is a pioneer in the Open-Access hosting service and academic event management. The aim of the firm is Accelerating Global Knowledge Sharing.

More information about the firm can be found on the homepage: http://www.iiste.org

CALL FOR JOURNAL PAPERS

There are more than 30 peer-reviewed academic journals hosted under the hosting platform.

Prospective authors of journals can find the submission instruction on the following page: http://www.iiste.org/journals/ All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Paper version of the journals is also available upon request of readers and authors.

MORE RESOURCES

Book publication information: http://www.iiste.org/book/

Academic conference: http://www.iiste.org/conference/upcoming-conferences-call-for-paper/

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digital Library, NewJour, Google Scholar