Haematological Alterations in Kuroiler Chicks Exposed to Cadmium Acetate

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Abstract The current study was designed to evaluate the effect of cadmium on the haematological profile of Kuroiler chicks. Kuroiler chick is a diseases resistance hybrid variety developed in India and is more promising than broilers etc., in terms of both the protein content of the meat and the benefits it has on the poultry industry. 10 days old Kuroiler chickens were exposed to a single sub lethal dose (7 mg/kg body weight) of cadmium acetate and different blood parameters were evaluated after 20 and 40 days of exposure. The different parameters were analyzed and calculated using standard methods and formula. It was noted that cadmium has an effect on all the blood parameters. Treated groups revealed significant suppression of total erythrocyte count, haemoglobin, packed cell volume, mean corpuscular haemoglobin concentration and neutrophils. The total leucocyte count, mean corpuscular haemoglobin, mean corpuscular volume, lymphocytes, eosinophils, monocytes and basophils were found to be increased in treated groups. The drastic alterations in studied parameters indicated that cadmium has long term effect on the blood profile of chicken and thus ultimately affect the health status of the chick, the quality of meat and also hampers the poultry industry.

Keywords Cadmium, Erythrocytes, Haematological Profile, Haemoglobin, Kuroiler Chick, Leucocytes

1. Introduction

Poultry is one of the most important and fastest growing segments of the agriculture sector in India. India is world's fifth largest egg producer and the eighteenth largest producer of broilers [1]. Indian poultry has shown spectacular progress transforming itself from backyard farming to a dynamic and sophisticated agro-based industry. Poultry industry is advancing by improvement of genetic potential of new fowls' strains [2] to provide the

high quality with low cost protein requirements of the human population worldwide. A large chunk of Indian population consumes poultry eggs and broiler chicken; therefore, the quality of meat and eggs should be maintained.

The Kuroiler is a hybrid of chicken developed in India. Introduced in the early 1990's this breed was created by Vinod Kapur of Kegg Farms Private, Ltd., and the name is a portmanteau of Kegg and Broiler. Kuroiler are derived from crossing either coloured broiler males with Rhode Island Red females, or White Leghorn males crossed with female Rhode Island Red. Kuroiler, a dual purpose breed producing meat and egg, can live on a diet of kitchen and agricultural waste, and produce around 150 eggs per year whereas native Indian hens lay only 40 eggs per year. Due to its unique genetic features, the Kuroiler is resistance to diseases and is a potential bio convertor of no cost agricultural, household and natural waste into human protein food and substantial incomes for rural household.

Kuroiler have been become popular in rural areas in India, including Uttar Pradesh, Jharkhand, West Bengal, Mizoram, Chhattisgarh, Meghalaya and Uttarakhand. The Govt. of Uganda had imported Kuroiler hatching eggs and the Kuroilers outperformed the indigenous birds in growth rate, body weight, eggs production, egg size and hatchability which transforms to a 133% increase in meat production, 462% increase in egg production and a 341% increase in income for rural poultry farmers [3]. Thus, Kuroilers seem to empower the poultry industry as well as the quality of meat.

Heavy metals are one of the most common forms of anthropogenic pollutants in the environment thus threatening human and animals' health through environmental and occupational exposure. Cadmium is an environmental pollutant and can be considered as the most toxic heavy metals [4]. As cadmium is non-biodegradable it is found widely in nature and present in air, all soils and aquatic systems [5, 6]. Natural and human related activities are responsible for cadmium pollution [7, 8]. Raised

concentration of cadmium in soil may be found as a result of industrial activities (sewage, phosphate fertilizers and pesticides) containing high concentrations of cadmium [9].

Under normal conditions, the intake of cadmium depends on the concentration in the sources as air, water, and land. Cadmium results in the formation of reactive oxygen species [10] causes various adverse effects such as disturbances of enzymes function [11], the enhancement of lipid peroxidation [12], nephrotoxicity [13], immunotoxicity [14], carcinogenesis and oxidative DNA damage [15].

Cadmium exposure in animals occurs due to inhalation of polluted air, ingestion of polluted food and drinking of polluted water [16-18]. It is absorbed from gastrointestinal tract to blood and is taken up from the blood into tissues. Reduced feed intake resulting in weight loss, decreased RBC and Hb values and anaemia occur in cadmium exposed animals [19, 20]. Anaemia is a major hematotoxic effect following long term exposure to cadmium in man [21, 22] and in laboratory animals [23, 24].

The importance of haematological biochemistry, population genetics and medical anthropology is well established. Recent speculations have proved that they may be used as valuable indicators of disease or stress in animals [25]. Biochemical profiles of blood can provide important information about the internal environment of the organism [26]. Blood parameters are probably the more rapid and detectable variations under stress and are fuel in assessing the health condition [27]. Investigation of haematological parameters is necessary for clinical diagnosis of a disease and pathological condition [28].

Measurement of haematological parameters provides valuable information and is routinely used in human and animal medicines, but unfortunately due to lack of information, blood profile has not been widely used in avian medicine [29, 30]. Several factors including physiological [31] and environmental conditions [32, 33], diet contents [34, 35] water and feed restriction [36-38], fasting [39], age [40-42] and administration of metals [43-44] affect the blood profiles of healthy birds.

WHO recommends the use of blood parameters for medical and nutritional assessment [45-46]. Exposure of cadmium alters different blood parameters, affects the body weight and induces immunopathological changes in thymus [47-49] thus directly affecting the health status. Therefore, investigating the effects of the administration of cadmium is necessary.

Thus the main objective of the present study was to analyze the haematological changes in Kuroiler chick exposed to cadmium acetate to give an insight of the health status of the chicks as well as the quality of meat.

2. Materials and Methods

2.1. Selection and Maintenance of Experimental Birds

Newly hatched Kruoiler chicks were procured from

Salim hatchery Meerut. Before experimentation the chicks were housed in clean wood and steel cages in animal house and acclimatized to laboratory conditions (temp. 36 ± 2 °C, light: dark period 18h: 6h period). They were fed on formulated chicks feed (Hindustan poultry feed ltd. Meerut India) and water ad libitum. The feeding was stopped 24h before commencement of experiments to avoid metabolic variations due to diet.

2.2. Chemicals

Cadmium acetate was obtained from BDH, India Ltd., Mumbai. All other chemicals and solvents used were of analytical grade.

2.3. Dose Selection

2.3.1. Determination of Lethal Dose 50 (LD₅₀)

Preliminary toxicity test was conducted under laboratory condition to determine the LD₅₀ value for 72h of cadmium by the standard method. Stock solution of the metal cadmium was prepared by simple dilution techniques. The concentration of cadmium below 30mg/100ml/kg body weight did not cause any mortality and the mortality rate increased with increase in toxicant dose. The mortality was 100% after 78mg/kg body weight. The percent mortality and probit mortality were statistically analyzed by the probit method of Finney [50] to calculate LD₅₀ value.

The 72h LD₅₀ values obtained were: - 70 mg/100ml/kg body weight.

2.3.2. Sub Lethal Dose Selected for Experiment

1/10 concentration of 72h LD₅₀ values were selected as sub lethal concentration i.e., 7 mg/100ml/kg body weight.

2.3.3. Dose Schedule

Single dose was administered via oral gavage in 10 days old chicks. The post- mortem examination was done after 20 and 40 days as per the protocol given.

2.4. Experimental Design

The 10 days old acclimatized chicks were divided into four groups with 6 to 8 chicks in each as shown in table 1.

Table 1. Table showing different experimental groups and nomenclature

Group	Name	Post-mortem examination
Group I A	Control Group (C-20)	After 20 days
Group I B	Cadmium treated group (Cd- 20)	20 days post treatment
Group II A	Control Group (C-40)	After 40 days
Group II B	Cadmium treated group (Cd -40)	40 days post treatment

After the decided experimental duration of different groups blood was collected from the heart of chicks and was analyzed for the various haematological parameters as in table 2.

Table 2. Table showing the various haematological parameters studied, their methodologies, formula used for calculation and the units in which the results are expressed

S. No.	Parameter studied	Methodology used	Formula used	Units
1	Total Erythrocyte Count (TEC)	Neubaur's haemocytometer chamber method [51]	No. of RBC counted x 10000	Millions/mm ³
2	Total Leukocytes Cells (TLC)	Neubaur's haemocytometer chamber method [51]	No. of RBC counted x 50	mm ³
3	Haemoglobin (Hb)	Acid haematin method [52]	Dilution of haemoglobin was read in terms of gram percentage	grams per 100 ml. of blood (gm/dl)
4	Packed Cell Volume (PCV)	Wintrobe's haemocrit tube [53]	The column of red blood in the Wintrobe's tube was read as percentage	%
5	Mean Corpuscular Haemoglobin (MCH)		$\frac{\text{Haemoglobin in gm/dl} \times 10}{\text{RBC in millions}}$	Pictograms (pg)
6	Mean Corpuscular Volume (MCV)		$\frac{\text{PCV (\%)} \times 10}{\text{RBC in millions}}$	Femtolitre (fl)
7	Mean Corpuscular Haemoglobin Concentration (MCHC)		$\frac{\text{Haemoglobin in gm/dl} \times 100}{\text{PCV (\%)}}$	Gram/dl
8	Differential Leukocyte Count (DLC)	[54]	Different cells were identified and counted	%

2.5. Statistical Analysis

Values are expressed as Mean $\pm$ SD and the results obtained were analyzed using one-way ANOVA. Inter group comparisons were performed by using the least significance difference (LSD) test. A probability value of $P < 0.05$, 0.01 was considered as statistically significant.

3. Results

The alterations in various haematological parameters are presented in the table 3. Single dose administration of cadmium resulted in significant changes in the haematological parameters screened 20 and 40 days after the exposure.

Effect on TEC: There was a significant decrease ($P < 0.05$) of about 33.79% in TEC in Cd-20 group and a significant decrease ($P < 0.01$) of 53.03% after 40 days in Cd-40 group as compared to the respective control values.

Effect on Hb: The Hb levels also decreased significantly ($P < 0.05$) by a percentage of 22.43 and 35.10 in Cd-20 and Cd-40 groups respectively as compared to control groups.

Effect on PCV: There was a significant decrease ($P < 0.05$) of about 12.43% and 13.71% in PCV values in Cd-20 and Cd-40 groups respectively as compared to control groups.

Effect on TLC: There was a significant increase ($P < 0.05$) of about 21.38% in TLC count in Cd-20 group and a significant increase ($P < 0.01$) of 39.89% after 40 days in Cd-40 group as compared to the respective control values.

Effect on MCV: The MCV values also increased significantly ($P < 0.01$) by a percentage of 32.26 and 83.75 in Cd-20 and Cd-40 groups respectively as compared to control groups.

Effect on MCH: The MCH values also increased significantly ($P < 0.05$) by a percentage of 17.15 and 38.18 in Cd-20 and Cd-40 groups respectively as compared to control groups.

Effect on MCHC: The MCHC values also decreased non significantly by a percentage of 11.43 and decreased significantly ($P < 0.05$) by 26.07% in Cd-20 and Cd-40 groups respectively as compared to control groups.

Effect on DLC: There was a significant decrease ($P < 0.01$) in the neutrophils whereas eosinophils, monocytes, lymphocytes and basophils (non significant) were found to increase significantly in Cd-20 and Cd-40 groups respectively as compared to control groups. The % decrease in neutrophils was 36.36 and 20 in the Cd-20 and Cd-40 groups respectively. The % increase in eosinophils, monocytes, lymphocytes and basophils in Cd-20 group was 125, 150, 80 and 200 respectively in Cd-20 group and 115, 40, 57.14 and 100 respectively in Cd-20 group.

Table 3. Table showing the various values of the parameters studied in the experimental groups. All the data are expressed as mean $\pm$ SD. a= compared to control group 20 day; b= compared to control group 40 day; ** P<0.01, *P<0.05, NS= non-significant

S.No.	Parameters	20 DAY		40 DAY	
		Control group (C-20)	Cadmium treated group (Cd-20)	Control group (C-40)	Cadmium treated group (Cd-40)
1	TEC (million/mm ³)	3.64 $\pm$ 0.8945	2.41 $\pm$ 1.2154 ^{a*}	3.94 $\pm$ 0.03	1.85 $\pm$ 0.025 ^{b**}
2	Hb (gm/dl)	11.50 $\pm$ 0.7453	8.92 $\pm$ 0.6144 ^{a*}	12.96 $\pm$ 0.316	8.41 $\pm$ 0.019 ^{b*}
3	PCV (%)	34.50 $\pm$ 0.5621	30.21 $\pm$ 0.4856 ^{a*}	38.12 $\pm$ 0.456	32.89 $\pm$ 0.327 ^{b**}
4	TLC (10 ³ /mm ³)	18000 $\pm$ 122.125	21850 $\pm$ 123.136 ^{a*}	18600 $\pm$ 121.8	25915 $\pm$ 161.03 ^{b*}
5	MCV (fl)	94.78 $\pm$ 2.2154	125.36 $\pm$ 2.784 ^{a**}	96.75 $\pm$ 0.081	177.78 $\pm$ 2.747 ^{b**}
6	MCH (pg)	31.59 $\pm$ 0.1276	37.01 $\pm$ 1.2554 ^{a*}	32.89 $\pm$ 0.046	45.45 $\pm$ 0.3742 ^{b*}
7	MCHC (gm/dl)	33.33 $\pm$ 1.8954	29.52 $\pm$ 1.2365 ^{aNS}	33.99 $\pm$ 0.400	25.57 $\pm$ 0.244 ^{b*}
8	Lymphocyte %	67 $\pm$ 1.1426	80 $\pm$ 1.8451 ^{a**}	56 $\pm$ 1.14	88 $\pm$ 0.5099 ^{b**}
9	Neutrophil %	22 $\pm$ 0.9784	14 $\pm$ 0.865 ^{a**}	45 $\pm$ 1.321	36 $\pm$ 0.7348 ^{b**}
10	Eosinophil %	4 $\pm$ 0.2158	9 $\pm$ 0.2567 ^{a*}	4 $\pm$ 0.7071	8.6 $\pm$ 0.3742 ^{b*}
11	Monocyte %	4 $\pm$ 0.4856	10 $\pm$ 0.4715 ^{a*}	5 $\pm$ 0.337	7 $\pm$ 0.3742 ^{b*}
12	Basophil %	3 $\pm$ 0.7453	9 $\pm$ 0.9442 ^{a*}	1 $\pm$ 0.2449	2 $\pm$ 0.3162 ^{bNS}

4. Discussion

Exposure of Kuroiler chicks treated with cadmium acetate produced marked alteration in hematological parameters. The present study clearly shows significant decrease in TEC, Hb, PCV, MCHC and neutrophils and increase in TLC, MCH after 20 and 40 days post treatment. Decrease in TEC, PCV and Hb indicates anaemic condition of the chick as also reported earlier due to oral and intraperitoneal (i.p.) administration of cadmium [5, 55]. In the present study cadmium was administered orally after 10 days of hatching and the anaemic condition as well as the haematotoxic effect of cadmium treatment was evident even after 40 days of post treatment. The mechanism for cadmium induced anaemia though not clear but has been attributed to iron-deficiency due to inhibition of iron absorption from the gastrointestinal tract [56], hyperplastic anaemia derived from the inhibitory effect of cadmium on the growth of erythroid progenitor cells [23, 57]; haemolytic anaemia due to red blood sequestration in spleen [58] which results in shorter life span and increased destruction of erythropoietin due to renal injury [59].

The decrease in MCHC and increase in MCV supports the view that iron deficiency contributes to the development of anaemia as also seen in cadmium treated mice [60].

Reduction in number of RBCs can be attributed to decreased rate of erythropoiesis [61] or loss of erythrocytes due to toxicant induced haemorrhage in the internal organs [62]. Decrease in Hb content, total erythrocyte count and PCV value under stressful condition could be an expected consequence of loss of erythrocyte.

Significant increase of WBC count in cadmium exposed groups indicates the hyper sensitivity of WBC to cadmium

which might be due to immunological reaction to produce antibodies to cope up with stress induced by the toxicant. Increase in WBC count can be correlated with an increase in antibody production which helps in survival and recovery from the toxic effect of cadmium.

Decrease in the Hb content as observed might be attributed to the rapid oxidation of Hb to methaemoglobin or release of O₂⁻ radical due to toxic stress of cadmium. Neutrophils were found to be decreased in treated groups. Eosinophils, Monocyte, lymphocytes and basophils were found to be increased in both treated groups as compared to the control group indicating the stressful status.

Haematological parameters have commonly been used as indicators of physiological conditions and nutritional deficiency in chickens. The changes in haemoglobin concentration, total erythrocyte count, haematocrit level and differential leukocyte count may indicate stress [63-64], while the changes in erythrocyte, haemoglobin and packed cell volume may reflect an alteration of energy status in chickens [65-66].

5. Conclusions

It is thus concluded that cadmium induced impairment of metabolism as chicks were observed to be under severe metabolic stress. The haematological alteration and variation in different enzymes can be used as good biomarkers of cadmium pollution in the terrestrial environment.

Further, research with toxicity testing method would give a more comprehensive picture which can be of great importance in monitoring the possible eco toxicological risk assessment of cadmium.

Moreover, in the present study, even after 40 days of exposure to a single dose of cadmium, the values of different hematological parameters didn't show much improvement indicating that cadmium is not excreted out easily from the body and its effect are both time and age dependent.

Furthermore, it is also concluded that the anaemic condition, altered haematological profile and metabolic stress in Kuroiler chickens would definitely alter the quality of meat lowering the protein content as well as weakening the poultry economy.

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