

Original Research Article

THE EFFECTS OF PROLONGED CHLORAMPHENICOL ADMINISTRATION ON HEAMATOLOGICAL PARAMETERS AND HISTOPHATOLOGY OF LIVER, KIDNEY, AND SPLEEN IN BROILER CHICKEN

Abstract

Chloramphenicol a broad spectrum antibiotic was tested on broiler birds to evaluate the effect of its prolonged administration on heamatological parameters, identification of histopathological changes on organs (Liver, Spleen and Kidneys) and to determine if prolonged effect has effects on weight gain and mortality rate.

One hundred and twenty day old chicks from Kamadex Ibadan were used for the experiment. The birds were assign to two (2) treatments and control group each replicated in a Complete Randomized Design (CRD), birds in treatment one (T1) were administered normal dose of Chloramphenicol (250mg/Kg), while treatment two T(2) were served with triple dose in medicated water served ad-lib for seven weeks, while the control (T3) were served with un-medicated water ad-lib. Samples (blood, liver, kidney and spleen) were collected and analyzed after 8 weeks of the experiment.

Birds showed significant variations in heamatological values across treatments. Lymphocysts count: treatment two (T2) was higher than the treatment one (T1) whereas that of (T3) was found to be less, compared to those of T1 and T2. Heterophils: T3 was higher than the T1 while that of T2 was less as compared to T3 and T1. Basophil: T1 was higher than T3 while that of T2 was less. A lower value was observed in weight gain with birds on T2 as compared to T1 and T3. The liver of birds on T1 and T2 were significantly larger than those on T3. High mortality was recorded in birds on T2 compared to those in T3 and T2.

The histopathological pictures of the liver, kidney and the Spleen depict a varying degree of necrosis and hemorrhagic foci on all the organs, the changes were much severe on T2 as compared to those of T1 and T3.

INTRODUCTION

Poultry like any other livestock is confronted with the problem of disease thus; hindering profitable poultry production, which bring huge lose to the poultry farmers. Poultry just like Dogs and Cats may become ill from many causes. There are few categories of disease that commonly occur in small poultry flocks. These include external and internal parasites,

respiratory diseases, nutritional problems, reproductive diseases, Bacteria (e.g fowl typhoid, Salmonella), fungal (e.g Aspergillosis), viral (e.g Gamboro, new castle and e.t.c) and protozoan (e.g coccidiosis) disease [1]. On other hand, Salmonella infection caused by a variety of salmonella species as one the most important bacterial diseases in poultry causing heavy economic losses through mortality and reduced productivity [2]. Avian salmonellosis infection may occur in poultry as either acute or chronic form by one or more member of genus *salmonella* under the family *Enterobacteriaceae* [3]. Besides motile *Salmonella* (paratyphoid group) infection cause salmonellosis in chicken and have zoonotic significance. Disease of poultry are of significance because of their zoonotic nature (e.g certain *Salmonella* infections, *Chlamydiosis* and *Erysipelas* caused by *E. insidiosa*

Avian *salmonella* infections are important as both a cause of clinical disease and as a source of food-borne disease to humans. Under the family of *Enterobacteriaceae*, the genus of *salmonella* is a facultative intracellular pathogen causing localized or systemic infections as well as chronic asymptomatic carrier state [4].

Salmonella strains of avian origin are also often resistant to various antimicrobials approved for use in poultry medications including; tetracycline [5 and 6], oxytetracycline [7], penicillin [8], aminoglycosides [9], sulfisoxazole [6] and fluoroquinolones [10]. On the other hand, [11], found several strains of multiple antibiotic-resistant *salmonella* strains in chicken

Among the drugs used to treat these conditions are Furazolisone, gentamycin sulfate, and sulfa drugs (Sulfadimethoxine, sulfamethaxine and sulfamerazane)

[12] reported that; Chloramphenicol is a broad spectrum antibiotic whose spectrum includes several gram positive and gram negative bacteria, spirochetes and Rickettsiae.

Chloramphenicol

Chloramphenicol is a bacteriostatic antimicrobial originally derived from the bacterium *Streptomyces venezuelae*, isolated by David Gortlieb, in 1947 and introduced into clinical practice in 1949, [13].

An antibiotic first isolated from cultures of *Streptomyces venezuelae* in 1947 but now produced synthetically. It has a relatively simple structure and was the first broad spectrum antibiotic to be discovered. It is mainly bacteriostatic but may be bactericidal when used in high concentrations or when used against highly susceptible organisms. Chloramphenicol is effective against a wide range of microorganisms. It acts by stopping bacterial growth by binding to the bacterial ribosome thereby blocking (peptidyltransferase) and inhibiting protein synthesis [13]. Chloramphenicol is lipid soluble, allowing it to diffuse through the bacterial cell membrane. It then reversibly binds to the L16 protein of the 50S subunit of bacterial ribosome, where transfer of amino acids to growing peptide chains is prevented (perhaps by suppression of peptidyltransferase activity), thus inhibiting peptide bond formation and subsequent protein synthesis, [13]. [14] reported that chloramphenicol has advantages over other antimicrobial agents. It has a broad spectrum of activity in combination with excellent penetrability into body tissues and its use in large animals has not been associated with any degree of toxic side activity.

Three common forms are used for systematic therapy depending on the route of administration, a free base form of chloramphenicol, chloramphenicol palmitate and chloramphenicol succinate other formulations are also available for topical use [15]. Chloramphenicol in animals is well absorbed by both oral and parenteral routes [16]. The usual dose of chloramphenicol is

5mg/kg/day in four divided doses. It is available as 25mg capsule or as a liquid (125mg/5ml) [17]. Chloramphenicol is metabolized by the liver to chloramphenicol glucuronate (which is active) and the dose must be reduced in liver Impairment. Majority of chloramphenicol is excreted by the kidney as the inactive metabolite, chloramphenicol glucuronate. Only a tiny fraction of the chloramphenicol is excreted by the kidney unchanged [18]

Heamatology profiles

Blood plays an important role in the transportation of nutrients, metabolic waste products and gasses around the body [19]. Moreover, blood represents a means of assessing clinical and nutritional health status of animals [20]. Heamatological profiles both in both in human and in animal science is an important index of the physiological state of an individual. The ability to interpret the state of blood profile in normal and diseased conditions is among its primary tasks. The full blood count examines mostly the cellular component of blood whereas biochemical testing focuses on its chemical constituents [21]). It has been shown that data from blood profiles could be exploited in the improvement of chicken [22]. In addition, blood parameters helps greatly in diagnosis of specific poultry hen pathologies and might serve as basic knowledge for studies in immunology and comparative avian pathology [23]. Flactuations or varriations in heamo-biochemical profiles have been reported in chickens of the same age and sex and reared under the same conditions but sampled at different times of theday [24]. It was observed that there is definite change in the profiles of the blood cells throughout life. Blood picture changes with the advancement in age, certain condition as stress, bacterial infections, viral infections and intoxication

Blood is a combination of plasma and cells that circulate through the entire body. It is a specialized bodily fluid that supplies essential substance around the body such as Sugars, Oxygen and hormones. Plasma makes up to 55 percent of blood content. The other 45 percent consist mainly of red blood and white blood cells and platelets. Each of these has a vital role to play in keeping the blood functioning effectively [25]. The blood of the domestic fowl contains erythrocytes, thrombocytes, granulated and agranulated leucocytes suspended in plasma.

MATERIALS AND METHOD

The following materials were employed in order to carry out this experiment:

Materials

1. One hundred and twenty (120) day – old broiler birds (F-from Kamadex)
2. Brooding materials:, wood shavings, lighting material (light bulbs, lanterns, bush lamp and etc), heating materials (Warmers, Stoves and etc), Saw dust, Newspapers and magazines and Ceiling boards.
3. Feeds: Broiler starter (commercial feed vital) was used from day old to four weeks, while broiler finisher was used from five (5) weeks to eight (8) weeks.
4. Drugs: Chloramphenicol 100%, Multivitamin (Vitalyte powder), Anti coccidian (Amprollium) and Antiseptic (Morigad)
5. Vaccines: Infectious bursal disease vaccine (IBDV), Newcastle Disease vaccine Lasota (NDVL)
6. Sample containers: Sample bottles, EDTA bottles, Formalin, Cotton wool, Cold box, Syringe and Needle and Digital scale.

7. Scalpel blades, Scissors, Disposable hand gloves, Forceps, Collection tray and Polyethane bags.
8. Miscellaneous equipment: Manual and Digital weighing scale, Syringes and needles, Teaspoon, Graduated cup, Bucket, Broom, Chart paper and marker.
9. **Pen:** Three pens were constructed; each pen has a dimension of 6.0 m² capable of stocking forty birds.
10. Feeders and Drinkers: Plastic drinkers (Chick tray) were used to serve feed to the birds from day old to four weeks and were replaced with metal feeders from five weeks of age up to 8 weeks. A plastic drinker of four-liter capacity was used to serve water to the birds

Methods

Study area

The study was conducted at College of agriculture and animal science, Ahmadu Bello University, Mando Road – Kaduna at the students experimental pen located at (11⁰, 10² 07² 38'E) in the Northern Guinea Savannah Zone of Nigeria.

Treatment and Sample Techniques

The birds were stabilized for seven days using; Enrofloxacin and multivitamin for three (3) and four (4) days respectively, they were fed and watered ad-libitum using broiler starter feed and clean water. They were vaccinated against Gamboro (infectious bursal) disease at seven (7) and twenty one days and Newcastle disease at fourteen (14) and twenty eight days (28) respectively. Anti stress was given after each vaccination. The birds were assigned to two (T₁ and T₂) treatments and control (T₃) each containing twenty (20) birds. Each treatment was replicated; Completely Randomized Design (CRD) was used for the experiment.

T₁ ----- (i.e 250mg/kg. bdw)

T₂----- Triple dose of the drug (i.e 750mg/kg.bdw) and;

T₃----- As control

Oral preparation of chloramphenicol (normal dose 250mg capsule) was used as the treatment. The dosage was determined based on body weight, which was obtained by taking the average weight three (3) times weekly. This procedure lasted for eight (8) weeks.

Data Collection and Handling

The weight of the birds were measured on the weekly basis and tabulated. The mortalities were also recorded from each treatment and control as they occurred. At the end of the experiment eight (8) weeks, three (3) birds were randomly picked from each replicate and blood samples were collected using the wing vein and emptied into EDTA (ethyl di-tetra Acetic acid) container/bottles for preservation and the bottles were immediately arranged inside a cold box containing ice packs and cotton wool.

The birds were finally slaughtered and the livers, spleens and kidneys were carefully cut and weighed using a digital scale, portion of each organs were swiftly immersed in a labeled bottle containing 75% concentration of formalin and were also arranged inside the cold box. The samples (blood, livers, spleens, and kidneys) were finally histopathologically analyzed.

RESULT AND DISCUSSION

The results of this experiment are highlighted under the following:

Effects of prolonged chloramphenicol administration on heamatological parameters.

Effects of prolonged chloramphenicol administration on weights of various organs.

Effects of prolonged chloramphenicol administration on mortality rate

Effects of prolonged chloramphenicol administration on histopathology of various organs.

Table 1: Effects of Prolonged Chloramphenicol Administration on Heamatological Parameters.

Parameters	T ₁	T ₂	T ₃
PCV (%)	29.2± 0.2	30.4±0.2	31.2±0.5
Hbg/Dl	7.3±1.2	8.1±0.3	8.4±0.1
RBC 10 ¹² /μ	1.9±0.1	2.0±0.1	2.1±0.0
LYMPHOCYTES (%)	26.9±0.5 ^b	28.0±0.6 ^a	25.0±0.4 ^b
MONOCYTES (%)	9.2±0.7	9.4±0.5	8.8±0.3
HETEROPHILS (%)	62.4±0.8 ^a	60.9±1.2 ^a	65.0±0.3 ^b
EOSINOPHILES (%)	1.9±0.7	1.5±0.2	1.3±0.2
BASOPHILS (%)	1.0±0.2 ^b	0.0±0.0 ^a	0.8±0.0 ^b

Note: Mean Values with the same superscript are not significantly different (P<0.05) across the same row.

From the above table(1), the PCV value agrees with [26], which falls between 29 – 30.5, while the hemoglobin value is relatively higher between 6 and 8. The RBC value closely related with [26], which falls between 2.0 and 3.0 for the leucocyte components, there is slight differences between the treatments (especially, treatment two (T₂) and control(T₃), which can be seen in lymphocytes, heterophils and basophil values.

Table 2: Effects of Prolonged Chloramphenicol Administration on Weight Gains

	Initial live Weight (Kg)	Final Weight Weight (Kg)	Weight Gain (Kg)
T1	0.13±0.1	1.9±0.1	1.8±0.0
T2	0.13±0.1 ^b	1.6±0.1 ^b	1.5±0.0
T3	0.13±0.1	1.7±0.1	1.6±0.0

Note: Mean Values with the same superscript are not significantly different ($P < 0.05$) across the same row.

Most of the values obtained are similar to the value of streptomycin (1.65kg) obtained by [27]. Also, there were significant differences between the values obtained in the result with treatment two (T_2) having less weight gain. Treatment one (T_1) has more weight gain compared to treatment two (T_2) and control (T_3).

Table 3: Effects of Prolonged Chloramphenicol Administration on Weight of Various Organs

ORGANS IN GRAMS (G)	T1	T2	T3
Liver	43.3±1.0 ^b	43.6±0.6 ^a	37.1±0.5 ^b
Spleen	1.5±0.4	1.6±0.3	1.4±0.1
Kidney	12.7±0.1	13.3±0.1	12.4±0.1

Note: Main Values with the same superscript are not significantly different ($P < 0.05$) across the same row.

The values obtained above disagree with the values obtained by [27] possibly because they were pullets and the treatment given was *Lablab purpureus* beans. For the liver, the control has lesser values compared to the two (T_2) treatments whereas, for the spleen, there is significant difference

among the treatments and control. The kidney shows significant difference in treatment two (T₂) only.

Effects of Prolonged Chloramphenicol Administration on Mortality Rate

The mortality rates were as follows; Normal dose (T₁) had no mortality, triple dose (T₂) had three (3) mortalities and control (T₃) had a single mortality.

The triple dose (T₃) showed a higher significant different mortality across treatments.

Effects of Prolonged Chloramphenicol Administration on Histopathology of Various Organs

The Liver

Based on the histopathological analysis, in treatment one (T₁), there were areas of coagulation, necrosis characterized by varying degrees of karyohexis and pyknosis of hepatocytes. There was moderate congestion of Vessels and few hemorrhagic foci sparsely spread. Also there was disoriented architecture associated with massive hemorrhage where as in other samples there were no microscopic changes seen.

However treatment two (T₂), on the other hand multiple foci of hemorrhages with sinusoids while some portions are necrotic characterized by karyohexis and different degrees of pyknosis of hepatocytes. Also in one of the samples, there was a marked (severe), hemorrhage within the sinusoids and moderate congestion of vessels and hepatocytes very prominent but normal. Consequently, the control (T₃) was having vascular congestion and cellular infiltration by mostly mononuclear cells and few heterophils. There was a definite circumscribed area heavily populated by microphages, lymphocytes and perivascular cuffing.

The spleen:

In the treatment one (T₁), there was no obvious microscopic change except for few foci of hemorrhages, whereas in treatment two (T₂) there was moderate proliferation of fibrous connective tissues around a blood vessel. The control (T₃) was having widespread degeneration of connective tissues and marked increase in fibrous connective tissues around a few arteries with closely parked concentric lamellae of collagen fibre surrounded by fragmented elastic lamellae and deposits of eosinophilic materials in the wall of the vessels – i.e “oion-skinning” systemic lupus erythematosus.

The kidney:

In treatment one (T₁), there was generalized disintegration of tissues architecture characterized by loss of glomeruli and disappearance of renal tubules. There was a marked and diffused area of hemorrhages as well as few patches of coagulation necrosis showing varying degree of karyohexis of the tubular cells.

On the other hand, treatment two (T₂) depicts picture of foci of necrosis and disruption of capillary loops in the glomerulus as well as shrinking of glomerular material – a turf with swelling of endothelia cells. There was severe tubular damage with vacuolation of most of the epithelia cells and also replacement of epithelia cells by flattened cells – segmented glomerulonephritis.

The control (T₃) contains few patches of hemorrhages diffusely dispersed and condensation of glomerular materials while there was a sharp area of demarcation in apparently encapsulated reminiscent to coagulation necrosis. There was thinning of tubular wall leading to enlargement of tubules and compaction of interstitial tissue.

CONCLUSION

From the foregoing, it was concluded that chloramphenicol which is a broad spectrum antibiotic has significant effects when overdosed on the hematological parameters and histopathology of visceral organs, so, it should not be overdosed for prolonged period.

Ethical Approval:

Animal Ethic committee approval has been collected and preserved by the author(s)

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