COMPARATIVE STUDY ON THE EFFECT OF DIRECT AND INDIRECT CYANIDE ORAL ADMINISTRATION ON THE HEALTH OF BROILER BIRDS IN RELATION TO ENERGY

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Abstract: The study aimed at comparing the effect of direct and indirect cyanide oral administration on the health of broiler birds in relation to energy levels. Eighty four day old chicks were used for this study. They were divided into seven groups. Group 1- served as control. Groups 2 – 4 was given sub -lethal dose of cyanide. 1, 2 and 3 mg CN/kg body weight by gavage, respectively in form of sodium cyanide (NaCN). Groups 5, 6 and 7 were exposed to sub lethal dose of 1, 2 and 3 mg CN/kg in form of NaCN respectively, mixed directly in their feed. The result shows that at the end of 12 weeks exposure to cyanide, regardless of the mode or duration of exposure serum levels of Glucose, Lactate dehydrogenase, uric acid, urea and creatinine increased significantly when compared with the control. However a significant decrease in Total Protein was indicated in all the treated Groups when compared with the control. In addition a less pronounced biochemical effects was produced in birds when cyanide was given in their diet compared with the effects when it was given directly. In conclusion the study indicates that cyanide is able to alter energy levels in birds as well as negatively impact the health status in broiler birds irrespective of the mode and duration of exposure.

Keywords: Creatinine, cyanide, glucose, domestic chicken, lactate dehydrogenase, urea

Introduction

Compounds which release hydrogen cyanide on hydrolysis by appropriate enzymes are known as Cyanogenic compounds (Cho *et al.*, 2013). These are found in foods such as Sorghum, bamboo, lima beans and cassava. The prevailing economic situation in developing countries has resulted in the use of cassava as a source of carbohydrate as well as other non-convectional foods in the production of animal feeds (Okeke *et al*., 1985). The cyanide content in some of the cassava products vary between 20 – 60 mg/kg wet weight (Okafor, 2005). Cyanide poisoning has also been implicated in the death of migratory birds in the areas surrounding a gold mine extraction facility as well as tailing pond (Clark & Hothem, 1991). It has also been suggested that drinking of lethal cyanide solutions by animals may not cause death immediately, however, a sufficiently low cyanide level may result in death later through the liberation of additional cyanide by the gastric acid (Clark & Hothem, 1991; Bhattacharya, 2018). Symptoms of cyanide poisoning in birds includes salivation, shortness of breath, weakness and blinking of the eyes. These symptoms occur between thirty seconds and five minutes in very sensitive species. At higher doses of cyanide the breathing becomes more laboured, then gasping and eventually death occurs (Wiemeyer *et al*., 1986; Rice *et al.*, 2018).

Total protein is the biochemical test used in the measurement of serum proteins. Albumin and globulin are serum proteins, and the concentration of these proteins in the serum is clinically important. Causes of high serum total protein include severe kidney disease, chronic infection liver dysfunction and cancer of the white blood cells (Slightam, 2012).

Lactate Dehydrogenase is present in most organisms, including plants and animals. It converts pyruvate to lactate in the absence of oxygen or when oxygen is in short supply and it also performs the reverse action in the liver (Nelson & Cox, 2000). Three different pathways by which pyruvate metabolic levels can be utilized in a cell are; LDH conversion of pyruvate to lactate, glucose generation by gluconeogenesis and acetyly-coA formation. Lactate on the hand is released into the bloodstream where it is converted to glucose by the red blood cells and the skeletal muscles. Hence, metabolic pathways are affected by the modulation of pyruvate or lactate cellular status (Plummer, 1999).

Creatinine, uric acid and urea are primarily used to assess kidney function. The kidney plays an important role in their metabolism and excretion. Serum creatine phosphokinase activities measurement is valuable in the diagnosis of skeletal and cardiac muscle disorders (Wyss & Kaddurah-Daouk, 2000). The study therefore aims at comparing the effect of direct and indirect cyanide oral administration on the health of broiler birds in relation to energy levels.

Materials and Methods

Toxicants

Sodium cyanide (NaCN) (96% maximum limit of impurities, chloride 0.1%, iron 0.02%, lead 0.002%, sodium 0.5%) produced by BDH chemicals Ltd Poole England was procured and used.

Preparation of stock solution and feed

Stock solution was prepared by dissolving sodium cyanide in distilled water in standard volumetric flask and the required volume was drawn from this stock solution; while the feed was prepared by mixing the required dose with the broilers feed The sub-lethal concentration of potassium cyanide used in this study was 1, 2 and 3 mg/L of distilled water and 1, 2 and 3 mg/kg of feed.

Experimental design

Eighty four (84) day old broiler chicks were used for this study. The birds were bought from Zartech Sapele, Delta state. The birds were kept in standard bird cages made with iron rods. All the birds were fed with starters feed for 4 weeks, growers mash from 4 to 8 weeks and finally finishers mash from 8 – 12 weeks; all feeds used were bought from top feed Nigeria. The broiler chicks were randomly assigned into seven groups consisting of four birds each. Treatments were administered as follows:

Group 1- served as normal control. They were given distilled water in their drinkers throughout the duration of the experiment.

Group 2 - given 1 mg CN/kg body weight orally by gavage as NaCN for 4weeks

Group 3 - given 2 mg CN/ kg body weight directly by gavage as NaCN for 8 weeks

Group 4 – given 3 mg CN/kg body weight directly by gavage as CN for 12 weeks

Group 5 – exposed to 1 mg CN/ kg feed as NaCN for 4 weeks

Group 6 – exposed to 2 mg CN/kg feed as NaCN for 8 weeks

Group 7 – exposed to 3 mg CN/kg feed as NaCN for 12 weeks

The chicks were weighed before cyanide administration every morning. 28 Chicks comprising of 7 chicks selected randomly from each group was given this treatment for 4 weeks, while the other 28 chicks was treated for 8 weeks and the last 28 chicks from each group was treated for twelve weeks. The total duration of the experiment was for 12 weeks

Collection of blood samples

At the end of each phase of the experiment (4, 8 and 12 weeks) four broilers from each group was sacrificed by cervical decapitation and blood samples collected into sterile tubes that did not contain anticoagulants. The blood was then centrifuged at a speed of 3000 g for 15 min and the resulting sera collected for biochemical analysis.

Biochemical analysis

The sera collected was labelled and stored in ice packs until needed. The level of Total protein Glucose and Lactate dehydrogenase activity was determined spectrophotometrically according to the method of Tietz (1990). Assay for Creatine activity was also by the use of spectrophotometer according to the method of Bartels & Bohmer (1952) and assay for Urea and Uric acid was also by the use of the spectrophotometer according to the method of Tietz (1995).

Results and Discussion

One of the most sensitive indicators of kidney injury is an increase in urea and creatinine levels in the serum. Serum uric acid and creatinine is also used as a rough index of Glomerular filteration rate. The levels of some biochemical parameters in the serum of birds exposed to cyanide are shown in Tables 1 and 2. A significant increase ($p<0.05$) in serum uric acid was observed in groups III, IV and VII relative to control for each duration of exposure. Also significant increase ($p<0.05$) in serum urea, glucose, creatinine and lactate dehydrogenase was also indicated in all the treated groups except groups II, V and VI after each period of exposure. Serum uric acid, urea, glucose, creatinine, and lactate dehydrogenase level in the birds administered 3 mg/kg body weight were significantly higher ($p<0.05$) than those of birds given lower doses of cyanide directly for each period of exposure. Similarly the levels of serum uric acid, urea, glucose, creatinine, and lactate dehydrogenase in birds administered 3 mg CN tainted food is significantly higher ($p<0.05$) than those of birds fed lower cyanide in their diet. However the serum uric acid, urea, glucose, creatinine, and lactate dehydrogenase levels of birds exposed to cyanide via food was lower than birds given corresponding levels of cyanide directly. The increase in serum uric acid and urea activity observed in this study indicates a damage to the kidney of birds this agrees with the findings of Elsaid and Elkomy (2006), who reported that cyanide induced nephrotoxicity was reflected by the observed increase in serum uric acid level, also increase in serum urea level has been reported to be due to some degenerative changes observed in the kidney of cyanide fed rabbits (Okolie & Osagie, 2000). However, it is contrary to that of Tulswani *et al*. (2005) who reported no significant change in the level of blood urea following cyanide exposure (7.0 mg/kg for 14 days) in rats. Increase in plasma creatinine levels is usually associated with impairment of renal function (Bishop *et al*., 2005). These findings to some extent are in line with those reported in rat (Elsaid & Elkomy, 2006) and pigs (Manzano *et al*., 2007), where sub-lethal cyanide exposure caused significant elevation of serum creatinine concentration.

Table 1: A comparative study on the effect of cyanide on the kidney of cyanide exposed birds

Groups / Parameters	Direct Exposure				Food		
	Group I Control	Group II +CN	Group III +CN	Group IV +CN	Group V +CN	Group VI +CN	Group VII +CN
4 weeks exposure							
Uric acid (umol/l)	118.09±16^{a}	121.11±12^{a}	133.26±17^{b}	148.89±13^{b}	119.15±11^{a}	122.23±14^{a}	127.05±11^{b}
Urea (mmol/l)	0.64±0.07^{a}	0.71±0.03^{a}	0.86±0.06^{b}	1.21±0.04^{d}	0.68±0.05^{a}	0.75±0.03^{a}	0.98±0.06^{c}
Creatinine (mg/dl	0.32±0.04^{a}	0.38±0.03^{a}	0.46±0.06^{b}	0.60±0.05^{c}	0.36±0.05^{a}	0.39±0.06^{a}	0.48±0.05^{b}
Total protein (g/L)	4.19±0.04^{a}	4.05±0.06^{a}	3.92±0.05^{a}	3.44±0.04^{b}	4.13±0.03^{a}	4.03±0.04^{a}	3.85±0.06^{b}
8 weeks exposure							
Uric acid (umol/L)	122.09±23^{a}	125.21±15^{a}	132.36±12^{b}	142.42±23^{c}	123.18±11^{a}	124.42±24^{a}	128.10±11^{b}
Urea (mmol/l)	0.79±0.04^{a}	0.82±0.08^{a}	1.07±0.05^{b}	1.56±0.03^{c}	0.78±0.06^{a}	0.87±0.04^{a}	1.31±0.08^{c}
Creatinine (mg/dl)	0.38±0.05^{a}	0.44±0.04^{a}	0.59±0.05^{b}	0.89±0.06^{c}	0.43±0.04^{a}	0.49±0.05^{a}	0.67±0.04^{b}
Total protein (g/L)	4.29±0.04^{a}	4.03±0.06^{a}	3.97±0.05^{a}	3.49±0.04^{b}	4.13±0.03^{a}	3.98±0.04^{a}	3.55±0.06^{b}
12 weeks exposure							
Uric acid (umol/L)	132.17±23^{a}	135.08±15^{a}	143.76±12^{b}	158.89±23^{c}	127.25±11^{a}	132.63±24^{a}	140.10±11^{b}
Urea (mmol/l)	0.86±0.08^{a}	1.12±0.05^{b}	1.16±0.04^{b}	1.81±0.05^{c}	1.01±0.03^{a}	1.09±0.04^{b}	1.45±0.08^{d}
Creatinine (mg/dl)	0.46±0.23^{a}	0.62±0.21^{b}	0.93±0.05^{c}	1.41±0.08^{d}	0.52±0.05^{a}	0.63±0.08^{b}	0.82±0.06^{c}
Total protein (g/L)	4.39±0.04^{a}	4.18±0.06^{a}	4.02±0.05^{a}	3.51±0.04^{b}	4.31±0.03^{a}	4.22±0.04^{a}	3.94±0.06^{b}

The results are expressed as mean± Standard Deviation (n=4). Values not sharing a common superscript on the same row differ significantly ($p<0.05$) from each other

Table 2: A comparative study on the effect of cyanide on the level of glucose and lactate dehydrogenase activity in cyanide exposed birds

Groups / Parameters	Direct Exposure				Food		
	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
	Control	+CN	+CN	+CN	+CN	+CN	+CN
4 weeks exposure							
Glucose (mg/dl)	170.01±13^{a}	176.75±15^{a}	189.49±15^{b}	210.03±16^{c}	180.43±13^{a}	188.58±15^{a}	223.23±12^{c}
Lactate Dehydrogenase (LDH) (IU/L)	95.62 ± 5^{a}	100.23±8^{a}	115.35±9^{b}	123.37±5^{b}	97.76±3.1^{a}	108.17±8^{a}	118.39±6.8^{b}
8 weeks exposure							
Glucose (mg/dl)	174.37±17^{a}	181.32±12^{a}	187.42±18^{b}	209.63±13^{c}	178.03±12^{a}	180.87±18^{a}	210.86±12^{c}
Lactate Dehydrogenase (LDH) (IU/L)	167.4±23^{a}	184.62±15^{a}	211.1±24^{b}	241.71±42^{b}	163.1±25^{a}	171.9±32^{a}	197.21±.26^{b}
12 weeks exposure							
Glucose (mg/dl)	176.01±13^{a}	184.75±17^{a}	201.49±15^{b}	220.03±26^{c}	180.43±13^{a}	196.58±15^{b}	213.23±12^{c}
Lactate Dehydrogenase (LDH) (IU/L)	148.06±12^{a}	157.61±22^{a}	173.70±11^{b}	195.75±26^{c}	154.92±12^{a}	159.57±10^{a}	182.21±22^{d}

The results are expressed as mean± Standard Deviation (n=4) Values not sharing a common superscript on the same row differ significantly ($p<0.05$) from each other

Cyanide has been reported to alter glucose metabolism (Way, 1984).In this present study the significant increase in serum glucose may be due to increased gluconeogenesis as a result of increased energy needs. The hypothesis is supported by the observed increase in activity of aminotransferases in the organs which is necessary for the provision of intermediates for gluconeogenesis. Similar increases in blood glucose were previously reported in fishes, swine and rats (Tulswani *et al*., 2005; Sadati *et al*., 2013). However, results in goats, rats and rabbits (Okolie & Osagie, 2000; Soto-Blanco *et al*., 2002) indicated no significant increased blood glucose concentration, after cyanide ingestion. Another probable reason for the increased glucose concentration in the cyanide treated birds may be due to increased gluconeogenesis as a result of increased energy needs.

Lactate dehydrogenase activity has been shown by several researchers to increase due to cyanide intoxication (Okolie & Osagie, 2000; Al-Ghanin & Mahboon, 2012). The results of the present study is in harmony with these findings as serum LDH activity increased irrespective of the mode and duration of exposure to cyanide. The increase in LDH activity reflects increase dependence on anaerobic respiration for energy needs, a fact that is attested to by the increased serum glucose concentration. This further confirms the findings that the toxic effect of cyanide is attributed to its disruption of energy metabolism (Petersen, 2002).

Excretion of protein is currently one of the most sensitive indices of renal dysfunction (Ologunde *et al*., 2008). The data obtained showed a significant decrease ($p<0.05$) in total protein in groups IV and VII (exposed to 3 mg cyanide directly and via the diet, respectively) when compared with the control for each of the period of exposure. Serum total protein in birds given 3 mg CN directly and via food was also significantly lower ($P<0.05$) than those of birds given lower doses of cyanide The decrease in plasma total protein level in this study indicates renal dysfunction and this is in agreement with the findings of Prasad & Math, (1995) and Ghodsi & Baghshani (2013). Reduction in plasma total protein may be due to hyperproteinemia which may be occasioned by renal damage caused by cyanide toxicity (Ologunde *et al*., 2008).

The present study revealed a less pronounced biochemical effects are produced in broilers when cyanide is given in their feed compared with the effects when it is given directly by gavage. The reason for this observation may not be unconnected with the fact that cyanide mixed with food will be completely absorbed thus reducing its bioavailability as well as toxicity.

Conclusion

The study indicates that cyanide is able to alter energy metabolism in broiler birds. Also, established in this study is that cyanide exposure caused significant increase in serum glucose level in birds, irrespective of the mode and duration of exposure. Therefore the use of cyanide, either in the form of plants or indiscriminate contamination of water should be avoided were broiler birds are concerned.

Recommendation

The use of plant sources high in cyanide as bird feed should be avoided or reduced to a minimum due to its negative impact on the health of the birds.

Conflict of Interest

The author declares that this work does not have any conflict of interest.

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