EFFECT OF CYROMAZINE INCLUSION AND LITTER DEPTH ON GROWTH PERFORMANCE, NUTRIENT UTILIZATION, HISTOPATHOLOGY AND ITS RESIDUES IN SELECTED TISSUES OF BROILER CHICKENS

BY

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DECLARATION

I hereby declare that this thesis was written by me and is a correct record of my own research work. It has not been presented in any previous application for a higher degree of this or any other university. All citations and sources of information are clearly acknowledged by means of references.

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CERTIFICATION

This thesis entitled "Effect of cyromazine inclusion and litter depth on growth performance, nutrient utilization, histopathology and its residues in selected tissues of broiler chickens" by **Adeleye, O.O** meets the regulation governing the award for the degree of Doctor of Philosophy of the Federal University of Agriculture, Abeokuta and is approved for its contribution to scientific knowledge and literary presentation.

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ABSTRACT

Increase in the number of poultry production enterprises and urban encroachment have resulted in increasing complaints on obnoxious odour pollution from local residents. Cyromazine has a distinctive quality to control fly larvae that would hatch on poultry manure thus preventing odour. Melamine (cyromazine metabolite) is a public health issue. This study was conducted to determine the effect of cyromazine inclusion and litter depth on performance, nutrient utilization, histopatology and residues in selected tissues of broiler chickens. Structured questionnaires were administered to determine the level of use of cyromazine among poultry farmers. A total of 320 day old broiler chicks (Arbor acre) were used for the feeding study. Diets were formulated to contain 4 levels of cyromazine (0.00, 0.25, 0.50 and 0.75 g/kg) fed to broilers on 2 litter depths (3cm and 5cm). Each dietary treatment had 40 birds and 4 replicates of 10 birds each. Cyromazine inclusion was stopped at 6 weeks to observe a withdrawal period of 7, 14, 21 and 28 days. Two birds per treatment were selected at random at the 6th week for metabolic trial. A bird per replicate was randomly selected, blood samples were collected for haematology and serum indices. At week 6, 7, 8, 9 and 10, these birds were slaughtered to harvest tissues for residue determination. Data were collected for feed intake, weight gain, feed conversion ratio, mortality, nutrient utilization, haematology and serum chemistry, histopathology, carcass yield and organ weights. Residues of cyromazine and melamine were also determined in drumstick and thigh. Data were analysed using descriptive statistics for questionnaires and feeding trials were analysed in a Completely Randomized Design in a 2 x 4 factorial arrangement. Result showed that majority (93%) of respondents indicated that cyromazine improved performance and 89.7% showed that it reduced odour. Average daily weight gain and FCR of the cyromazine treated groups were significantly improved ($p<0.05$) for the dietary treatments while carcass weight did

not vary significantly ($p>0.05$). Litter depth 3cm showed significant ($p<0.05$) difference in growth performance. Birds on 3cm litter depth had higher ($p< 0.05$) CP digestibility (81.39%) than 5cm litter depth (66.07%). Although haematology and serum indices showed no specific pattern in all the weeks, birds fed cyromazine levels above the recommended value (0.50g/kg) elicited an increased erythrocyte and reduced Aspartate aminotransferase levels. The histopathology of kidney was characterised by focal area of interstitial infiltration cells with tubular necrosis and desquamation. The liver was characterised by focal area of lymphoid aggregate with disseminated necrosis of the hepatocytes and inflammatory cells. The spleen also indicated sinuses, trabecular arteries with hyperemia. The melamine residue in meat (thigh and drumstick) were higher ($p<0.05$) in the treated groups than the control group, which also contained residue of cyromazine. In conclusion, cyromazine inclusion significantly enhanced growth performance; it adversely affected the kidney, liver and spleen and also left residues of cyromazine and melamine in the tissues of broiler chickens even up to 28 days withdrawal period. Therefore for public health implications its use should be monitored and controlled.

DEDICATION

This thesis is dedicated to the Almighty God, the Alpha and Omega, the beginning and the end, who was and is to come. Awesome God, my Defender, Shield and Buckler. You are the source of my wisdom, patience and strength.

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CHAPTER ONE

1.0 Introduction

Nigeria, a country with over 167 million people (NPC, 2011) has vast areas of land, but saddled with many problems ranging from widespread poverty, food problems resulting in serious malnutrition and hunger among others. Olorunfemi *et al.*, (2009) reported the daily recommended minimum animal protein intake per head in developing countries like Nigeria as 70.4g/head/day for an adult, of this at least 26-35g should be of animal origin. However, low animal protein intake has remained a major human nutritional problem in Nigeria, especially for low income earners (Amaefule *et al.*, 2009). To meet this increasing demand for dietary animal protein, a short gestation monogastric animal especially poultry has been suggested as possible solution (Nworgu *et al.*, 2000). Poultry has been ranked the highest in percentage rate of meat production among all other livestock in the past 35 years (Larry, 1993).

Litter is an integral element in providing the proper environment for efficient poultry production. Litter functions as a medium for faecal decomposition, moisture absorption and as insulation between the ground and live birds (Monira *et al*., 2003). Poultry litter is the mix of bedding material, manure and feathers that result from intensive poultry production. Broiler litter makes up the vast majority of litter produced in Nigeria. About 1.72kg of litter per broiler is produced every seven weeks. (Runge *et al* 2007).

Increase in the number of poultry production enterprises and urban encroachment have resulted in increasing complaints on obnoxious odour pollution from local residents (Power *et al.*, 2005). It has been reported that poultry litter odour occur as a result of aerobic and anaerobic microbial activities within the litter (Lacey *et al*., 2004; Rappert and Muller, 2005). In most cases, the offensive characteristic odour increased with the

accumulation of bird waste in the bedding material over the chicken's growth cycle (Powers *et al.*, 2005). In anaerobic environments, the decomposition of organic compounds results in the production of odorous volatile compounds that are metabolic intermediates or end products of microbial processes (Zhu, 2000). Many of these compounds are then carried by ventilation air, airborne dust, and other particles and dispersed into the atmosphere. Odour emissions from animal production sites are one of the most important factors to consider when determining setback distances from neighbours since the human nose can readily detect odours. However, odours are often perceived as indicators of airborne pollutants. Though seen as pollutant, it is also important to know that degradation of these poultry wastes is of economic importance to the environment.

Recently the use of Cyromazine as feed additive to curb the menace of obnoxious smell arising from poultry birds litter is a subject of research. Cyromazine has been reported to prevent the degradation of poultry litter to avoid air pollution (WHO, 2008). It has been reported that the end product of cyromazine metabolism (melamine) resulted in infant mortality in China after six babies died and 294,000 were hospitalized after drinking melamine tainted infant formula (WHO, 2008). More than 1000 dogs and cats have died in various countries due to renal failure caused by accumulated kidney stones as a result of melamine in pet food (WHO 2008).

The prevention of litter degradation by the use of cyromazine can be seen as negatively mitigating the environment because the decomposition of livestock waste is needed for sustainability of the environment. The minimum dosage of cyromazine in poultry with its resultant residue in poultry meat safe for human consumption is also yet to be determined.

There is little or no information on the dosage of cyromazine safe enough to be consumed by poultry birds and humans consuming poultry products because of residues. There is also a dearth of information on the influence of varying levels/dosage of cyromazine consumption on litter degradability and quality of underground water. Therefore, this study aims at determining the response of broiler chickens to cyromazine inclusion and its effects on selected organs and meat cut parts.

1.1 Justification

Cyromazine is an organophosphate insecticide which has been used over years, yet in a report by Russell (1990) stated "there is urgent need to improve estimation of the aquatic toxicity of current organophosphates". Although, not only is the aquatic environment but indeed all facets of the environment is vulnerable to chemicals, but the problem is further compounded by human misuse of such chemicals. Reasons for concern in the application of Cyromazine as feed additive arose after it was hypothesized that the ingested Cyromazine could be metabolized by the birds to melamine, which might be deposited in eggs and body tissue intended for human consumption. A residual maximum of 50ng/g for cyromazine in the edible parts of eggs and poultry meat are allowed as stipulated by the US Code of Federal Regulations (1987). However, residual levels in consumer products and inclusion levels in animal diets differ between countries depending on their application as a veterinary drug. Animal nutritionists are not to rely on the so called safe inclusion levels, but that feed should rather contain undetectable levels of melamine. However there are few information on the degradation of cyromazine and environmental implications on its use. Several methods such as spraying of litter with chemicals have been used to reduce the population of housefly which create nuisance in poultry pens, before cyromazine was added to feed to interfere with the pupal stage of insect development. In a bid to reduce the odour of poultry litter, the health of the consumers

and exposure of the neighbours to environmental hazards should be of great concern. In view of this, Lee et al. (2011) suggested that environmentalists, resource managers, regulatory bodies use toxicity data in the formulation of various standards designed to protect the environment and make it sustainable. According to this author, pollution control should be practised for the well – being of humans and not the protection of an ecosystem. Also, the relationship between cyromazine and melamine was mentioned since melamine is a metabolite of cyromazine as reported in literature (Brown, 2007; Caldas, 2007; Hilts *etal.*, 2008; Reimschuessel *et al.*, 2008; WHO, 2008; Hau *et al.*, 2009; Sebastian, 2009; Kobayashi *et al.*, 2010 and WHO, 2010). The study reveals the inclusion of cyromazine to feed and its residue on broiler chicken.

1.2 OBJECTIVES

1.2.1 Broad Objective

To determine the effects of varying levels of Cyromazine inclusion on broiler chickens and its effects on selected organs and meat cut parts

1.2.2 Specific Objectives

1. To carry out a survey on the number of farms using cyromazine around Abeokuta, Ogun state.
2. To determine the growth performance of broiler chickens fed diets containing varying inclusions of cyromazine.
3. To determine the effect of varying levels of cyromazine inclusions on the nutrient digestibility of broiler chickens
4. To determine the safe withdrawal period of 7, 14, 21 and 28 days of varying levels of cyromazine on experimental birds.

5. To determine the effect of varying levels of cyromazine on haematological and serum indices on experimental birds
6. To determine the effects of varying levels of cyromazine on carcass yield of experimental birds and residues of both cyromazine and melamine in selected cut parts of broiler chicken
7. To determine the histhopathology on selected organs of broiler chickens (Kidney, Liver and Spleen)

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Litter materials

Availability of dry organic materials dictates the type and depth of litter material poultry farmers will use. Litter source material usually varies according to regions (Monira *et al*., 2003: Skrbic *et al*., 2012). Garcia *et al*., (2010) reported that ideally, bedding material has to be absorbent, have a reasonable drying time and be innocuous to poultry and farmers. The most commonly used materials on floor are sawdust, wood shavings, rice hulls, straw and paper product (Swain and Sundaram, 2000). The litter material is speed approximately 5cm deep and can serve several flocks, although single flock clean out is very common in Nigerian broiler production. Litter quality may be the origin of environmental and management problems in commercial poultry industry if not properly selected and managed (Karamaclis *et al*., 2008, Garcia *et al*., 2010). Litter has to meet hygienic requirements and ensure controlled ammonia concentrations throughout production cycle (Villagra *et al*., 2011). Litter quality plays a vital role in the performance, health and welfare of broilers and as such the depth of the litter is important. Litter quality such as manipulation of pH and H_2O activity of litter will modulate microbiota content and also contribute to improving the competence of the immune system of birds (Lee *et al*., 2011). On the depth of litter in broiler house, Obeng, (1987) suggested that cool sand should be 2cm deep and any other litter should be 8cm deep while EPA, (2002) reported the usual litter depth for wood-shavings to be 5 and 10cm for chopped straw. Laseinde, (1999) recommended fresh bedding to be spread over entre floor area of poultry house at about 7.5 – 10cm depth for wood shavings.

2.2 Livestock / poultry waste

Animal production operations are a source of numerous airborne contaminants including gases, odour, dust, and microorganisms. Gases and odours are generated from poultry manure decomposition shortly after it is produced, during storage and treatment and during land application. (Rappert and Muller, 2005).

Human and animal health may be negatively affected by the concentration of contaminants of livestock and poultry buildings. Most of these health concerns are associated with chronic or long term exposure to gases, dust, or microorganisms. However, acute or short-term exposures to high concentrations of certain constituents can also have a negative effect on both human and animal health. For example, the agitation and pumping of liquid manure inside a livestock building can generate concentrations of hydrogen sulphide that are lethal to humans and animals. Microorganisms that populate the gastro-intestinal systems of animals are present in freshly excreted manure hence the generation rates of odour, manure gases, microorganisms, particulates and other constituents vary with weather, time, species, housing, manure handling system, feed type, and management system. Therefore, predicting the concentrations and emissions of these constituents is extremely difficult (Kennet *et al* 2000).

Organic wastes from intensive animal production (e.g. poultry,swine, dairy farms) provide excellent habitats for the growth and development of house fly (Thomas & Skoda 1993). The house fly, *Musca domestica* L., is a vector of many metaxenic pathogens and can cause serious sanitary problems because of its high reproductive potential, feeding habits and ability to disperse (Del Ponte 1958, Aberg-Cobo *et al*. 1959).

In relation to abiotic conditions, improper management of organic wastes and high temperatures (spring and summer) generates favourable conditions for populations of this pest to increase. Most poultry farmers use chemical larvicides and adulticides as the primary means of nuisance fly control. Improper use of these products combined with the housefly's short life cycle (< seven days) (Larsen &Thomsen 1940) and high biotic potential, produce conditions conducive to the development of resistance to insecticides.

2.3 Cyromazine

Cyromazine (N-cyclopropyl - 1, 3, 5 – triazine - 2, 4, 6 - triamine) is an insect growth regulator commonly used to control immature houseflies on poultry farms (Hogsette 1979, Miller and Corley 1980, Miller *et al.* 1981, Awad and Mulla 1984). Cyromazine is formulated as a pre-mix (1%), which is added to poultry feed; it is also formulated as a water soluble granule and a soluble powder (50%) for topical application to manure containing fly larvae (Royal Society of chemistry 1993) Cyromazine produces irreversible morphophysiological changes, which culminate in the death of the insects (Hogsette 1979, Awad and Mulla 1984). The effect varies according to the developmental stage of the insects. When housefly larvae are exposed to cyromazine, deformations may be observed in the pupal stage, which result from interference with chitin digestion and synthesis. When applied at the prepupal stage, cyromazine produces morphogenic aberrations in the adults, like absence of wings and underdevelopment of the genitalia in both males and females (Cerf and Georghiou, 1974). Housefly developed resistance to Cyromazine, resulting from the overuse and improper use of the product. This overuse was first reported in Florida about the same time the product was registered in the U.S. (Bloomcamp *et al.* 1987). Earlier reports by Sheppard *et al* (1989), Geden *et al.*(1992), Sheppard *et al.*(1992) indicate that resistance or increased tolerance is

widespread in the U.S. and Europe. Although Crespo *et al.* (2002) reported that the control of house fly populations resistant to cyromazine is possible through an integrated management scheme that includes cultural and biological strategies with adulticides and concentrated larvicides used in a rational manner.

2.3.1 **Chemistry and application of cyromazine**

Cyromazine (*N*-Cyclopropyl - 1, 3, 5 – triazine - 2, 4, 6 - triamine) is a triazine with a crystalline appearance. The molecular formula of this substance is $C_6H_{10}N_6$ and it has a molar mass of 116.19 g/mol (Merck, 2001). Cyromazine is a cyclopropyl that is a melamine derivate and alternative names include Larvadex®, Trigard®, Vetrazin® and CGA-72662 (Merck, 2001). The distinctive utility of Cyromazine is to alter the formation of the chitin layer of fly larvae that would hatch in poultry manure. Typically it is added to layer feed (marketed under the Larvadex label), when flies start to negatively affect bird comfort over time. The ingested Cyromazine will then pass the digestive system into the excreta, exposing larvae to the product without any additional labour. Conventional methods involved a technique where manure was sprayed with a similar product by hand, which is considered to be more labour intensive than the addition of Cyromazine as feed additive.

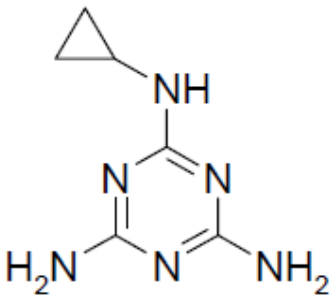


Figure 1: The chemical structure of Cyromazine (Merck, 2001).

2.3.2 The metabolism and distribution of cyromazine

Cyromazine is a derivate of Melamine and therefore it was thought that cyromazine is metabolized similarly to melamine in the body. Similar to melamine, cyromazine is also rapidly excreted by rats, mainly in urine within 24 hours and 95% of all ingested cyromazine is excreted within 72 hours (Simoneaux *et al.*, 1978).Cyromazine is almost completely absorbed after ingestion and distributed to all organs and body tissues (Caldas, 2007). Brake *et al.* (1984) showed evidence of liver abnormalities in broiler breeders fed 1000 mg/kg, thereby implying damage to the metabolic system. Wilson *et al.* (1983) recovered fatty livers from their treatment necropsy tests; however, it could not be associated with any of the treatments. Renal injury has been suggested by Brake *et al.* (1989) after wet litter were recorded forturkeys receiving cyromazine levels of 1000 mg/kg and more. It was proposed that the kidneys eliminate cyromazine and related substances from the body and have a metabolizable threshold after which the renal system will not be able to manage toxin elimination sufficiently. From the study by Capps (1990) on rodents, the primary emission route is through renal excretion where 52-78% of the ingested cyromazine were excreted within 24 hours, which is contradictory to the 97% reported by Caldas (2007). The reports of the two researchers, however, agreed that the excreted components were 72% unchanged cyromazine, 9% hydroxyl-cyromazine, 7% melamine and 2% 1-methyl-cyromazine. According to Keiding, (1999) 2-14% of ingested cyromazine was metabolized to melamine. In the study reported by Caldas, (2007), laying hens received a diet containing 5 mg/kg cyromazine for seven days. Almost all of the residuals were recovered (99.8%) of which the excreta contained the highest residual level (99.1%) followed by egg albumin (0.4%), yolk (0.2%), tissue (0.1%) and expired CO_2 (0.1%). The birds were euthanized and average cyromazine distribution levels measured for body tissue were the highest

for liver (0.31%) whilst renal tissue and breast muscle were 0.019% and 0.010% respectively. Supposing that 7% of the deposited cyromazine is metabolized to melamine (Caldas, 2007), the calculation could be based on the findings above that 0.00133 mg/kg melamine was potentially deposited in the renal tissue and 0.0007 mg/kg in breast muscle tissue for the study of Caldas, (2007) due to cyromazine ingestion.

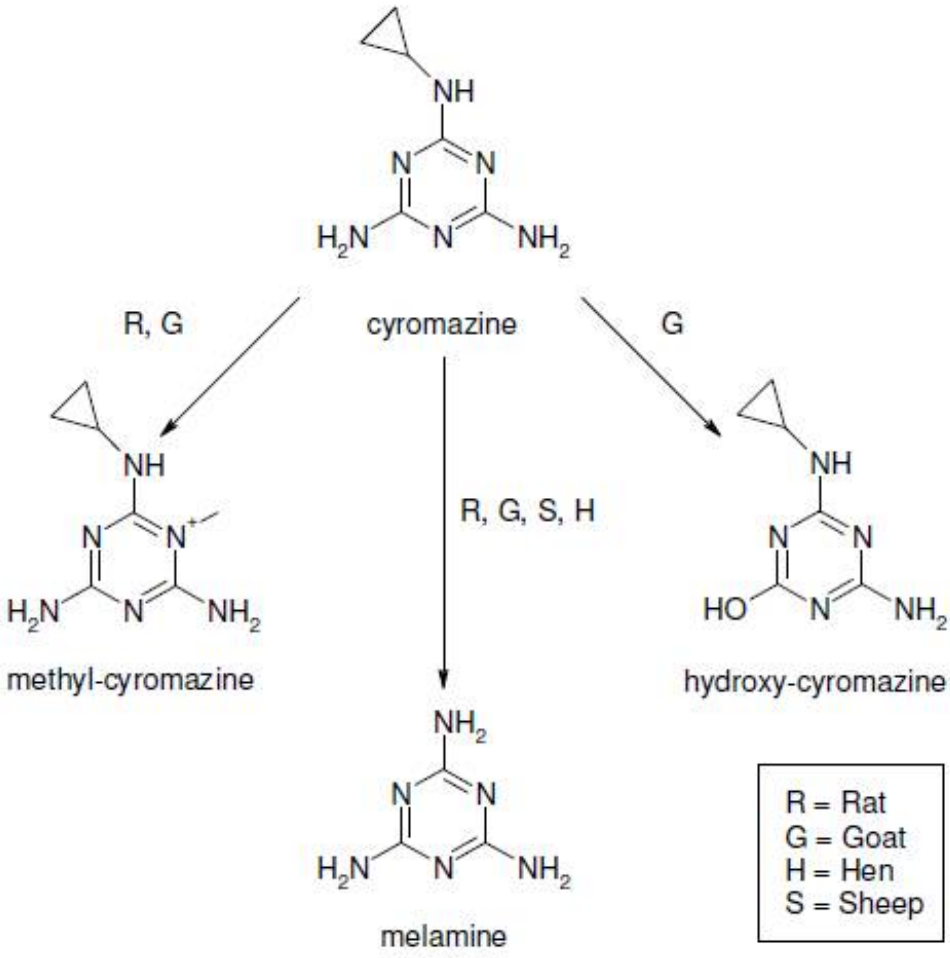


Figure 2: Proposed pathway of cyromazine metabolism in animals

Cyromazine, which is the active ingredient of Larvaside® which is included in laying-hen diets to control flies, may be metabolized to melamine in the animal, resulting in the possibility of melamine being detected in the meat or eggs. A residual maximum of 50

ng/g for cyromazine in the edible parts of eggs and poultry meat are allowed as stipulated by the US Code of Federal Regulations (1987); however, residual levels in consumer products and cyromazine inclusion levels in animal diets differ between countries depending on their application as a veterinary drug. There were approximately 94.7 million tons of poultry meat (U.S. Department of Agriculture, 2009) and 60.678 million tons of eggs produced worldwide during 2009 (FAOSTAT, 2010). From these facts it is evident that massive economica losses could be experienced when tainted poultry products have to be destroyed due to products containing melamine residues that exceed legal baseline levels.

2.4 **Melamine**

Melamine (1, 3, 5-triazine- 2, 4, 6 - triamine) with chemical formula $C_3H_6N_6$, is an organic compound and a trimer of cyanamide. Melamine (MEL) is commonly found as a white solid and has a molar mass of 126.12g/mL, a small molecule with high nitrogen content (Osborne *et al.*, 2008). It is used in the manufacture and processing of a variety products ranging from plastics, coatings, leather, paints, laminates, flame retarding agent and table-tops and in recent years it has been added to certain food products. Some bacteria have the ability to metabolize MEL; however, the same is apparently not true for mammals and therefore it holds no nutritional value for animals (Osborne *et al.*, 2008).

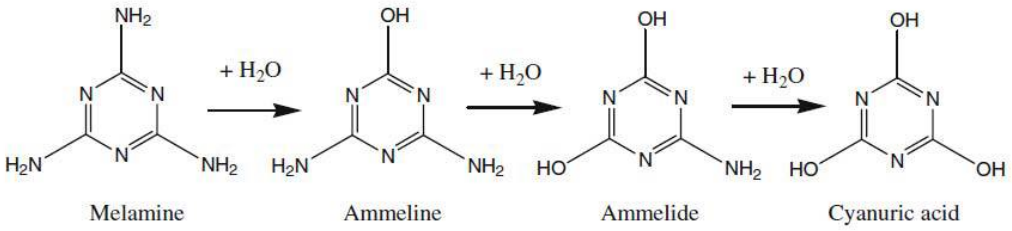


Figure 3: The chemical process in the formation of MEL related compounds (Tyan *et al.*, 2009).

Cook *et al.* (1981) stated that melamine is biodegradable as illustrated in Figure 1 and Jutzi *et al.* (1982) confirmed the derivative pathway of melamine with four different methods. Melamine is commercially synthesized from urea (Hau *et al.*, 2009) and the by-products ammeline, ammelide and cyanuric acid originate during hydrolysis (Figure 1). The melting point of MEL is 345°C, which explain why some plastic ware melts after high heat exposure (Tyan *et al.*, 2009). Melamine is not very soluble in water (3240 mg/L at 20°C) and according to Chapman *et al.* (1943) less than 1% melamine is required to saturate an aqueous solution after being diluted in water at 20°C.

Melamine is not an effective source of non-protein N for ruminants due to the slow and incomplete hydrolysis of melamine (Newton & Utley, 1978). Furthermore, in a rat study by Mast *et al.* (1983) it has been shown that more than 90% of ingested melamine was excreted within 24 hours, which further accentuated the insignificant nutritional contribution of melamine. Despite the theoretical desirable qualities that melamine displays, the discovery of alternative feed additives should rather be explored on other biological fields, such as entomology. Melamine has not been permitted to be used as a direct feed additive; however, traces may be detected in feed due to crops fertilized with melamine related products, or as a breakdown product from cyromazine (CYR) that has been included as a veterinary drug. Gossner *et al.* (2009) reported that the levels of melamine in contaminated animal feeds, ranged from 3.3 mg/kg to 21 000 mg/kg during 2008 and 2009. These practices are currently considered as unethical and should not be tolerated by authorities while the public should be properly informed of the current situation and possible ill symptoms.

The general position of animal nutritionists and veterinarians are not to rely on so-called safe inclusion levels, but that feed should rather contain undetectable levels of melamine.

It has been accepted, however, that the presence of melamine in the environment is inevitable and may contribute to a background level of contamination in feeds (WHO, 2008). Therefore, restrictions on maximum melamine inclusion levels in various feedstuffs have been set as an alternative resort. The World Health Organisation (WHO) has set the tolerable daily intake (TDI) levels for humans at 0.2 mg/kg body weight (Setiogi, 2008), and the maximum allowable melamine concentration of human foods at 2.5 mg/kg, while the maximum allowable level for infant formula was set at 1 mg/kg. For animal feeds, the industry has also accepted 2.5 mg/kg melamine as the maximum allowable melamine level. However, products containing excessive melamine residues could still surface in countries with no restriction policies.

It was reported in 2007,by the new York Times, that the addition of melanine scrap into fish and livestock feed to give the false appearance of a higher level of protein in many part of chaina mainland was an open secret (Baroza and Barrionueevo 2007). Few days after the report, it was discovered that despite the open ban of melamine use in vegetable protein, at least some chemical manufacturers are still selling it for use in animal feed and in products for human consumption (Baroza and Barrionueevo 2007). (Martin 2007) also found out that melamine was purposely added as binder to fish and animal feed in Ohio and Colorado. Several companies, including Nestle were involved in scandal involving milk and infant formula which was adulterated with melamine that led to the death of six infants and more than 50,000 being hospitalized (McDonald 2008, Macartney 2008, WHO 2009). In 2008, fresh brown eggs imported to Hong Kong from Hanwei Group in Dalian in North eastern China, were found contaminated with nearly twice the legal limit of melamine. This led to the testing of all mainland Chinese pork, farmed fish, animal feed, chicken meat, eggs and offal products for melamine. (BBC News 2008)

2.4.1 **Chemistry and application of melamine**

Melamine (1, 3, 5 - triazine - 2, 4, 6 - triamine) with chemical formula $C_3H_6N_6$, is an organic compound and a trimer of cyanamide. Melamine is commonly found as a white solid and has a molar mass of 126.12g/mL, a small molecule with high nitrogen content (Osborne *et al.*, 2008). It is used in the manufacture and processing of a variety of products ranging from plastics, coatings, leather, paints, laminates, flame retarding agent and table-tops and in recent years it has been added to certain food products. Some bacteria have the ability to metabolize MEL; however, the same is apparently not true for mammals and therefore it holds no nutritional value for animals (Osborne *et al.*, 2008).

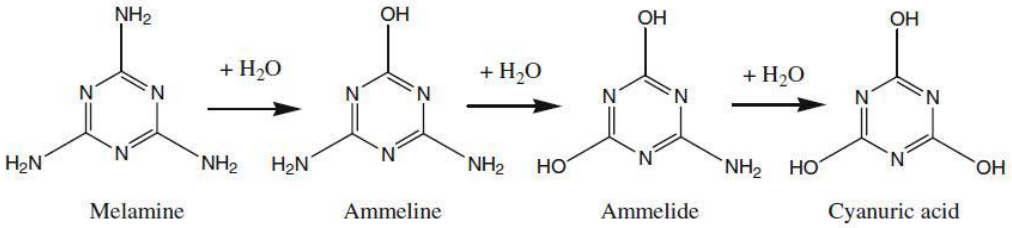


Figure 4: The chemical process in the formation of melamine related compounds (Tyan *et al.*, 2009).

2.4.2 **The relationship between melamine and cyanuric acid**

It is still inevitable to mention the interaction of cyanuric compound with melamine and how it is related to nephrotoxicity in combination with melamine. According to Puschner *et al.* (2007), intake of melamine and cyanuric acid alone seems to be less of a problem than ingesting a combination of the two chemicals (melamine-cyanurate) which has repeatedly been reported to induce kidney disorders. Similar to melamine, cyanuric acid has a low solubility in water (2000 mg/L at 25°C) and the simultaneous addition of the two substances result in an isomeric interaction, creating an insoluble (2 mg/L) crystalline structure in the distal nephrons (Hau *et al.*, 2009). The motive for the inclusion of cyanuric acid in animal feed remains uncertain. It is speculated that cyanuric

acid has been added as an adulterant similar to melamine in feed due to its high nitrogen content. Another theory is that bacterial melamine metabolism could have occurred in the toxic pet food, creating cyanuric acid as a byproduct (Osborne *et al.*, 2008). Melamine and its derivatives can be stored in the body and when these compounds are present in diets for food animals, they represent risk to human health as well. Suchy *et al.* (2014) reported that in laying hens melamine is not only stored in their tissues, but it was biotransformed into cyanuric acid which had not been reported in poultry before. Bai *et al.* (2010) and Novak *et al.* (2012) also reported that the administration of melamine contaminated diets to laying hens resulted in the residues melamine into eggs.

2.4.3 **The metabolism and distribution of melamine and cyanuric acid**

Very little is presently known about the exact mechanism of melamine metabolism and nephrotoxicity, however, it appears as if melamine is quickly metabolized by the body. Allen *et al.* (1982) reported that humans excreted 98% of ingested cyanuric acid within 24 hours in urine. Approximately 90% of all ingested melamine in a rat study was excreted in the urine within 24 hours (Mast *et al.*, 1983). Melamine primarily causes severe damage to the kidneys which ultimately may result in death if the animal receives excess levels of melamine. These levels vary between different species. It is said that the mechanism of kidney failure observed in animals due to melamine ingestion can be compared to uric acid nephropathy that occur in humans because both induce mechanical obstructions which is similar to humans enduring gout (Reimshuessel *et al.*, 2008). Therefore, renal failure due to melamine toxicity is the result of intrarenal crystal obstruction causing increased renal pressure which reduces renal blood flow (Reimshuessel *et al.*, 2008). Approximately 22-25% of the blood flow generated by cardiac output in a human body enters the two kidneys (Sebastian, 2009). Toxins are removed by the kidney via glomerular filtration, tubular excretion through passive

diffusion and active tubular secretion (Sebastian, 2009). Sebastian (2009) explains that the most common site for toxin related injury is in the proximal convoluted tubule. Reasons for this is that some toxins are activated by enzymes (cytochrome P450 and cysteine conjugate β-lyase) localized in the epithelial cells of the proximal tubular. Also the epithelium cells of the proximal tubules are more loosely arranged than the distal tubular cells, making toxin intrusion effortless. The proximal convoluted tubular epithelial cells seem to be most susceptible to injuries related to decreased blood flow due to obstruction of the tubule. When feeding high levels of melamine or cyanuric acid alone, stone formation occurs mainly in the proximal tubules (Kobayashi *et al.*, 2010). Although, histopathological evaluations showed complications in the distal tubule as well as in the collection ducts caused by MEL-cyanurate crystals (Dobson *et al.*, 2008; Kobayashi *et al.* 2010). A theory which is currently globally accepted has been developed to explain these findings. It states that the glomerular filtrate has a pH of 7.4 after which it decreases to approximately 6.8 in the proximal tubules due to H+ secretion and further H+ secretion in the collecting ducts via luminal proton ATPases can decrease the urinary fluid pH to under 6 (Barac-Nieto, 2004). Bhalla *et al.* (2009) reported that MEL-cyanurate stone formation occurs at pH 5.8 which may be the reason for stone formation in the distal tubules and therefore low urinary pH may be encouraging crystal formation and ultimately causing acute renal failure. Infants and very small children seem to be more severely affected by MEL than adults due to several reasons including that infants have to take in more feed according to their body weight compared to adults; more frequent feedings; differences in intestinal absorption and immature kidney functioning. However, the main reason why infants are more susceptible is that their blood serum and urine contain considerably higher levels of uric acid compared to adults and older children, increasing the possibility for uric acid-MEL precipitation in the renal

tubules. Also it is said that infants have lower solutes (citrate and phosphate) to compete against MEL for binding sites, making susceptibility even more severe (WHO, 2010). In theory these findings might also apply to the avian species due to their higher uric acid blood serum levels compared to mammals. Layer chickens have lower uric acid levels than non-layer birds and uric acid serum levels decrease with age in most species while low protein diets are also thought to initiate low uric acid levels (Bowes *et al.*, 1989). The lowest daily intake of MEL that would initiate bladder stone development was reported by Melnick *et al.* (1984) to be 750 mg/kg for rodents after ingesting MEL for 13 weeks. Renal stones collected from patients who ingested MEL alone are primarily composed of MEL and uric acid. These two substances are bonded at a 1:1 ratio in rats and for humans more or less 1:2 due to the higher uric acid levels in humans (Reimshuessel *et al.*, 2008). To identify the origin of the crystals, they are stained with Oil Red O. Crystals composed of calcium phosphate and calcium oxalate did not stain with this reagent; however, MEL-containing crystals will stain with Oil Red O and therefore they are different from the typical calcium stones (Sebastian, 2009). Unlike calcium oxalate crystals, MELcyanurate crystals cause renal failure due to stone obstruction in the renal tubules (Kobayashi *et al.*, 2010). Calcium oxalate crystals on the other hand will begin to stick to tubular epithelial cells and will grow by expressing stone matrix proteins (Tawada *et al.*, 1999).

There are limited documented research results pertaining to melamine in the production of animal feed (Cruywagon *et al*., 2009

2.5 **Melamine distribution and withdrawal in animal products**

2.5.1 **Poultry meat and eggs**

According to Thompson *et al.* (2008), melamine and its compounds are rapidly eliminated by the kidneys and do not accumulate in the body. Comparison between the findings of different researchers regarding melamine distribution and withdrawal rates in eggs is shown in Table 1.

Table 1 Comparison between maximum melamine distribution (mg/kg) and melamine residuals in eggs after withdrawal from the feed.

[1]**Day**	**Author**	**Inclusion level (mg/kg)**									
		2	5	30	50	60	100	250	500	1000	2000
	Distribution										
	Bai *et al.* (2010)	-	-	-	-	-	1.6	3.0	6.7	11.7	28.7
	Chen *et al.* (2010)	-	0.3	-	1.0	-	1.9	-	-	-	-
	Maff (2010)	-	-	0.7	-	1.6	-	-	-	-	-
	Yuchang *et al.* (2010)	0.1	-	-	-	-	2.4	-	-	-	-
	Withdrawal										
10	Bai *et al.* (2010)	-	-	-	-	-	ND	ND	ND	ND	ND
7	Maff (2010)	-	-	ND	-	0.04	-	-	-	-	-

[1]Days after MEL exposure has been discontinued

ND – not detected

From Table 1 it is evident that there exists an upward trend for MEL distribution as the administered dose increases and that a negligible amount of MEL is present after a seven day withdrawal period. Bai *et al.* (2010) provided MEL feed (0, 100, 250, 500, 1000 and 2000 mg/kg) to layers for 34 days. In eggs MEL levels peaked at a range of 1.6-28.7 mg/kg at day four for all treatments and remained at a plateau for as long as the birds had access to the MEL feed. Similar results were reported by Chen *etal.* (2010) where distribution levels did not differ in eggs from day 1-15, confirming the statement by Thompson *et al.* (2008) that MEL accumulation did not occur. The distribution levels increased (Bai *et al.*, 2010) as the MEL content of the different feeds increased and a similar distribution trend were reported for all treatments. After the withdrawal period was initiated on day 34, MEL levels rapidly decreased in eggs and no MEL was detected for treatments receiving 0, 100, 250 and 1000 mg/kg MEL already on day three, followed by the treatments receiving 500 and 2000 mg/kg MEL on day four (Bai *et al.*, 2010). Chen *et al.* (2010) established a linear equation (Y = 0.08491 + 0.01473X) to calculate the MEL distribution rate in eggs after receiving a certain level of contaminated feed. It states that if a feed containing more than 164 mg/kg MEL are fed to layers, it is predicted that the residual levels in eggs will exceed the maximum permitted level of 2.5 mg/kg (Setiogi, 2008). 2.5 mg/kg is the maximum limit of any veterinary drug that may be traced as a residual in animal tissue or products (Zhu *et al.*, 2000). Lu *et al.* (2009b) showed that there is a difference in the distribution and metabolism of MEL in eggs and other body tissues. Residues were first detected in egg albumin and the kidney, shortly followed by plasma, liver, drumstick and lastly the breast muscle and egg yolk. Melamine was rapidly metabolized and eliminated within the 14 day withdrawal period from the plasma, liver, kidney and muscle compared to the albumin and yolk. On day 21 no samples revealed MEL levels above 0.30mg/kg. A few studies have been conducted

to determine the rate of MEL distribution and withdrawal from body tissues (kidney, liver, muscle) and the available data has been combined in Table 2 and Table 3 to compare the reported results.

Table 2 Comparison between residue results for melamine distribution in poultry meat.

Inclusion level (mg/kg)

[1]Day	species	Sample type and author	Inclusion level (mg/kg)						
			2	**100**	**200**	**250**	**500**	**1000**	**2000**
		Kidney							
42	Broilers	Lu *et al.* (2009)	-	1.7	3.2	-	4.1	9.2	-
34	Layers	Bai *et al.* (2010)	-	1.3	-	1.6	2.7	8.0	21.7
40	Broiler	Yuchang *et al.* (2010)	0.1	4.5	-	-	-	-	-
		Liver							
42	Broilers	Lu *et al.* (2009)	-	ND	ND	-	1.3	2.7	-
34	Layers	Bai *et al.* (2010)	-	0.5	-	0.5	1.5	2.8	6.9
40	Broiler	Yuchang *et al.* (2010)	-	-	-	-	-	-	-
		Muscle							
42	Broilers	Lu *et al.* (2009)	-	ND	ND	-	1.7	3.7	-
34	Layers	Bai *et al.* (2010)	-	0.4	-	0.8	1.6	3.7	9.3
40	Broiler	Yuchang *et al.* (2010)	0.1	1.7	-	-	-	-	-

[1]Day refer to animal age when sample were collected by each author

Table 3 Comparisons regarding the withdrawal rate of melamine from poultry meat.

[1]Day	Species	Sample type and author	Inclusion level (mg/kg)						
			2	**100**	**200**	**250**	**500**	**1000**	**2000**
		Kidney							
7	Broilers	Lu *et al.* (2009)	-	ND	ND	-	ND	ND	-
10	Layers	Bai *et al.* (2010)	-	ND	-	ND	ND	ND	ND
		Liver							
7	Broilers	Lu *et al.* (2009)	-	ND	ND	-	ND	ND	-
10	Layers	Bai *et al.* (2010)	-	ND	-	ND	ND	0.06	0.40
		Muscle							
7	Broilers	Lu *et al.* (2009)	-	ND	ND	-	ND	ND	-
10	Layers	Bai *et al.* (2010)	-	ND	-	ND	ND	ND	ND

[1]Day refer to animal age when sample were collected by each author

Lu *et al.* (2009) noted that MEL were detected on day 28 in breast samples above 200 mg/kg and liver samples above 100 mg/kg; however, on day 42 no MEL could be detected in the 200 mg/kg treatments and MEL levels were lower for the 500 and 1000 mg/kg group on day 42 compared to day 28 proving that MEL do not accumulate in the body and that some sort of mechanism might trigger more rapid elimination in the bird. No illness symptoms or production defects were observed for all treatment birds, proving that MEL levels up to 1000 mg/kg are tolerated by birds without affecting production. No MEL was detected for treatment groups receiving less than 100 mg/kg. Various tissue samples were also analyzed for the study of Bai *et al.* (2010) on day 34 where renal tissue displayed the highest concentrations (1.3-21.7 mg/kg) and MEL was also detected in the liver, muscle and reproductive organs. Liver tissue had higher distribution rates compared to muscle for the low dose groups; however, more MEL was gradually deposited in muscle than the liver as the dose level increased. After a ten day withdrawal time no MEL was detected in the kidneys and muscle with low levels of 0.06 and 0.4 mg/kg in the liver for the treatments receiving 500 mg/kg and 2000 mg/kg MEL respectively. No MEL was detected for any tissue on withdrawal day 20. Similar findings were reported by Yu *et al.* (2009).

2.5.2 **Other species and products**

After feeding layer ducks different MEL inclusion diets (0, 1, 5, 25, 50 and 100 mg/kg), an increased trend in liver and kidney weights were observed as the MEL dosage increased (Yuchang *et al.*, 2010). Melamine levels peaked around day two for all treatments and the MEL concentration in eggs were higher for the groups that received 25 mg/kg and more compared to the 0-5 mg/kg groups. The half life time of MEL in eggs after withdrawal was found to be 18 hours. Depleted levels were detected four days after withdrawal for all inclusion levels. The 25, 50 and 100 mg/kg treatments reached

the 0.05 mg/kg detection level after 3, 4 and 8 days respectively. For the study of Yuchang *et al.* (2010) higher MEL levels were found in poultry eggs compared to kidney and muscle tissue.

In a fish study by Andersen *et al.* (1986) catfish, trout, tilapia and salmon received 400 mg/kg body weight a day in three trials of which MEL or Cyanuric acid (CYAN) or MEL-cyanurate were fed for three days. Melamine levels of 0.04-0.12 mg/kg were detected in the trout and salmon control fish, due to contaminated feed that was fed to the fish six months prior to the initiation of this study. For trial one, fish only received MEL and residual levels were detected in the meat for catfish (210 mg/kg), tilapia (177 mg/kg), salmon (94 mg/kg) and trout (80 mg/kg). After a six day withdrawal period, tilapia had considerably lower residual levels (0.02 mg/kg) compared to catfish (81 mg/kg), salmon (58 mg/kg) and trout (34 mg/kg). It could be that tilapia has the ability to excrete MEL more efficiently than the other fish species. Or it was likely due to the fact that tilapia emitted some of the MEL containing gel food which resulted in decreased ingestion; however, high levels were comparable to catfish. Trial two was similar to trial one except that MEL was substituted with CYAN in the diets fed. It was interesting to note that even though no MEL was detected in the catfish feed, MEL was found in the muscle tissue (0.006-0.012 mg/kg). Salmon also revealed small muscle concentration levels (0.019-0.083 mg/kg); however, their feed had been found to be contaminated with 6.7 mg MEL /kg. No MEL traces were found in trout and tilapia. In trial three all fish received the MEL-cyanurate contaminated diet for three days after which a 14 day withdrawal period followed. In all fish species MEL-cyanurate crystals were observed in the kidneys, however the level of crystal formation was considerably different from and between species. Melamine was also detected in muscle tissue of individuals from

different species and could be attributed to the level of crystallization where more crystals made less MEL available to be deposited in muscle.

It was reported that during 2007 contaminated pet food were used as a supplement in some swine feed and contained 30-120 mg/kg MEL (US FDA, 2007). Even though these levels were declared to cause no harm to humans, the public remained cautious of consuming pork. The half-life excretion rate of MEL was reported to be 2.7 hours for rats (Mast *et al.*, 1983) and in pigs 4.04 hours (Baynes *et al.*, 2008) with a renal clearance of more or less 27 mL/min for pigs, giving a renal clearance difference of about 1.5 between the two species. This is due to the greater renal clearance mechanism of rats which is about five times more efficient. The observed renal clearance findings of pigs as found by Baynes *etal.* (2008) suggests that the distribution of MEL mainly occurs in the extracellular fluid rather than being deposited or metabolized by organs, except for the kidney where MEL clearance is made. They also reported that the elimination tempo of MEL from pigs were 1.5 times longer than for rats due to different glomerular filtration rates. The bottom line is that 99% of the MEL occurring in pig blood is excreted within 28 hours which is expected to be below the safe level of 50 μL/mL and due to this quick excretion rate, distribution of MEL in the muscle and organ tissue (except for the kidneys) is expected to be low and should be considered to be safe for consumption.

In goat milk MEL could still be detected after 84 hours of 40 mg/kg ingestion (Baynes *et al.*, 2010) and a long plasma half-life (11.12 hours) were reported for small ruminants like goats. Although the half-life of MEL in milk was established to be a quick 9.44 hours, detectable levels were still above the 1mg/kg safe level for three days. Therefore MEL elimination in milk could be considered to be of higher importance than tissue

residues. In a Holstein cattle study conducted by Cruywagen *et al.* (2009) dairy cattle receiving MEL contaminated feed containing 1142 mg/kg for eight days revealed MEL levels of 10-15.7 mg/kg in milk for as long as exposure consisted. Excretion in milk represented 2% of the total dose; however, for the goat study by Baynes *et al.* (2010) only 0.3% of the oral dose was recovered in the goat milk, which could be likely due to the lower milk production relative to body weight of dairy goats compared to dairy cows. This statement has been verified by the higher percentage of ingested MEL in the milk of high producing goats compared to the low production goats. For both studies MEL could be detected in milk for 4-7 days after the last time of exposure.

In another dairy study, cows received feed containing 50 mg/kg and 100 mg/kg MEL respectively for 28 days (Maff, 2010). Milk MEL levels peaked at 0.9 mg/kg and 1.6 mg/kg for the low and high groups respectively within two days and rapidly decreased to below 0.01 mg/kg within seven days after withdrawal (Maff, 2010). Tissue samples were also collected on day 28 for muscle (0.46-0.69 mg/kg), fat (0.25-0.63 mg/kg), liver (0.58-1.0 mg/kg) and kidneys (2.3-3.4 mg/kg). After feeding lambs 2-100 mg/kg MEL, maximum levels were reported for muscle (0.435 mg/kg) and liver (0.469 mg/kg) samples on day 49 (Lu *et al.*, 2010). Kidney maximum levels (1067 mg/kg) were observed on day 53 which differs from other species. Treatment groups receiving 2 mg/kg had tissue distribution levels below the so called safe level of 0.05 mg/kg. When MEL intake was discontinued, detectable levels below 0.020 mg/kg were reported for all tissue samples within 4.5 days after withdrawal. Except for fish, MEL residues in tissue samples of cattle, swine, chicken, sheep and duck exposed to 100 mg/kg a day could not be detected after a four day withdrawal period. For fish, five days for muscle and 14 days for renal tissue were necessary for complete depletion. Yuchang *et al.* (2010) reported higher residual levels in chicken muscle (1.86 mg/kg) compared to pig (1.36 mg/kg),

sheep (0.53 mg/kg) and cattle (0.47 mg/kg) after feeding 100 mg/kg MEL per day. Also eggs had more MEL residuals (2.366 mg/kg) compared to milk (0.487 mg/kg); however it was interesting to note that chicken, duck and layer muscle tissue samples had the fastest depletion rate of 24 hours compared to pigs (48 hours), cattle (65 hours) and sheep (98 hours).

2.5.3 **Toxicity and production impact of Melamine**

Melamine when swallowed, inhaled or absorbed through the skin is described as harmful. Chronic exposure may cause cancer or reproductive damage. It causes eye, skin and respiratory irritation. However, the short-term lethal dose is equal to common table salt (Bradley 2008) US Food and Drug Administration (FDA) scientists explained that when melamine and cyanuric acid are absorbed into the bloodstream, they concentrate and interact in the urine-filled renal tubules, then crystallize and form large numbers of round, yellow crystals, which in turn block and damage the renal cells that line the tubes, causing the kidneys to malfunction. (Weise 2007). The European Union set a standard for acceptable human consumption (Tolerable Daily Intake) of melamine at 0.2 mg per kg of body mass, (Harrigton 2010) (previously 0.5 milligrams), Canada declared a limit of 0.35 mg and the US FDA's limit was put at 0.063 mg daily (previously 0.63 mg). The World Health Organization's food safety director estimated the amount of melamine a person could stand per day without incurring a bigger health risk "tolerable daily intake" (TDI), was 0.2 mg per kg of body mass (Endreszl, 2008). Toxicity of melamine can be moderated by intestinal microbes. In culture, Klebsiellaterrigena, which rarely colonizes mammalian intestines, (Lauran, 2013) was shown to convert melamine to cyanuric acid directly. Rats colonized by *K. terrigena* showed greater melamine-induced kidney damage compared to those not colonized. (Zhang, 2013).

2.6 Cyromazine distribution and withdrawal in animal products

2.6.1 Poultry meat and eggs

There has been some concern that cyromazine (CYR) could be metabolized in poultry breeds to melamine which could be deposited in meat and eggs, since it has been reported that 10% of the ingested cyromazine will convert metabolically to melamine (USEPA, 2007). Chou *et al.* (2003) tested chicken, egg, beef, mutton and pork samples for any cyromazine and melamine residues. None of the samples revealed detectable melamine levels (> 0.02mg/kg) and only one beef sample contained 0.04 mg/kg cyromazine. Cyromazine were detected (100 mg/kg) in layer eggs after feeding 2.5 mg cyromazine/kg feed/day for five weeks (Miller & Corley, 1980) and no traces were found in liver or muscle tissue. It was then concluded that there is a positive correlation between induced dose levels and residues in eggs. Cyromazine was also detected in the liver, muscle and faeces after an increase in inclusion level. In a study by Anderson *et al.* (1986), cyromazine inclusion in diet (0.00044 mg/kg), traces were found in only three out of 32eggs with detection levels of <0.01, 0.11 and 0.22 mg/kg respectively and no traces were found in the thigh and breast portions and the liver, gizzards and fat. These findings might not be considered accurate since tissue samples were harvested from only two birds. However, these results have the same trend as the previous mentioned trial of Miller and Corley (1980).

Cecil *et al.* (1981) determined the amount of cyromazine distribution in the liver, fat and muscle of layers after feeding a CYR derivate known by the industry as CGA-19255 at four different levels (2.5, 12.5, 25 and 125 mg/kg). CGA-19255 is metabolized in the body to CYR. Cyromazine were not traced in the first group and increasing levels were found in the liver and muscle as a result of increased administration levels. Cyromazine deposition in fat was only detected after 125 mg/kg ingestion of CGA-19255. No CYR

were detected after only a week of withdrawal in all tissues for all treatment levels and the authors concluded that these inclusion levels did not suppress any production performance qualities. No MEL was detected in any egg or meat samples in the study of Boone *et al.* (1985) after feeding layers 0-5 mg/kg CYR. The CYR maximum levels were already reached on day three in eggs and day 14 in meat samples. Withdrawal times were rapid and within one and two days no CYR were detected in meat and eggs samples respectively.

Even though cyromazine is rapidly eliminated from the body, undeniable evidence published by Brake *et al.* (1991) shows that contaminated faeces were passed several weeks after birds were withdrawn from a diet containing cyromazine. The higher the administered dose, the longer the residual toxicity duration persisted. Brake *et al.* (1991) reported that when feeding layers 25mg/kg/day cyromazine for the first 20 weeks of age, residuals could be found in the droppings 13 weeks after cyromazine withdrawal and it was suggested that residues were deposited in body tissues. The recycling of cyromazine might have occurred since the birds were reared on the floor and according to the later studies of Caldas (2007) 97% of ingested cyromazine (5 mg/kg) are excreted within 24 hours. Although the inclusion levels in the study of Caldas (2007) were only 5 mg/kg which might have relieved some renal pressure to manage cyromazine excretion compared to the 25 mg/kg applied by Brake *et al.* (1991), the delay in cyromazine excretion reported by Brake *et al.* (1991) is questionable since contamination could repeatedly occur by ingesting faeces containing cyromazine.

2.6.2 **Other species and products**

In studies on other species, findings similar to the poultry trials were reported. In a lactating goat study by Simoneaux *et al.* (1984), a low (4.8 mg/kg) and high (48 mg/kg)

cyromazine concentration were administered to two groups. Approximately 90.4% and 82.2% of the recovered residues were excreted via urine and 7.5% and 5.7% in the faeces respectively for the low and high doses. Less than 2% of the administered dose was recovered in body tissue. Again the liver had the highest residue levels (0.791mg/kg and 1.522 mg/kg) followed by kidney tissue (0.043 mg/kg and 0.437 mg/kg) and loin cuts (0.009 mg/kg and 0.104 mg/kg). Cyromazine detected in the milk accounted for 0.2% of the total recovery with an average of 0.017 mg/kg for the low dose and 0.35 mg/kg for the high cyromazine dose. In a similar study by Tortora (1991), 150 mg/kg were administered to dairy goat which is higher than the dose provided by the previous researcher. Also Tortora (1991) had a lower recovery rate of 73.9% compared to Simoneaux *et al.* (1984) which reported 102% and 90.4% for the low and high dosed animals respectively. The highest recoveries were in the kidneys (3.78mg/kg) followed by the liver (0.93mg/kg) and loin cut (0.79 mg/kg) which is contradictory to the higher liver recovery rates of Simoneaux *et al.* (1984). Milk contained 0.76 % of the total recovery (0.656 mg/kg). These studies added to the confirmation that higher administered cyromazine doses will result in higher residual traces as mentioned earlier. Melamine was also detected in the kidney (1.24 mg/kg), liver (0.16 mg/kg) and loin cut (0.03 mg/kg) as a result of cyromazine metabolism *in vivo*. No MEL was detected in fat and cyromazine levels were 0.11 mg/kg. In a small study on sheep, 5 mg/kg cyromazine were administered for nine days on only one sheep (Simoneaux *et al*., 1981). The results were similar to the goat studies. The liver yielded the highest. cyromazine recovery (0.174 mg/kg) followed by the kidneys (0.048 mg/kg) and leg muscle (0.013 mg/kg). It was interesting to note that a higher level of melamine was detected in the liver (0.645 mg/kg) compared to cyromazine, which might be explained by the relatively low recovery rate of 67% that could have influenced accurate detection.

No detectable cyromazine residues were found in rat tissue after feeding 0.5 mg/kg cyromazine (Simoneaux *et al.*, 1978), except for the liver (0.007 mg/kg); however, the author discarded this value due to a lack of accuracy. Caldas (2007) proposed a possible pathway for cyromazine metabolism in different animal species. In the rat, goat, hen and sheep cyromazine can be metabolized to melamine. However, very little data on these reports are available. Only in rats and goats the residual methyl- cyromazine were observed and goats were the only animal investigated to have hydroxyl- cyromazine present as a cyromazine metabolite.

2.7 **Toxicity of cyromazine**

Few literatures are available for cyromazine toxicity. The adverse effect of oral administration of 1000mg/kg of cyromazine was weight loss due to decreased feed intake and there is quick recovery after cyromazine was withdrawn in rats (Pfeifer, 1993) and dogs receiving more than 3000mg/kg (Jessy *et al*., 1979). Goldenthal *et al*., 1979 also reported a decreased liver weight for rats' recovery more than 1000mg/kg cyromazine. Some reproductive complications were noticed in rats receiving the same dose (Blair *et al.*, 1989). Pfeifer (1993) reported that cyromazine can be considered to have a low acute toxicity levels. Brake *et al*, 1989, reported broiler breeder to be more sensitive to cyromazine than layers and turkeys. In another study by Brake *et al*.,(1983 and 1984), 3000mg/kg cyromazine was reduced to 1000mg/kg due to high mortality rate and poor feed conversion ratio(FCR), weight loss and reduced reproductive and progeny performance with high mortalities were noticed with levels above 1000mg/kg. Decreased fertility, hatchability and egg production were also reported. Cyromazine was reported to be non-toxic to young layers when ingesting 10 000 mg/kg/day for four weeks and 1000 mg/kg for 20 weeks (Brake *et al*., 1985); however, severe weight loss was recorded for both dosage levels. According to the findings of Brake *et al.* (1989) administered levels

of 2000 mg/kg cyromazine or less are non-toxic to turkeys; however, a marked decrease in feed intake and growth were observed in turkeys receiving levels between 500-2000 mg/kg cyromazine. Despite the occurrence of suppressed body weights and poor reproductive performance, these production traits were reversible after cyromazine was withdrawn from the diets of broiler breeders (Brake *et al.*, 1984), layers (Brake *et al.*, 1985) and turkeys (Brake *et al.*, 1989). It is interesting to note that cyromazine inclusion of 300 mg/kg in layer diets resulted in increased egg production compared to 0 mg/kg and 30 mg/kg over a period of 32 weeks; however, a decline in egg weight was reported for week 32 where shell weight was significantly decreased compared to the 0mg/kg treatment (Brake *et al.*, 1983).

For turkeys however no significant difference in egg production ($P > 0.05$) were found after feeding different levels of up to 2000 mg/kg, emphasising the hardiness of turkeys compared to broiler breeders against cyromazine. Inclusions of 1000 mg/kg in layer and broiler breeder diets had adverse effects on egg production and egg weight, but broiler breeders revealed an excellent feed conversion ratio (FCR) compared to the 0 mg/kg group. Decreased shell weights after chronic exposure to 1000 mg/kg cyromazine were measured; however, no significant ($P > 0.05$) fertility or hatchability difficulties were observed in the egg. Inclusion levels of 300 mg/kg or less had no deleterious effects on reproductive and progeny performance (Brake *et al.*, 1984; Brake *et al.*, 1985; Buhr *et al.*, 1983; Cecil *et al.*, 1981). Some residual effects were visible in the progeny of the birds and lower body weights were reported for the 1000 mg/kg treatments. The poorer performance for the 1000 mg/kg group could be explained by the decrease in egg weight; however, the 300 mg/kg treatment also had decreased egg weights but the chick weight was not negatively affected compared to the 0 mg/kg and 30 mg/kg group with the highest egg weights. According to Brake *et al.* (1984), metabolic damage is caused by

cyromazine ingestion and therefore nutrient deposition could be retarded in the egg, explaining the decrease in chick body weight without inhibiting liveability.

Contradictory results were reported by Wilson *et al.* (1983) where layer breeder hens who received 1000 mg/kg cyromazine had the highest egg production rate compared to 0, 50, 100 and 500 mg/kg with no suppressed feed intake and zero mortality. However, in a second experiment for the same study the treatment that received 1000 mg/kg cyromazine did not differ from the other treatments regarding egg production and was indeed the lowest of the treatments with the highest mortality rate. Even though no toxic effects can be identified after ingesting cyromazine, the US EPA (1999) prescribes that the inclusion of cyromazine may not exceed 5 mg/kg in poultry feed and that a 72 hours withdrawal period must be allowed before slaughter.

2.7.1 Cyromazine – Environmental Toxicity

According to the Vetrazin® Technical Manual (Anon, 1979) Vetrazin (cyromazine) is a non – irritant and is relatively safe to wildlife and species of birds. The major degradation product of cyromazine, melamine, was suspected to be a carcinogen (Toth and Bardalaye, 1987). However, Lim et al. (1990) claimed that this apparent carcinogenicity may be due to secondary effects resulting from the formation of renal bladder stones. These authors also claimed that melamine was not mutagenic. Cyromazine potentials for contaminating water on a local basis through effluent discharges. However, little is known of its toxicity to aquatic organisms. Walker 1964; Mecek et al 1976b, Schober and Lampert, 1977; Dewey. (1986) reported that the chlorotriazines are highly toxic to aquatic insects. While it is possible that the chlorine moiety has a great deal to do with its toxicity. Walker (1964) was one of the first authors to note aquatic toxicity effects for simazine and triazine.

Aquatic environment is more vulnerable to pesticides, because of its polarity (water solublility), stability to hydrolysis, photolysis and heat. In 1988, the friend of the UKs potable water sources had levels of pesticides that exceeded the European Community (EC) drinking water directives (Beck, 1989). Out of 150 streams surveyed in 10 midwestern states of the USA, 55% possessed detectable quantities of triazines (Anon, 1989). These are but two examples of the mounting evidence suggesting unacceptable contamination of aquatic ecosystem.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Studies carried out include:

- Experimental 1: Survey of cyromazine users in farms in Abeokuta metropolis.
- Experiment 2: Effect of inclusion of Cyromazine on the growth performance, nutrient retention and carcass yield of broiler chicken
- Experiment 3:Determination of residuals in the blood and tissue of broiler chickens

3.2 Experiment 1: Survey of Cyromazine users in farms in Abeokuta metropolis

3.2.1 Study Area

The study was carried out in Abeokuta the capital city of Ogun State, South-west Nigeria. It is located within latitudes 3°30'N-4°30'N and longitudes 6°30'E-7°30'E. The state has a total of 20 Local Government Areas. Abeokuta has the highest population and it is the most urbanized city in the state. It is mostly populated by civil servants, artisans, traders, transport workers, student's etc. The state covers a land area of 16,762 km2 with a population of 3,728,098 according to the 2006 population census. Odeda local government area of Abeokuta is the study area.

3.2.2 Sampling Technique

Sample units were randomly selected across different locations in the study area. They were chosen carefully in order to cut across various farms. The farms were selected using a systematic random sampling. A total of 100 structured questionnaires were distributed but only 60 were returned. Out of 60 returned, 29 were used for analysis as 31 were discarded due to incomplete information.

3.2.3 Method of Data Collection and Data Sources

Primary data were used in this study. These were collected by personal interview and recording with the aid of structured questionnaire. Questionnaires were administered at Veterinary shops, farm settlement and farmers meeting. Data were collected on socio-economic characteristic of household (such as household head age, income, Education etc), type of management, number of birds, age at which larvacide is introduced, how often larvacide is used, how often litter is changed, disposal of litter, benefit in terms of performance among several other variables needed to carry an analysis of larvacide usage.

3.2.4 Method of Data Analysis

Descriptive statistics with the use of frequencies and percentages were used to describe the socio-economic characteristics of respondents, location, used of larvacides e.t.c.

Experiment 2: Effect of inclusion of Cyromazine on growth performance, nutrient digestibilities and carcass yield of broiler chickens

3.3 Experimental location

The experiment was carried out at the Poultry Unit of the Directorate of University Farms (DUFARMS), Federal University of Agriculture Abeokuta, Nigeria. The area is located in the sub savannah region with an average temperature of 38^{o}C and a relative humidity of 80^{0}C. It lies in the region 76m above sea level and falls within latitude 75^{0}C "N- 708"N and longitude 30 11.2" E-30 2.5 E. It receives a mean precipitation of 1037mm per annum (Google earth, 2014)

3.3.1 Feed additive / Test ingredient

The feed additive used in these studies was a Lavacide (containing cyromazine). The product was obtained from a company in Ogun state Nigeria. The Larvacide product contains 99% inert products and only 1% cyromazine (Drugs.com, 2010). It was recommended by the manufacturer that Larvacide should be included at 250 – 500g/tonne.

3.3.2 Experimental birds and Management

A total of three hundred and twenty (320) day old Arbo Acre broilers were purchased from a commercial hatchery in Ibadan for this experiment. The brooder house and equipment were thoroughly disinfected prior to the arrival of the birds. The brooding lasted for 14days during which the temperature of the brooding environment was maintained to ambient temperature of the birds. Birds were intensively managed on deep litter. They were allotted to different dietary treatments and the feeding trials started immediately. Litter depth was filled to 3cm and 5cm mark from the floor of the pen. Vaccination schedule and medication programmes were strictly adhered to in this study. Feed and water were supplied *ad libitum.* The basal compositions of experimental diets are shown in Tables 4 and 5.

Table 4: Basal composition (gkg^{-1}) of experimental diets (0 – 4 weeks)

	Starter diets			
Ingredients	(0.00g/kg)	(0.25g/kg)	(0.50g/kg)	(0.75g/kg)
Maize	494.50	494.50	494.50	494.50
Soybean meal	120.00	120.00	120.00	120.00
Groundnut cake	140.00	140.00	140.00	140.00
Wheat offal	185.00	185.00	185.00	185.00
Bone meal	20.00	20.00	20.00	20.00
Oyster shell	30.00	30.00	30.00	30.00
Common salt	2.50	2.50	2.50	2.50
Vitamine/Mineral Premix	2.50	2.50	2.50	2.50
Methionine	3.00	3.00	3.00	3.00
Lysine	2.50	2.50	2.50	2.50
Determined analysis (%)				
Dry matter	87.08	90.07	89.00	93.95
Crude protein	20.27	21.58	24.13	26.56
Ether Extract	9.43	10.88	10.20	9.77
Crude fibre	12.33	12.80	11.67	12.33
Ash	5.60	6.92	6.93	9.75
*M E (Kcal/kg)	2989.75	3025.65	3990.86	3144.5

Vit. A: 4000000IU, vit.D: 800000 IU, vit E: 40000mg, vitk_3: 800mg, vitB_1: 1000mg, vit B_2: 6000mg, vit. B_6: 5000mg, vit B_{12}: 25mg, Niacin: 60000mg, Panthotenic Acid: 20000mg, Folic Acid: 200mg, Biotin: 8mg, Manganese: 300000mg, Iron: 80000mg, Zinc: 20000mg, Copper: Nill, cobalt: 80mg, Iodine: 400mg, Selenium: 40mg, Choline: 800000mg

T1 – control, T2 – 0.25g/kg, T3 – 0.50g/kg, T4 – 0.75g/kg

ME – Metabolizable energy

Table 5: Basal composition (gkg^{-1}) of experimental diets (4 –8 weeks)

	Finisher diets			
Ingredients	0.00	0.25	0.50	0.75
Maize	550.00	550.00	550.00	550.00
Soyabean meal	70.00	70.00	70.00	70.00
Groundnut cake	20.00	20.00	20.00	20.00
Wheat offal	300.00	300.00	300.00	300.00
Bone meal	20.00	20.00	20.00	20.00
Oyster shell	30.00	30.00	30.00	30.00
CommonSalt	2.50	2.50	2.50	2.50
Vitamine/ Mineral Premix	2.50	2.50	2.50	2.50
Methionine	2.50	2.50	2.50	2.50
Lysine	2.00	2.00	2.00	2.00
Total	1000.00	1000.00	1000.00	1000.00
Determined analysis (%)				
Dry matter	90.10	87	88.67	87.17
Crude protein	13.94	17.23	17.97	20.10
Ether extract	8.03	8.67	17.67	14.33
Crude fibre	11.30	9.2	12.0	11.50
Ash	5.5	5	6.5	7.5
ME(Kcal/kg)	3286.97	2715.22	2837	2906

Vit. A: 4000000IU, vit.D: 800000 IU, vit E: 40000mg, vitk_3: 800mg, vitB_1: 1000mg, vit B_2: 6000mg, vit. B_6: 5000mg, vit B_{12}: 25mg, Niacin: 60000mg, Panthotenic Acid: 20000mg, Folic Acid: 200mg, Biotin: 8mg, Manganese: 300000mg, Iron: 80000mg, Zinc: 20000mg, Copper: Nill, cobalt: 80mg, Iodine: 400mg, Selenium: 40mg, Choline: 800000mg

T1 – control, T2 – 0.25g/kg, T3 – 0.50g/kg, T4 – 0.75g/kg

3.5.2 Dietary treatments and experimental design

At day old, 320 birds were weighed and randomly divided into two groups of different litter depth (3 cm and 5 cm). Each group was sub divided into four treatments of 40 birds each. The birds were randomly assigned to four dietary treatments (0 g/kg, 0.25 g/kg, 0.5 g/kg and 0.75 g/kg cyromazine) in a 2 x 4 factorial arrangement. The birds were fed starter diets from 0 - 4 weeks and finisher diets from 4 - 6 weeks. Same commercial diets were fed to the two groups throughout the period of the experiment. Cyromazine was pre - mixed with small quantity of feed before properly mixed with known quantity of feed.

3.6 Data Collection

3.6.1 Proximate analysis of experimental diet

Proximate analysis of the samples was determined according to the standard procedure of the AOAC (2000). Samples were analysed for dry matter (DM), Crude fibre (CF), Ether extract (EE) and ash the nitrogen fraction of the samples were determined by using the Kjedahl method and Crude Protein(CP) was determined by multiplying the N value by 6.25. True protein analysis of the feed was also carried out.

3.6.2 Weighing of birds

Birds in each replicate were weighed at the beginning of the experiment and subsequent weighings were done weekly. All weighings were done on a Five Star® weighing scale (maximum = 50kg, minimum 25g). Weight gain was determined by the difference in the body weights of two consecutive weightings for each replicate group.

3.6.3 Performance characteristics

(i) Average Feed intake

Known quantity of feed was supplied to each replicate group each day and the left over by the following day was subtracted from the amount supplied, divided by the number of chicken. The difference was recorded as the feed consumed.

(i) Average feed intake = $\frac{\text{feed supplied (g) – feed leftover (g)}}{\text{Number of birds}}$

(ii) Average weight gain (g) = $\frac{\text{final weight (g) – initial weight (g)}}{\text{Total number of birds in the group}}$

(iii) Feed conversion ratio = $\frac{\text{Amount of feed consumed (g)}}{\text{Weight gain (g)}}$

3.6.4 Metabolic Trial

At the end of 6th week (withdrawal day of the test ingredient), a bird per replicate was randomly selected and transferred into the metabolic cages fitted with individual feed troughs and facility for separate excreta collection. Weighed quantity of feed was supplied and excreta were collected over a period of five days. A three day acclimatization period was allowed prior to the collection of droppings. Thedroppings per bird per day (for three days) were oven dried at 60^{o}C for 24 hours. Efforts were made to ensure that the excreta was not contaminated with feathers nor feed particles. The dried excreta for each group were pooled together and milled (0.1mm), weighed and stored in well labelled, sealed sample bottles for proximate analysis using the procedures described by AOAC (2005).

3.6.5 Formulae used for calculations in metabolic trial

Dry matter digestibility (%) = $\frac{\text{\% Dry matter feed intake – Dry matter output x 100}}{\text{Dry matter feed intake}}$

Nutrient intake = % Nutrient in diet x Feed intake

Nutrient voided = Nutrient in faeces x Amount of faeces voided

$$\text{Nutrient Digestibility coefficient (\%)} = \frac{\text{Nutrient intake} - \text{Nutrient voided} \times 100}{\text{Nutrient Intake}}$$

3.7 Carcass yield evaluation

At six, seven, eight, nine and ten weeks, a bird per replicate whose weight were close to the mean replicate weight were randomly selected, weighed, slaughtered via neck slit, allowed to bleed for two minutes and then scalded at 60^{o}C following standard commercial procedures (Jensen, 1984). Cut parts such as thigh, drumstick, back, wings and breast were weighed and visceral organs (gizzard, liver, heart, and kidney) and abdominal fat were eviscerated and weighed with a sensitive scale and expressed as percentage of the live weight. This was done to establish a 7, 14, 21, 28 days withdrawal period.

$$\text{Dressing Percentage} = \frac{\text{Empty carcass weight} \times 100}{\text{Live weight}}$$

3.8 Mortality

Number of dead birds per replicate was recorded to calculate the % mortality

$$\text{Mortality \%} = \frac{\text{Number of dead birds} \times 100}{\text{Total number of birds}}$$

3.9 Experiment 3: Determination of cyromazine residues in meat of broiler chickens

3.9.1 Measurements and sampling

To determine the residual rate of cyromazine and melamine in body tissues, four birds were weighed and slaughtered to harvest the tissues (thigh and drumstick) from each treatment on the 2nd, 4th and 6th week which is the termination period of cyromazine ingestion. By 7th, 8th, 9th and 10th week this was also done to establish a cyromazine

withdrawal period of 7, 14, 21 and 28 days respectively. The harvested parts were stored in refrigerator at constant temperature.

3.9.2 Cyromazine residue determination

Harvested tissue samples that were frozen (thigh and drumstick) were thawed overnight and then cut into pieces to increase evaporation area. Extraction of the meat was done by blending 30g of the meat sample with 1mL NaOH for 2 minutes. Sample was weighed with a sensitive scale. 1g of the blended sample was then transferred to 100ml centrifuge tube for the sample to be homogenized. It was homogenized in 70ml with ammoniacal acetonitrile, (20%) NH_4OH in acetonitrile. This was centrifuged at 10000g for 10 minutes.

This was filtered through a Whatman number one filter paper into a beaker. The filtrates were then evaporated to small volume for 10 minutes. These were later transferred to separating funnels and 50mlof n hexane was added and shaken properly. They were allowed to separate and the upper n hexane layer was discarded. The acetonitrile phase was evaporated into dryness in water bath. It was then made up of 1ml with acetonitrile and kept in the fridge for High Performance Liquid Chromatography analysis.

The standards (cyromazine and melamine) were purchased from Sigmoid Aldrich in US. The samples were analyzed for melamine and cyromazine using HPLC at Biochemistry Department of Bowen University Iwo, Osun State.

3.8.3 Haematology and serum chemistry

3.8.4 Collection of Blood samples

Blood samples were collected from the birds by the 2^{nd}, 4^{th} and 6 weeks (42 day) and when the cyromazine treatment was terminated and at 7th, 8^{th}, 9th and 10th week to

establish a 7, 14, 21 and 28 days withdrawal period. About 2.5ml of blood was collected from a bird per replicate into tube containing Ethylene Di-amine Tetra Acetate (EDTA) and another 2.5ml was collected into a hypodermic syringe. The blood in EDTA bottles were used to determine haematological measurements(haemoglobin concentration (Hb), red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), haemaglobulin and white blood cell differential (heterophils, lymphocytes, eosinophils, basophils and monocytes) according to standard methods (Schalm, 1986). Haemoglobin (Hb) concentration was determined using improved Neubauer haemocytometer (Coles, 1986). While those in hypodermic syringe were used to determine serum measurements (Serum total protein, Albumin, Globulin, Urea and Creatinine). Serum glucose was determined by using the commercial diagnostic kits. Plasma was separated from the blood samples in EDTA bottles with a micro pipette into a test tube for triglyceride and cholesterol analyses. The blood sample in the hypothermic syringe was allowed to clot before refrigerating for 6 hours and later spun in a centrifuge at 900rpm for minutes.

3.9 **Histopathology**

Each bird per replicate was slaughtered at 2nd, 4th and 6th week of inclusion to collect the kidney, liver and spleen for histopathology. This was repeated at 7, 8, 9 and 10 weeks to establish a withdrawal period of 7, 14, 21 and 28 days. The organs collected were stored in sample bottles that contain 10% formalin.

3.10 Statistical Analysis

Data collected were subjected to 2 x 4 factorial arrangements while the main effect (3cm and 5cm litter depth) and levels of cyromazine were used to evaluate the factors associated. Significant means were separated using Duncan's Multiple Range Test (Duncan, 1955) and was compared by using SAS (1999) at 5% level of probability.

Orthogonal analysis was used to determine the trend of response of Cyromazine inclusion.

Statistical model:

$Y_{ijk} = \mu + L_i + S_j + (SL)_{ij} + \sum_{ijk}$

where

Y_{ijk} = Observed value of dependent variable

μ = population mean

L_i = the effects of litter

S_j = the effects of cyromazine

$(LS)_{ij}$ = the interaction effect of litter and cyromazine

$\sum_{ijk}$ = residual error

CHAPTER FOUR

4.0 RESULTS

4.1: Distribution of respondents by Location

Table 6 showed the distribution of respondent by the location of their farm. Odeda showed the highest (13.8) population of respondents, followed by Oshiele (10.3), While the least were shown in Alabata, Camp, Aregbe, Obada Adatan, Itaoshin, Oshara, Odo-Eran.

Table 6 Distribution of Respondents by Location

Variables	Frequency	%
Odeda LG		
Alabata	2	6.9
Aregbe	2	6.9
Camp	2	6.9
Idera	2	6.9
Kotopo	1	3.4
Odeda	4	13.8
Odo eran	1	3.4
Odoran	1	3.4
Olodo	1	3.4
Osara	1	3.4
Oshara	1	3.4
Oshiele	3	10.3
Osiele	1	3.4
Itoko	1	3.4
Ojoo	1	3.4
Egbeda	1	3.4
Labuta	1	3.4
Malaka	2	6.9
Ipaja	1	3.4
Total	29	100

Source: Field Survey (2016)

4.2: Distribution of Questionnaires in farms in Abeokuta metropolis

The distribution of respondents by social economy characteristics is presented in table 7 The study showed that majority of the household heads are male (72.4%). Expectedly, majority (65.5%) of the household heads were educated up to the secondary school level. Average age of household heads was about 45 years with an average of 5 persons in the household (79.3%). Further analysis of the age of household heads revealed that majority (about 34.5%) were still below the age of 50. 72.4% were Yorubas, 17.2% Igbo and 10.2% Hausa. Majority (58.6% are Christians while 37.9% were Muslim and only 3.4% are for other religion.

Table 7 Distribution of Respondents by socio economic characteristics

Variable	**Frequency**	**%**
Gender of head		
Male	21	72.4
Female	8	27.6
Marital Status		
Married	19	65.5
Single	4	13.8
Widow	2	6.9
Divorcee	4	13.8
Educational level		
No formal education	2	6.9
Primary	3	10.3
Secondary	15	51.7
HND/BSc	8	27.6
MSc	1	3.4
Age		
< 30 yrs	5	17.2
31 – 40	9	31.0
41 – 50	10	34.5
51 – 60	2	6.9
61 – 70	3	10.3
70 years	29	100
Religion		
Christianity	17	58.2
Islam	11	37.9
Others	1	3.4

Source: Field Survey (2016)

4.3 The distribution of farmers by larvacide Usage

Table 8 shows the distribution of farmers by larvacide usage. (72.4%) of the respondents had less than 1000 birds. 58.6% respondents were raising layer birds, 13.8% were raising layers and broilers while 13.8% were raising broilers alone. 72.4% of the respondents were using larvacide. 51.7% used larvacide always. 69.0% respondents noticed flies on the droppings always, 10.3% noticed flies on faeces fortnightly. 13.8% notice flies on droppings quarterly while 6.9% does not notice flies. A total of 34.5% change their litter every month, 6.9% of respondents change their litter once in every two months while 13.8% does not change litter at all until after the birds were disposed.

Result also shows 82.8% dump litters in a nearby land, 6.9% dump it in nearby river while about (3.4%) dumped their refuses in the pit. 44.8% of respondents recorded/showed that litter used to decompose while 24.1% says litter does not decompose.

In terms of performance, 93.1% recorded that larvacide improves performance while 6.9% claimed it does not have effect on the performance. Out of the 93.1% that showed that it improves performances, 53.4% shows it improves egg size,24.1% indicated that it improve shelf life, 24.1% for number of cracks, 6.9% for weight of birds, 20.7% the quantity of feed eaten. 89.7% showed that larvacides reduces odour and 3.4% says it does not affect odour. While 55.2% recorded that odour is perceived after the use of larvacide is stopped for 2months while 34.5% recorded 4 months and 10.3% after 6 months.

Table 8 Distribution of Respondents by larvacide usage

Management System		
Deep Litter	26	89.7
Cage system	3	10.3
Number of Birds		
1000 or less	21	72.4
1001- 2000	7	24.1
4001-5000	1	3.4
Types of birds		
Broiler/ Layers	4	3.4
Broilers	1	13.8
Cockerel	17	3.4
Layers	4	58.6
Layers/ broilers	1	13.8
Layer/ Cockerel	2	3.4
Use of larvacide		
No	8	27.6
Yes	21	72.4
Age of Birds		
Growers	1	3.4
Point of cage	13	44.8
Point of Lay	15	51.7
How often		
Always	20	69
Forthnightly	3	10.3
Quarterly	2	6.9
Not at all	4	13.8
Once used how often it returns		
After a month	4	13.8
Two months interval	10	34.5
Quarterly	10	34.5
Six month interval	3	10.3
Yearly	2	6.9
Where do you dump your litter		
Nearby river bank	2	6.9
Nearby land	24	82.8
Pit	1	3.4
Flowing river	2	6.9
Do you notice the litter decompose		
No	10	34.5
Yes	19	65.5
Does larvacide improve performance		
Yes	2	6.9
No	27	93.1
If yes , in which of these		
Egg size	1	3.4
Shelf life of egg	7	24.1
Number of cracks	7	24.1
Weight of bird	2	6.9
Quantity of feed eaten/ day	6	20.7
Do you believe lavacide reduces odour		
Yes	26	89.7
No	3	10.3
Once used, how long does it take to perceive odour again		
Two months	16	55.2
Four months	10	34.5
Not Specific	3	10.3

Source: Field survey (2016)

4.4: Proximate Composition of the Experimental Diets

The proximate composition of the experimental diets is shown in Table 9. Nutrient values were significantly ($P<0.05$) different except for ash. There was increase in Crude Protein value as the level of inclusion of cyromazine increased in both starter and finisher diets. Crude protein recorded the highest value (24.13%) at 0.75 g/kg and least value (19.17%) at the control (0 g/kg) level. Treatment 2 recorded the highest value (10.88%) of EE while Treatment 1 recorded the lowest value (9.43%) in the starter phase. Crude fibre recorded the highest value (12.80%) at 0.25 g/kg cyromazine levels and least value (11.67%) at 0.5 g/kg CYR. The control had the lowest value for ash (5.60%) and dry matter (87.08%) in the starter phase, which increased with the level of inclusion of cyromazine. However in the finisher phase, there was no significant difference in all the parameters. The control had the highest dry matter (90.5%). In both phases, NFE value (43.77% and 48.3%) was highest for the control group, which also increased with increase in inclusion level of cyromazine.

For the starter, the highest TP value (10.47%) was recorded at 0.75 g/kg cyromazine inclusion while lowest value (4.86%) was recorded for the 0 g/kg level (control). The finisher also shows the same trend with the highest value (10.50%) at 0.75 g/kg and least value (4.05%) for the control.

Table 9: Proximate Composition and True Protein of the experimental diets

	Starter						Finisher					
Parameters (%)	0.00	0.25	0.50	0.75	SEM	P value	0.00	0.25	0.50	0.75	SEM	P-value
Dry Matter	87.08^{b}	90.07^{b}	89.00b	93.95^{a}	1.56	0.006	90.10	87	88.67	87.17	0.88	0.35
CP	19.17^{d}	20.27^{c}	21.58^{b}	24.13^{a}	0.318	0.00	13.94^{c}	17.23^{b}	17.97^{b}	20.10^{a}	0.68	0.001
Ether Extract	9.43^{c}	10.88^{a}	10.20^{b}	9.77^{c}	0.153	0.00	8.03^{c}	8.67^{c}	17.67^{a}	14.33^{b}	0.33	0.0001
Crude Fibre	12.33^{ab}	12.80^{a}	11.67^{b}	12.33^{ab}	0.333	0.139	11.30	9.2	12.0	11.50	0.76	0.35
Ash	5.60	6.92	6.93	9.75	2.428	0.184	5.5^{c}	4.83^{d}	6.3^{b}	7.57.3^{a}	0.14	0.0001
NFE	43.77^{a}	38.10^{b}	39.60^{ab}	40.40^{ab}	2.76	0.103	51.23	48.76	36.20	35.73	0.75	0.0001
True protein	4.86^{c}	7.93^{b}	8.07^{b}	10.47^{a}	0.60	0.0001	4.05^{d}	6.42^{c}	7.62^{b}	10.50^{a}	0.70	0.0001

^{abcd} Means on the same row having the different superscripts are significantly (P<0.05) different

S1 – 0 inclusion (control), S2 – 0.25 g/kg inclusion, S3 – 0.50 g/kg inclusion, S4 – 0.75 g/kg inclusion, F1 – 0 inclusion, F2 - 0.25 g/kg inclusion, F3 – 0.50 g/kg inclusion, F – 0.75 g/kg inclusion

4.2 Experiment 2

4.2.1 Effect of inclusion of Cyromazine and Litter depth on the production performance of broilers (2 and 4 weeks)

The main effect of cyromazine and litter depth on the performance of 2 - 4 weeks broiler chickens is shown in Table 10. The result showed that feed intake was significantly ($P<0.05$) influenced by the cyromazine inclusion. Birds fed diet containing cyromazine recorded higher values of feed intake. Birds on 0.75 g/kg consumed more feed ($P<0.05$) compared to those on control diet. Other parameters measured were not significantly ($P>0.05$) influenced by cyromazine inclusion. For litter depth, birds on 3cm litter depth showed higher ($P<0.05$) values (336.94, 298.19 and 21.30) of final weight, weight gain and daily weight gain compared to those on 5 cm litter depth. Birds on 3 cm litter depth showed a better FCR (0.69) than (0.79) of 5 cm litter depth.

At week 4, the final weight of birds on the 3 cm litter depth was higher ($P<0.05$) than the birds on 5 cm litter depth with 541.5 g in the 3 cm as against 508.70 g in the 5 cm litter depth. Birds on 0.5 g/kg cyromazine recorded a higher weight gain, daily weight gain and feed intake compared to those on 0.25 g/kg cyromazine inclusion. For litter depth, birds on 3 cm litter depth showed a significantly ($P<0.05$) higher value (541.57) than (508.70) of birds on 5 cm litter depth.

The interaction effect of cyromazine inclusion and litter depth on production performance of broiler chickens is shown in Table 11. The result showed that interaction of cyromazine and litter depth at 2 weeks significantly influenced ($P<0.05$) for all parameters measured except mortality. The final weight obtained in total and daily weight gain in birds on 3 cm litter depth, fed diets with 0.25, 0.50 and 0.75 g/kg cyromazine inclusion had higher values compared to those on other treatments. Birds fed 0.5 g/kg cyromazine on 5 cm litter depth recorded the least ($P<0.05$) values of these

parameters. The feed intake of birds raised on 5 cm litter depth and fed diets containing 0.75 g/kg cyromazine was higher ($P<0.05$) than birds raised on 5 cm litter depth and fed diet containing 0.00 g/kg and 0.50 g/kg cyromazine levels. The best FCR was recorded for birds on control diet raised on 5 cm litter depth.

At 4 weeks, birds raised on 3 cm litter depth and fed diet containing 0.5 g/kg cyromazine had the highest ($P<0.05$) final weight. Those fed diets containing 0.75 g/kg raised on 5 cm litter depth had the level of ($P<0.05$) final weight values. The final weight gain and daily weight gain values were higher. Birds fed diets containing 0.50 g/kg cyromazine diet raised on 5 cm litter depth compared to those on other diets. Birds on 0.75 g/kg cyromazine diet, raised on 3 cm litter depth had a significantly higher ($P<0.05$) feed intake compared to those on 0.25 g/kg cyromazine diet raised on 3 cm litter depth. The feed conversion ratio was better on bird fed 0.5 g/kg cyromazine diet raised on 5 cm litter depth.

Table 10: Main effect of cyromazine and litter depth on the performance of 2 and 4weeks broiler chicken

	Levels of cyromazine						Litter depth		
Parameters	**0.00**	**0.25**	**0.50**	**0.75**	**SEM**	**P value**	**3cm**	**5cm**	**SEM**
Weeks 2 (0 – 14) days									
Initial weight (g/bird)	35.00	37.50	40.00	42.50			38.75	38.75	
Final weight (g/bird)	311.88	304.82	292.08	330.76	31.18	0.33	336.94[a]	282.83[b]	14.75
Total weight gain (g/bird)	276.88	267.32	252.08	288.26	31.17	0.38	298.19[a]	244.08[b]	14.38
Daily weight gain(g/bird)	19.78	19.09	18.01	20.59	2.23	0.38	21.30[a]	17.43[b]	1.03
Feed intake	12.76[b]	13.85[ab]	13.55[ab]	15.03[a]	0.84	0.05	14.24	13.35	0.59
FCR	0.68	0.73	0.81	0.75	0.07	0.20	0.69[b]	0.79[a]	0.04
Mortality (%)	5.56	5.56	5.56	6.25	6.25	0.10	0.00	11.46	5.13
Weeks 4 (15 - 28) days									
Initial weight (g/bird)	311.88	304.82	292.08	330.76			336.94	282.83	
Final weight (g/bird)	506.50	507.92	565.76	520.36	17.59	0.04	541.57[a]	508.70b	13.24
Weight gain (g/bird)	194.62[b]	203.10[b]	273.68[a]	189.59[b]	30.94	0.01	204.62	225.87	19.85
Daily weight gain (g/bird)	13.90[b]	14.51[b]	19.55[a]	13.54[b]	1.47	0.01	14.62	16.13	1.42
Feed intake (g/bird)	35.38[ab]	34.00[b]	35.88[a]	36.63[a]	0.73	0.03	35.56	35.38	0.63
FCR	2.65	2.54	2.05	2.74	0.30	0.17	2.56	2.42	0.19
Mortality (%)	0.00	0.25	0.00	0.00	0.00	0.41	0.00	0.13	0.13

Table 11: Interaction effect of inclusion levels and Litter depth on the performance of broilers (2 and 4 weeks)

	3cm				5cm					
PARAMETERS	**0.00**	**0.25**	**0.50**	**0.75**	**0.00**	**0.25**	**0.50**	**0.75**	**SEM**	**P value**
Weeks 2 (0 - 14) days	35.00	37.50	40.00	42.50	35.00	37.50	40.00	42.50		
Initial weight (g/bird)	286.25^{c}	331.25^{abc}	367.50^{a}	362.78^{ab}	337.50^{abc}	278.39^{c}	216.67^{d}	298.75	42.79	0.00
Final weight (g/bird)	251.25^{c}	293.75^{abc}	327.50^{a}	320.28^{ab}	302.50^{abc}	240.89^{c}	176.67^{d}	256.25^{bc}	42.79	0.00
Weight gain (g/bird)	17.95^{c}	20.98^{abc}	23.39^{a}	22.88^{ab}	21.61^{abc}	17.21^{c}	12.62^{d}	18.30^{bc}	3.06	0.00
DWG (g/bird)	13.17^{ab}	14.52^{ab}	14.75^{ab}	14.52^{ab}	12.35^{b}	13.18^{ab}	12.35^{b}	15.53^{a}	1.52	0.01
Feed intake (g/bird)	0.79^{bc}	0.69^{bcd}	0.64^{cd}	0.64^{cd}	0.57^{d}	0.78^{bc}	0.98^{a}	0.85^{ab}	0.11	0.00
FCR	0.00	0.00	0.00	0.00	1.11	1.11	1.11	12.50	12.50	0.77
Mortality (%)										
Weeks 4 (15 -28) days										
Initial weight (g/bird)	286.25	331.25	367.50	362.78	337.50	278.39	216.67	298.75		
Final weight (g/bird)	506.39^{bc}	507.92^{bc}	591.96^{a}	560.00^{ab}	506.61^{bc}	507.92^{bc}	539.56^{abc}	480.71^{c}	39.12	0.03
Weight gain (g/bird)	220.14^{b}	176.67^{b}	224.46^{b}	197.22^{b}	169.11^{b}	229.52^{b}	322.89^{a}	181.96^{b}	39.12	0.04
DWG (g/bird)	15.72^{b}	12.62^{b}	16.03^{b}	14.09^{b}	12.08^{b}	16.40^{b}	23.06^{a}	13.00^{b}	1.89	0.04
Feed intake (g/bird)	36.00^{ab}	33.00^{c}	35.50^{abc}	37.75^{a}	34.75^{bc}	35.00^{bc}	36.25^{ab}	35.50^{abc}	1.29	0.06
FCR	2.38^{ab}	2.69^{a}	2.44^{ab}	2.72^{a}	2.91^{a}	2.39^{ab}	1.65^{b}	2.75^{a}	0.54	0.25
Mortality (%)	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.41

^{abcd} Means on the same row having the different superscripts are significantly ($P<0.05$) different.

4.2.2 Effect of inclusion of cyromazine and litter depth on growth performance of broiler chicken at 6 weeks

The main effect of inclusion of cyromazine and litter depth on the growth performance of broiler chickens at 6weeks is presented in Table 12. The result showed that the inclusion of cyromazine significantly ($P<0.05$) influenced final weight, weight gain and daily weight gain. Feed intake, feed conversion ratio and mortality were not significantly ($P>0.05$) influenced by the diets. Final weight of birds on 0.75 g/kg showed higher final weight, weight gain and daily weight gain values compared to those on other diets. The litter depth levels of 3 cm and 5 cm were not significantly ($P<0.05$) influenced by all parameters measured.

Table 13 shows the Interactive effect of cyromazine inclusion and litter depth on growth performance of broilers at 6 weeks. The result showed significant increase ($P < 0.05$) in final weight of birds fed 0.05 g/kg raised on 3 cm litter depth. However, the total weight gain and daily weight gain were higher ($P<0.05$) in birds fed 0.75 g/kg cyromazine raised on 5 cm litter depth, though these values were not different from birds fed 0.5 g/kg diet raised on 3 cm litter depth. Birds fed 0.5 g/kg cyromazine diet raised on 3 cm litter depth had higher ($P<0.05$) feed intake compared to those on 0.5 g/kg cyromazine diets raised on 5 cm litter depth. The feed intake values recorded for birds on diets were similar ($P>0.05$). The feed conversion ratio and % mortality were not significantly influenced ($P>0.05$) by the interaction of cyromazine inclusion and litter depth.

Table 12: The main effect of cyromazine and litter depth on the performance of broiler chicken at 6 weeks

Parameters	0.00	0.25	0.50	0.75	SEM	P value	3cm	5cm	SEM	P -value
Initial weight (g/bird)	506.50	507.92	565.76	520.36	22.26		530.8	508.70		
Final weight (g/bird)	920.34^{b}	920.63^{b}	1042.48^{a}	1075.67^{a}	36.82	0.00	1012.48	972.08	30.23	0.16
Weight gain (g/bird)	423.84^{b}	412.71^{b}	476.72^{ab}	555.32^{a}	47.04	0.01	470.91	463.38	35.04	0.80
DWG (g/bird)	30.27^{b}	29.48^{b}	34.03^{ab}	39.67^{a}	3.36	0.01	33.64	33.10	2.50	0.80
Feed intake (g/bird)	72.71	91.65	84.61	96.41	11.41	0.29	89.63	84.06	8.51	0.56
FCR	2.44	3.12	2.49	2.50	0.28	0.20	2.66	2.62	0.21	0.88
Mortality (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

^{abcd} Means on the same row having different superscripts are significantly ($P<0.05$) different

Table 13: Interaction effect of cyromazine inclusion and Litter depth on the performance of broilers at 6 weeks

	3 cm				5 cm					
PARAMETERS	0.00	0.25	0.50	0.75	0.00	0.25	0.50	0.75	SEM	P-value
Initial weight (g/bird)	506.39	507.92	565.76	560.00	506.61	507.92	539.56	480.71		
Final weight (g/bird)	904.72^{d}	935.50^{cd}	1170.24^{a}	1039.44^{bc}	955.95^{cd}	905.75^{d}	914.72^{d}	1111.90^{ab}	70.75	0.00
Total weight gain (g/bird)	398.33^{c}	427.58^{c}	578.27^{ab}	479.44^{bc}	449.33^{c}	397.84^{c}	375.17^{c}	631.19^{a}	3.04	0.00
Daily Weight Gain (g/bird)	28.45^{c}	30.54^{c}	41.31^{ab}	34.25^{bc}	32.10^{c}	28.42^{c}	26.80^{c}	45.09^{a}	0.47	0.00
Av. feed intake (g/bird)	64.44^{ab}	92.08^{ab}	107.83^{a}	94.17^{ab}	80.97^{ab}	91.22^{ab}	61.39^{b}	102.65^{ab}	20.23	0.12
FRC	2.24	3.03	2.62	2.74	2.64	3.21	2.37	2.26	0.47	0.59
Mortality (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

^{abcd} Means on the same row having the different superscripts are significantly ($P<0.05$) different

4.2.3 Effect of inclusion of cyromazine and litter depth on the performance of broiler chickens at 8 weeks

The main effect of inclusion of cyromazine and litter depth on the growth performance of broiler chickens at 8 weeks is presented in Table 14. Final weight (1933.82 g) of birds on 0.50 g/kg inclusion level was significantly ($P<0.05$) higher than other inclusion levels with birds on 0.00 g/kg having the least ($P<0.05$) value (1645.70). Birds on 0.50 g/kg showed higher value of weight gain and daily weight gain (891.34 g and 63.67 g) compared to birds on other diets. Birds on 0.50 and 0.25 g/kg cyromazine diets had a significantly higher ($P<0.05$) feed intake values compared to birds on other diets.

For litter depth levels, birds on 5cm litter depth showed higher ($P<0.05$) values of final weight (180.09 g), weight gain (829.01 g), daily weight gain (59.22) and feed intake (147.81 g) than those on 3 cm litter depth.

The interactive effect of cyromazine inclusion and liter depth is shown in Table 15. The result showed that final weight, total weight, daily weight gain and average feed intake are significantly ($P<0.05$) influenced by the interaction of cyromazine and litter depth. Birds fed 0.5 g/kg cyromazine diet raised on 3 cm litter size had highest final weight value, however this value was not different ($P>0.05$) from the final weight of birds fed 0.50 and 0.75 g/kg cyromazine raised on 5 cm litter depth. The least feed intake values were recorded for birds fed control diet and 0.75 g/kg cyromazine diets raised on 3 cm litter depth. Birds fed 0.50 g/kg cyromazine diets raised on 5 cm litter depth had the highest ($P<0.05$) total weight gain and daily weight gain values. Those on 0.75 g/kg cyromazine diet raised on 3 cm litter depth had the least ($P<0.05$) than those on other dietary treatments. FCR and mortality were not significantly influenced ($P>0.05$) by the interaction of cyromazine inclusion and litter depth.

Table 14: The main effect of cyromazine and litter depth on the performance of broiler chicken at 8 weeks

Parameters	0.00	0.25	0.50	0.75	SEM	P value	3cm	5cm	SEM	P value
Initial weight (g/bird)	930.34	920.63	1042.48	1075.67	51.93	0.00	1012.48	972.08		
Final weight (g/bird)	11645.70^{b}	1720.92^{b}	1933.82^{a}	1745.41^{b}	68.21	0.00	1721.83	1801.09	53.10	0.08
Weight gain (g/bird)	715.36^{b}	800.29^{ab}	891.34^{a}	669.74^{b}	55.38	0.01	709.35^{b}	829.01a	39.04	0.01
DWG (g/bird)	51.10^{b}	57.16^{ab}	63.67^{a}	47.84^{b}	3.96	0.01	50.67^{b}	59.22^{a}	2.79	0.01
Feed intake (g/bird)	131.56	148.32	134.78	131.90	11.59	0.23	125.48^{b}	147.81^{a}	5.16	0.00
FCR	2.70	2.66	2.13	4.83	0.30	0.21	2.56	2.60	0.20	0.87
Mortality (%)	2.50	0.00	1.79	0.00	1.79	0.57	0.00	2.14	1.49	0.17

^{abcd} Means on the same row having different superscripts are significantly ($P<0.05$) different

Table 15: Interaction effect of cyromazine inclusion and Litter depth on the performance of broilers at 8 weeks

	3cm				5cm					
PARAMETERS	0.00	0.25	0.50	0.75	0.00	0.25	0.50	0.75	SEM	P value
Initial weight (g/bird)	904.72	935.50	1170.24	1039.44	955.95	905.75	914.72	1111.90		
Final weight (g/bird)	1584.88^{c}	1673.50^{bc}	2006.70^{a}	1622.25^{c}	1706.52^{bc}	1768.33^{bc}	1860.95^{ab}	1868.57^{ab}	75.48	0.03
Total weight gain (g/bird)	680.15b^{c}	738^{abc}	836.46^{ab}	582.81^{c}	750.57^{abc}	862.58^{ab}	946.22^{a}	756.67^{abc}	80.99	0.88
Daily Weight Gain (g/bird)	48.58^{bc}	52.71^{abc}	59.75^{ab}	41.63^{c}	53.61^{abc}	61.61^{ab}	67.59^{a}	54.05^{abc}	5.77	0.88
Av. feed intake (g/bird)	118.58^{cd}	131.49^{bcd}	140.95^{abc}	110.89^{d}	144.54^{abc}	165.15^{a}	128.61^{bcd}	152.92^{ab}	14.99	0.03
FRC	2.55	2.53	2.36	2.82	2.86	2.80	1.91	2.84	0.52	0.67
Mortality (%)	0.00	0.00	0.00	0.00	5.00	0.00	3.57	0.00	3.57	0.57

^{abcd} Means on the same row having the different superscripts are significantly (P<0.05) different

4.2.4 **Effect of inclusion of cyromazine and litter depth on nutrient utilization of broilers at 6 week**

The main effect of cyromazine inclusion and litter depth on digestibility of nutrients at 6 week of age is as shown in Table 16. The result showed that the level of cyromazine inclusion had a significant ($P<0.05$) effect on CF, EE and Ash digestibility. While DM, CP and NFE were not significantly ($P>0.05$) affected. Dry matter, Crude protein and Ash digestibility increased with inclusion levels. Dry matter digestibility value ranged from 55.83 - 60.45%. CP digestibility ranged from 65.67 in 0 g/kg cyromazine inclusion to 84.20% in 0.75 g/kg. The control recorded the lowest value in all parameters measured. Crude protein digestibility was significantly higher ($P<0.05$) in birds on 3 cm litter depth. Litter depth had no significant ($P>0.05$) effect on all other parameters considered. CF and Ash digestibility were higher in birds placed on 3 cm litter depth.

The interactive effects of cyromazine inclusion and litter depth on digestibility of birds at 6weeks of age is as shown on table 15. It was observed that CP and Ash digestibility were significantly ($P<0.05$) influenced by the interaction effects of cyromazine inclusion and litter depth. Crude protein digestibility of birds fed 0 g/kg, 0.25 g/kg and 0.75 g/kg on 3 cm litter depth and birds of 0.5 g/kg and 0.75 g/kg on 5 cm litter depth showed higher values. Ash digestibility for birds on 0.75 g/kg of 3 cm litter depth showed the highest value (42.02) while the control (0 g/kg) of 5 cm showed the least value (11.16)

Table 16: Main effect of inclusion of cyromazine and litter depth on nutrient digestibility of broilers at 6 weeks

	Levels of cyromazine						Litter depth			
Parameter (%)	0g/kg	0.25g/kg	0.50g/kg	0.75g/kg	SEM	P value	3cm	5cm	SEM	P- value
DM	55.83	61.53	62.18	60.45	2.504	0.71	59.09	60.90	5.648	0.63
CP	65.67	74.10	70.96	84.20	3.495	0.13	81.39[a]	66.07[b]	12.245	0.05
CF	76.126[b]	79.671[ab]	85.090[a]	75.033[b]	3.55	0.09	80.317	77.643	5.24	0.32
EE	74.15[b]	80.11[ab]	74.10[b]	86.14[a]	1.599	0.05	74.727	82.527	5.24	0.09
Ash	15.23[b]	35.02[a]	20.22[b]	34.40[a]	4.84	0.01	29.73	22.70	7.33	0.12
NFE	70.35	71.73	78.65	72.44	4.93	0.73	68.70	77.88	10.43	0.26

[abcd] Means on the same row having the different superscripts are significantly ($P < 0.05$) different
DM – dry matter
CP – crude protein
CF – crude fibre
EE – ether extract
NFE nitrogen free extract

Table 17 Interaction of inclusion levels and Litter depth on nutrient digestibility of broilers at 6 weeks of age

	3cm				5cm					
Parameter (%)	0.00	0.25	0.50	0.75	0.00	0.25	0.50	0.75	SEM	P- value
Dry matter	54.11	57.78	63.09	61.39	57.54	65.28	61.27	59.51	1.68	0.883
CP	86.41^{a}	83.47^{a}	69.28^{ab}	86.41^{a}	64.93^{b}	64.72^{ab}	72.64^{a}	81.98^{a}	3.94	0.047
CF	77.27	81.00	85.35	77.65	74.78	78.34	84.83	72.42	2.23	0.200
EE	68.06	73.43	72.10	85.32	80.24	86.80	76.11	86.96	1.53	0.409
Ash	19.30^{bc}	38.51^{ab}	19.11^{bc}	42.02^{a}	11.16^{c}	31.53^{abc}	21.34^{abc}	26.78^{abc}	2.99	0.073
NFE	66.33	57.42	84.72	66.34	74.37	86.04	75.57	78.54	3.64	0.589

^{abcd} Means on the same row having the different superscripts are significantly ($P < 0.05$) different

CP - crude protein

CF - crude fibre

EE - ether extract

NFE – nitrogen free extract

4.2.5 **Effect of cyromazine and litter depth on haematological and serum indices of broilers at 2 weeks of age.**

The main effects of cyromazine and litter depth on haematological parameters and serum indices of broilers at 2 weeks of age are shown in Table 18. The result showed that WBC, Lymphocyte, Albumin, AST, Creatinine and TP were significantly ($P<0.05$) different. While PCV, RBC, Neutrophil, Monocyte, eosinophil, Basophil, Hb, Glucose, Aric Acid, MCV, MCH, and MCHC are not significantly ($P>0.05$) different. PCV values ranged from 32.63 to 37.38%. The highest mean value of WBC (13.46 x 10^9/l) was obtained from birds on treatment 4 while the birds on 3cm litter depth obtained a higher value (14.14%) than birds on 5cm litter depth. Numerically, the control recorded lower values for PCV (32.63 %), RBC (2.875x10^6/l), Eosinophil (2.375 %) and Hb (10.86) while Neutrophil and Monocyte showed a higher value (28.75) for the control than the other treatment groups. WBC was lowest (7.24 x 10^9 /l) in birds fed 0.25g/kg while highest values were recorded in birds fed with 0.75g/kg (13.40). Birds on 0.75g/kg recorded the highest value for Albumin (5.35) while the control showed the least value (3.23). Birds on 0.25g/kg recorded the highest value for AST (49.00), followed by birds on 0.50g/kg (48.25mg/dl) and the control showed the least value (40.13). The control showed the highest value of creatinine (2.40) while treatment 2 showed the least value (1.39).

The result of interaction effects of cyromazine and litter depth is shown in Table 19. It interaction between Cyromazine and litter depth was significant for PCV, Hb, Lymphocyte, Monocyte, Albumin, AST, Creatinine, TP and glucose while interaction was not significant ($P> 0.05$) for RBC, Hb, Neutrophil, Eosinophil, Basophil, Uric acid, ALT, MCV, MCH and MCHC. 0.75g/kg Cyromazine of 3cm litter depth showed a higher PCV value (33.75%) while 0.25g/kg cyromazine 5cm showed the least value. Birds fed

0.75g/kg cyromazine on 3cm showed a higher value of WBC (19.9 x10^9/l).While 0.25g/kg Cyromazine of 5cm showed the least value (6.75 x10^9/l). 0.75g/kg CYR of 3cm showed higher value of PCV (33.75%), WBC (19.9), lymphocyte (76.5 %), Albumin (5.43 g/l) and TP (4.8 g/l) while treatment 4 of 5cm showed a least value of PCV (34 %), WBC (7.02 %). The highest glucose level 202.5 mg/dl, Albumin (5.28 g/l) was recorded in the control and 0.75g/kg CYR of 5cm depth while the least value was recorded in 0.50 g/kg CYR of 3cm depth. The control of both 3cm and 5cm litter depth recorded the highest creatinine value (2.4 mg/dl) while the least value (1.25 mg/dl) was recorded in 0.25 g/kg of 3cm depth.

Table 18: Main effects of cyromazine and litter depth on haematology and serum indices of broilers at 2 weeks

	Level of cyromazine						Litter depth			
Parameters	0.00	0.25	0.50	0.75	SEM	P- value	3cm	5cm	SEM	P- value
Heamathology										
PCV (%)	32.63	33.00	37.38	33.88	5.32	0.965	34.38	34.06	1.51	0.93
RBC($x10^6$/l)	2.875	2.896	3.305	3.004	2.14	0.45	3.04	4.02	0.55	0.54
Monocyte (%)	1.625	1.375	1.500	1.250	0.46	0.09	1.31	1.50	0.18	0.32
Eosinophil (%)	2.375	3.500	2.625	2.875	0.63	0.60	2.69	2.81	0.25	0.67
Basophil (%)	1.25	1.00	0.750	1.375	0.32	0.53	0.75	1.19	0.15	0.55
MCV(fl)	11.35	9.97	11.30	10.13	1.42	0.57	11.30	10.08	0.45	0.57
MCH(pg)	3.78	3.48	3.73	3.36	0.52	0.87	3.76	3.42	0.16	0.87
MCHC(g/dl)	33.29	30.49	33.03	32.24	4.58	0.94	33.24	31.29	2.29	0.94
Serum indices										
Albumin (g/dl)	3.23^{d}	3.67^{c}	4.14^{b}	5.35^{a}	0.10	0.00	4.15	4.05	0.16	0.00
Glucose (mg/dl)	200.63	199.13	194.25	200.63	2.65	0.16	196.3	201.00	1.27	0.16
Creatinine(mg/dl)	2.40^{a}	1.39^{b}	1.65^{b}	1.58^{b}	0.20	0.02	1.71	1.80	0.10	0.02
TP (g/dl)	3.79^{b}	3.68^{b}	3.68^{b}	4.41^{a}	0.25	0.03	3.98	3.79	0.10	0.03
Uric acid(mg/dl)	2.60	2.93	2.89	3.33	0.37	0.78	2.96	2.91	0.15	0.78
ALT (u/l)	20.25	25.38	22.88	21.71	2.87	0.53	22.81	22.33	0.95	0.53
AST (mg/dl)	40.13^{b}	49.00^{a}	48.25^{a}	47.63^{a}	2.09	0.03	46.56	45.94	0.97	0.00

[a][bcd] Means on the same row having the different superscripts are significantly (P<0.05) different

PCV: Packed Cell Volume, RBC: Red Blood Cell, White Blood Cell, Hb: Haemoglobin, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin

Table 19: Interractive effects of cyromazine on haematology and serum indices of broilers at 2 weeks

	3cm				5cm					
Parameters	**0.00**	**0.25**	**0.50**	**0.75**	**0.00**	**0.25**	**0.50**	**0.75**	**SEM**	**P value**
Heamathology										
PCV (%)	33.00^{ab}	34.75^{c}	36^{bc}	33.75^{a}	32.23^{bc}	31.25^{c}	38.75^{bc}	34.00^{c}	10.71	0.97
RBC($x10^{6}$/l)	2.92	3.06	3.21	2.98	2.83	2.73	3.40	3.03	0.41	0.47
WBC ($x10^{9}$/l)	16.08	7.73	12.85	19.90	10.00	6.75	12.03	7.90	2.17	0.003
Hb(g/dl)	11	11.55	11.93	11.23	10.72	11.38	12.78	11.38	1.62	0.98
Neutrophil (%)	27.25	33.25	26.00	19.50	30.25	21.00	26.00	29.00	3.98	0.31
Lymphocyte (%)	68.75	60.50	69.25	76.50	62.75	48.50	68.25	64.00	6.39	0.19
Monocyte (%)	1.50	1.75	1.75	0.25	1.75	1.00	1.25	2.25	0.52	0.32
Oesinophil (%)	1.75	3.75	2.50	2.75	3.00	3.25	2.70	3.00	0.77	0.67
Basophil (%)	0.75	0.75	0.50	1.00	1.75	1.25	1.00	1.75	0.39	0.55
MCV(fl**)**	11.30	11.35	11.23	11.31	11.40	8.59	11.37	8.96	2.86	0.57
MCH(pg)	3.77	3.77	3.72	3.76	3.79	3.18	3.74	2.96	1.11	0.87
MCHC(g/dl)	33.33	33.23	33.12	33.25	33.25	27.75	32.94	31.22	2.42	0.94
Serum indices										
Albumin (g/dl)	3.50^{cd}	3.50^{cd}	4.16^{b}	5.43^{a}	2.95^{d}	3.84^{bc}	4.13^{b}	5.28^{a}	0.36	0.03
Glucose(mg/dl)	195.75^{ab}	199.75^{ab}	191^{b}	198.75^{ab}	205^{a}	198.5^{ab}	197.5^{ab}	202.5^{a}	6.52	0.16
Creatinine(mg/dl)	2.4^{a}	1.25^{b}	1.48^{b}	1.7^{ab}	2.4^{a}	1.53^{b}	1.82^{ab}	1.45^{b}	0.33	0.02
TP (g/dl)	3.73^{b}	3.55^{b}	3.85^{b}	4.8^{a}	3.85^{b}	3.8^{b}	3.5^{b}	4.03^{b}	0.36	0.03
Uricacid(mg/dl)	2.58	2.7	3.1	3.48	2.63	3.15	2.68	3.18	0.65	0.77
ALT (u/l)	20	28	21.75	21.5	20.5	22.75	24	22	4.76	0.53
AST(u/l)	40.75^{bc}	48.75^{a}	49^{a}	47.75^{ab}	39.5^{ab}	49.25^{a}	47.5^{ab}	47.5^{ab}	2.90	0.03

^{abcd} Means on the same row having the different superscripts are significantly ($P<0.05$) different

PCV: Packed Cell Volume, RBC: Red Blood Cell, White Blood Cell, Hb: Haemoglobin, TP: Total Protein AST: Aspartate amino transferase, ALT: Alanine amino transferase, AST: Aspartate amino transferase, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin.

4.2.6 Effect of cyromazine and litter depth on haematological and serum indices of broilers at 4weeks

Table 20 shows the main effect of cyromazine and litter depth on haematological parameters and serum indices of broilers at 4 weeks of age. The result showed Albumin, Glucose, AST and MCH were significantly different ($P<0.05$). 0.75 g/kg CYR showed the highest value for RBC (3.12 %), followed by 0.50 g/kg CYR treatment and control group which recorded the same value (2.32 x 10^6/l) and the least value in 0.25 g/kg CYR (1.85%). The control and 0.75 g/kg groups showed the higher value of 218.13 mg/dl and 212.38 mg/dl for glucose while treatment 2 recorded the least value (199.75 mg/dl). The control group also recorded the highest value for AST (75.38 u/l) and the least value was recorded in treatment 4. The highest value of Albumin (7.87 g/l) was recorded in treatment 4 and decreased with decrease in inclusion levels. Treatment 4 also recorded the highest value for MCH (1.12 g/dl) with the least value (0.43 g/dl) in treatment 2. ALT was not significant in inclusion level but was significant in litter depth with 5 cm showing the higher value (123.00 u/l).

The result of the interactive effects between the cyromazine inclusion levels and the litter depth is presented in Table 21. Interaction effect was significant ($P<0.05$) for albumin, glucose, ALT and AST while there was no significant ($P>0.05$) difference for PCV, RBC, WBC, Hb and MCHC. Higher PCV values were recorded for 5 cm litter depth. 3 cm litter depth recorded higher values than 5 cm depth for RBC. The highest WBC was recorded in treatment 4 of 5 cm depth (4.11 x 10^9/l). The same trend was also observed in Hb, MCH and MCHC. The control in 3 cm depth recorded the highest value for glucose and AST.

Table 20: Main effect of cyromazine and litter depth on haematological and serum indices of broilers at 4 weeks

Parameters	0.00	0.25	0.50	0.75	SEM	P value	3cm	5cm	SEM	P value
Heamatological										
PCV (%)	26.63	23.38	25.63	26.25	2.20	0.08	23.44	27.55	1.19	0.44
RBC($x10^6$/l)	2.39	1.85	2.32	3.12	0.74	0.14	2.14	2.70	0.40	0.23
WBC ($x10^9$/l)	12.08	13.93	13.53	13.35	2.33	0.12	14.74^{a}	11.70^{b}	1.28	0.05
Hb (g/dl)	8.83	6.65	8.39	10.53	2.24	0.26	7.70	9.49	1.25	0.41
Neutrophil (%)	26.38	26.13	33.38	31.13	4.09	0.19	27.19	31.31	2.54	0.34
Lymphocyte (%)	69.13	56.63	61.88	65.00	8.36	0.15	67.69^{a}	58.63^{b}	4.16	0.36
Monocyte (%)	1.50	1.50	1.63	2.63	0.53	0.43	1.81	1.81	0.36	0.42
Oesinophil (%)	2.5	2.5	2.25	1.88	0.57	0.29	2.63	1.94	0.35	0.22
Basophil (%)	0.63	1.00	1.00	0.50	0.27	0.50	0.88	0.69	0.20	0.78
MCV(fl)	11.17	9.49	11.10	9.76	1.46	0.59	10.86	9.99	0.84	0.66
MCH (pg)	0.78^{ab}	0.43^{b}	0.69^{ab}	1.12^{a}	0.42	0.04	0.58	0.93	0.22	0.06
MCHC (g/dl)	33.23	28.66	32.78	38.57	5.48	0.35	32.84	33.78	3.62	0.27
Serum indices										
Albumin (g/dl)	4.40^{d}	5.90^{c}	6.71^{b}	7.87^{a}	0.52	0.00	6.97^{a}	5.47^{b}	0.46	0.00
Glucose(mg/dl)	218.13^{a}	199.75^{b}	200.75^{b}	212.38^{a}	4.85	0.02	206.06	209.44	3.78	0.30
Creatinine(mg/dl)	48.25	56.63	54.75	52.63	4.55	0.39	56.31	49.81	3.20	0.53
TP (g/dl)	13.34	14.89	13.38	14.36	1.61	0.43	14.68	13.30	0.87	0.40
UA (mg/dl)	85.65	85.93	80.96	86.86	13.58	0.83	82.26	87.87	3.29	0.77
ALT(ul)	110.38^{a}	113.88^{ab}	121.00^{ab}	113.50^{b}	5.77	0.006	103.38^{b}	123.00^{a}	5.02	0.004
AST (mg/dl)	75.38^{a}	60.63^{ab}	61.38^{ab}	51.13^{b}	8.74	0.05	65.13	59.13	5.36	

^{abcd} Means on the same row having the different superscripts are significantly ($P<0.05$) different

PCV: Packed Cell Volume, RBC: Red Blood Cell, White Blood Cell, Hb: Haemoglobin, TP: Total Protein, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin

PCV- Packed cell volume, RBC – Red blood cell, WBC- White blood cell, AST- Aspartate amino transaminase

Table 21: Interactive effect of cyromazine and litter depth on haematology and serum indices of broilers at 4weeks

	3 cm				5 cm					
Parameters	**0.00**	**0.25**	**0.50**	**0.75**	**0.00**	**0.25**	**0.50**	**0.75**	**SEM**	**P value**
Heamatology										
PCV (%)	25.00	20.00	25.25	23.50	28.25	26.75	26.00	29.00	1.87	0.76
RBC ($x10^{12}$/l)	2.25	1.89	2.29	2.13	2.53	1.81	2.34	4.11	1.37	0.14
WBC ($x10^{9}$/l)	11.20	17.08	12.60	18.08	12.95	10.76	14.45	8.63	2.08	2.08
Hb (g/dl)	8.28	6.65	8.13	7.75	9.38	6.65	8.65	13.30	4.24	0.26
Neutrophil (%)	25.50	27.50	30.00	25.75	29.00	24.75	36.25	34.25	3.925	0.523
Lymphocyte (%)	69.25	67.50	64.75	69.25	67.75	45.75	59.00	61.25	6.191	0.410
Monocyte (%)	1.00	1.25	2.00	3.00	1.75	1.75	1.50	2.25	0.65	0.59
Oesinophil (%)	3.59	3.00	2.25	1.75	1.50	2.00	1.58	1.75	0.59	0.29
Basophil (%)	1.00	1.25	1.00	0.25	0.00	0.75	1.25	0.50	0.35	0.24
MCV (fl)	11.11	10.29	11.02	11.01	11.22	8.69	11.17	8.51	2.96	0.59
MCH (pg)	3.68	3.52	3.55	3.64	3.71	3.67	3.70	3.24	0.17	0.41
MCHC (g/dl)	33.10	33.21	32.07	32.99	33.36	24.12	33.49	44.14	1.79	0.35
Serum indices										
Albumine (g/dl)	4.40^{e}	6.52^{c}	8.05^{b}	8.9^{a}	4.40^{e}	5.27^{d}	5.36^{d}	6.85^{c}	0.29	0.001
Glucose (mg/dl)	222.50^{a}	197.25^{cd}	195.25^{d}	209.25^{abcd}	213.75^{abc}	202.50^{bcd}	206.25^{abcd}	215.50^{ab}	7.30	0.01
Creatinine (mg/dl)	46.75	61.25	58.25	59.00	49.75	52.00	51.25	46.25	7.25	0.39
TP (g/dl)	13.56	13.90	13.05	16.23	13.13	13.88	13.70	12.50	3.05	0.43
UA (mg/dl)	84.35	79.98	77.60	87.13	86.95	91.88	85.43	86.60	10.12	0.83
ALT (u/l)	110.75^{bc}	85.50^{c}	122.00^{ab}	107.25^{bc}	110.00^{bc}	142.25^{a}	120.00^{ab}	119.75^{ab}	13.49	0.01
AST(u/l)	85.75^{a}	73.00^{ab}	60.50^{abc}	41.25^{c}	65.00^{abc}	48.25^{bc}	62.25^{abc}	61.00^{abc}	10.63	0.06

^{abcd} Means on the same row having the different superscripts are significantly ($P<0.05$) different

PCV: Packed Cell Volume, RBC: Red Blood Cell, White Blood Cell, Hb: Haemoglobin, TP: Total Protein, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin

4.2.7 Effect of cyromazine and litter depth on haematology and serum indices of broilers at 6 weeks of age.

The main effects of cyromazine levels and litter depth on haematology and serum indices of broilers at 6 weeks of age is shown on Table 22. WBC, Neutrophil, Lymphocyte, Monocyte, Eosinophil, Basophil, AST, ALT and MCHC were significantly affected ($P < 0.05$). Treatment 4 recorded the highest value of (45.32%) for WBC with treatment 3 having the least value (22.49%) Birds on 5 cm litter depth recorded the highest value 42.64 for WBC. Control group showed the highest Neutrophil 26.63 % while treatment 4 showed the least (15.59%) which is significantly different from other inclusion levels. Lymphocyte follows the same trend. Treatment 2 and 3 showed the same highest value of 1.75% for Monocyte. Treatment 2 showed the highest value for Basophil (1.25%). The control showed the highest value of 93.88 for AST u/l while Treatment 4 showed the least (49.75 u/l). Treatment 4 showed the least value (58.25 u/l) of ALT which is significantly different from other inclusion levels. MCHC also follows the same trend as ALT but with significant difference in litter depth.

The result of the interaction effects between the inclusion levels and the litter depth is presented in Table 23. The result showed that RBC, Neutrophil, Monocyte, Eosinophil, Glucose, AST, TP, ALT were significantly different. 5 cm litter depth recorded the higher values with the highest value (64.51%) for RBC in treatment 4. For Neutrophil, both depth recorded similar values. Similar value was recorded for Monocyte with least value in treatment 4 of 5 cm litter depth. The same trend goes for Glucose. 3 cm litter depth recorded higher values for AST with 119.25 u/l for highest value in control and the least in 59.00 in treatment 4 of 5 cm. TP g/l recorded the highest value 18.63 in treatment 2 of 3 cm depth while the least value 9.58 was recorded in treatment 4 of 5 cm. ALT

showed the highest value of 119 u/l and least value of 27.25 u/l which is significantly different from others.

Table 22: Main effect of cyromazine and litter depth on haematology and serum indices of broilers at 6 weeks

	Levels of cyromazine						Litter depth			
Parameters	0.00	0.25	0.50	0.75	SEM	P-value	3cm	5cm	SEM	P-value
Heamathology										
PCV (%)	36.75	36.25	39.63	39.25	3.26	0.56	37.38	38.56	1.70	0.33
WBC(x10^9/l)	38.93^{ab}	26.55^{ab}	22.49^{b}	45.32^{a}	9.27	0.02	24.00^{b}	42.64^{a}	5.83	0.25
RBC(x10^6/l)	5.32	3.40	3.21	3.42	2.18	0.51	4.33	3.34	1.09	0.38
Hb (g/dl)	12.24	12.08	13.17	12.48	1.08	0.41	12.46	12.53	0.57	0.14
Neutrophil (%)	26.63^{a}	23.75^{a}	24.50^{a}	15.59^{b}	1.29	0.05	22.69	22.54	1.80	0.97
Lymphocyte(%)	67.63^{b}	66.75^{b}	69.75^{b}	80.75^{a}	3.02	0.001	71.38	71.06	1.94	0.88
Monocyte (%)	0.88^{b}	1.75^{a}	1.75^{a}	0.38^{b}	0.23	0.02	1.25	1.13	0.20	0.94
Eosinophil (%)	2.63^{b}	4.25^{a}	2.50^{b}	2.63^{b}	0.50	0.02	2.94	3.06	0.28	0.98
Basophil (%)	0.88	1.25	1.00	0.63	0.31	0.59	0.88	1.00	0.15	0.94
MCV(fl)	11.08	10.69	12.39	11.56	1.54	0.30	11.26	11.61	0.80	0.11
MCH (pg)	29.62	33.31	33.24	31.78	3.68	0.45	31.48	32.49	1.84	0.26
MCHC (g/dl)	33.31^{a}	33.31^{a}	33.24^{a}	31.76^{b}	0.82	0.00	33.32^{a}	32.49^{b}	0.44	0.00
Serum indices										
Albumin (g/dl)	8.40	9.20	10.38	8.95	1.63	0.74	9.00	9.46	1.08	0.38
Creatinine(mg/dl)	55.56	56.25	56.25	56.25	54.50	4.73	52.13	59.13	0.30	2.96
TP (g/dl)	14.45	16.33	15.66	13.26	2.50	0.21	16.11	13.74	1.20	0.13
UA (mg/dl)	90.85	82.13	84.15	77.21	11.49	0.29	84.26	82.91	6.21	0.10
ALT(ul)	116.88^{a}	113.75^{a}	124.38^{a}	58.25^{b}	22.13	0.00	106.31	100.31	13.10	0.02
AST (mg/dl)	93.88	65.25^{ab}	63.63^{ab}	49.75^{b}	17.93	0.09	75.75	60.50	10.42	0.11
Glucose (mg/dl)	170.38^{bc}	165.00^{c}	176.88^{b}	196.13^{a}	32.14	0.05	174.31	179.88	20.43	0.10

^{abcd} Means on the same row having the different superscripts are significantly (P<0.05) different

PCV: Packed Cell Volume, RBC: Red Blood Cell, White Blood Cell, Hb: Haemoglobin, TP: Total Protein, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin

Table 23: Interactiveeffect,t of cyromazine and litter depth on haematological and serum indices of broilers at 6weeks

Parameters	0.00	0.25	0.50	0.75	0.00	0.25	0.50	0.75	SEM	P-value
Haematological										
PCV (%)	33.00	36.25	39.25	41.00	40.50	36.25	40.00	37.50	4.95	0.33
RBC ($x10^6$/l)	28.20^{b}	26.55^{bc}	15.15^{c}	26.13^{bc}	49.65^{ab}	26.55^{bc}	29.85^{bc}	64.51^{a}	13.53	0.03
WBC ($x10^9$/l)	3.49	3.40	3.25	3.18	3.14	3.40	3.18	3.67	4.17	0.25
Hb (g/dl)	10.10	12.08	13.08	13.67	13.48	12.08	13.25	11.30	1.65	0.14
Neutrophil	26.00^{ab}	23.75^{abc}	24.50^{abc}	16.50^{bc}	27.25^{a}	23.75^{abc}	24.50^{abc}	14.68^{c}	20.80	0.04
Lymphocyte (%)	69.00	66.75	69.75	80.00	66.25	66.75	69.75	81.50	4.61	0.88
Monocyte (%)	1.00^{a}	1.75^{a}	1.75^{a}	0.50^{b}	0.75^{b}	1.75^{a}	1.75^{a}	0.25^{b}	0.41	0.04
Oesinophil (%)	2.50^{b}	4.25^{a}	2.50^{b}	2.50^{b}	2.75^{b}	4.25^{a}	2.50^{b}	2.75^{b}	0.75	0.05
Basophil (%)	0.75	1.25	1.00	0.5	1.00	1.25	1.00	0.75	0.48	0.94
MCV (fl)	9.27	10.69	9.27	12.89	12.89	10.69	12.61	10.24	10.24	0.11
MCH (pg)	33.32	33.31	33.33	33.33	33.29	33.31	33.14	30.22	30.22	0.26
MCHC (g/dl)	25.96	33.31	33.31	33.33	33.29	33.31	33.40	30.22	30.22	0.23
Serum indices										
Albumin (g/dl)	8.40	9.95	7.98	9.68	8.40	8.45	12.78	8.23	3.33	0.38
Glucose (mg/dl)	173.75	78.73	127.50	142.50	114.75	138.00	121.25	151	13.21	0.10
Creatinine(mg/dl)	52.00	64.75	58.50	62.00	59.00	48.50	54.00	47.00	8.42	0.16
TP (g/dl)	14.83^{ab}	18.63^{a}	14.05^{ab}	16.95^{ab}	14.08^{ab}	14.03^{ab}	17.28^{a}	9.58^{b}	3.22	0.03
Uric acid(mg/dl)	89.05	79.75	75.88	92.38	92.65	84.50	92.43	62.05	8.98	0.10
ALT(ul)	119.00^{a}	89.50^{a}	127.50a	89.25^{a}	114.75^{a}	138.00^{a}	121.25^{a}	27.25^{b}	13.21	0.09
AST(mg/dl)	119.25^{a}	78.50^{ab}	64.75^{b}	40.50^{b}	68.50^{b}	52.00^{b}	62.50^{b}	59.00^{b}	28.18	0.01

^{abcd} Means on the same row having the different superscripts are significantly $p<0.05$) different

PCV: Packed Cell Volume, RBC: Red Blood Cell, White Blood Cell, Hb: Haemoglobin, TP: Total Protein, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin

4.3.8 Effect of cyromazine inclusion and litter depth on carcass characteristics of broiler chicken at 6weeks of age

Table 24 shows the main effects of cyromazine and litter depth on carcass characteristics of broilers at 6 weeks of age. All measurements were not significantly (P>0.05) influenced by the inclusion levels of cyromazine. The relative cut parts weights of the thigh range from 7.1 g in birds fed 0.75g/kg to 7.71 g in birds fed the control diet. Drumstick range from 7.08 – 7.56 while breast range from 12.46 – 22.74%. The organs also showed no significant (P>0.05) difference in all the inclusion levels. Gizzard weight range from 3.18 to 3.72%, kidney range from 0.39 to 0.49% and liver range from 2.21% in bird fed 0.75 g/kg to 2.97% in birds fed 0.25 g/kg.

Interaction effect of cyromazine and litter depth on carcass characteristic of broiler chicken at 6 weeks (end of cyromazine) is shown in Table 25. The result showed that there was no significant (P>0.05) interraction between the inclusion levels and litter depth for all the relative weights of all the cut parts and the organs.

Table 24: Main effect of cyromazine and litter depth on carcass characteristics of broiler chicken at 6week (end of cyromazine)

Parameters	0.00 g/kg	0.25g/ kg	0.50 g/kg	0.75 g/kg	SEM	P-value	3cm	5cm	SEM	P-value
Live weight (g)	950	1050	950	912.5	109.36	0.83	996.9	934.4	77.30	0.57
Dressweight(g)	700	737.5	700	750	127.53	0.99	712.5	731.3	90.17	0.88
Dressing (%)	65.29	72.66	76.40	72.37	14.70	0.94	71.96	71.40	8.34	0.97
*Cut parts (%)										
Head	2.79	3.74	3.06	2.69	0.56	0.52	2.97	3.17	0.39	0.70
Neck	3.74	3.78	3.58	3.37	0.69	0.97	3.48	3.76	0.49	0.67
Wing	6.82	6.08	6.78	6.68	1.30	0.97	6.45	6.73	0.92	0.83
Back	10.83	11.13	11.46	10.93	2.25	0.99	10.49	11.68	1.59	0.59
Shank	4.08	3.97	3.94	3.72	0.69	0.99	4.03	3.82	0.49	0.76
Breast	12.73	13.04	22.74	12.46	6.22	0.54	18.13	12.35	4.40	0.35
Thigh	7.64	7.56	7.71	7.17	1.40	0.99	6.95	8.09	0.99	0.41
Drumstick	7.08	7.56	7.38	7.08	1.30	0.99	7.49	7.06	0.92	0.73
*Organs (%)										
Heart	0.52	0.63	0.60	0.55	0.67	0.87	0.58	0.58	0.08	0.98
Kidney	0.44	0.49	0.39	0.48	0.11	0.88	0.50	0.40	0.07	0.33
Liver	2.77	2.96	2.96	2.21	0.48	0.63	0.79	2.67	0.34	0.80
Spleen	0.20	0.22	0.54	0.15	0.20	0.49	0.37	0.18	0.14	0.33
Proventiculus	0.60	0.67	0.75	0.55	0.11	0.56	0.63	0.66	0.07	0.79
Gizzard	3.18	3.52	3.72	3.27	0.60	0.91	3.30	3.55	0.43	0.67

abcdMeans on the same row having the different superscripts are significantly ($P<0.05$) different

*Expressed as the percentage of live weight

Table 25: Interraction of cyromazine and litter depth of carcass characteristic of broiler chicken at 6 weeks (end of cyromazin)

Parameters	3cm				5cm					
	0.00g/kg	0.25g/kg	0.50g/kg	0.75g/kg	0.00g/kg	0.25g/kg	0.50g/kg	0.75g/kg	SEM	P value
Live weight(g)	900	900	1187.5	1000	1000	1200	712.5	825	154.66	0.09
Dress weight (%)	750	675	825	600	650	800	575	900	180.35	0.45
Dressing %	69.97	73.07	83.62	61.19	69.19	72.25	60.62	83.56	28.14	0.76
*Cut part (%)										
Head	3.58	3.34	2.46	2.47	2.54	4.13	3.13	2.89	0.85	0.59
Neck	3.67	4.07	3.55	2.62	3.50	3.48	3.94	4.13	1.04	0.71
Wing	7.76	5.55	6.42	6.06	5.80	6.61	7.21	7.29	1.97	0.77
Back	12.43	10.69	10.19	8.66	10.48	11.57	11.47	13.20	3.40	0.77
Shank	4.62	4.26	3.91	3.34	3.26	3.69	4.25	4.10	1.05	0.68
Breast	33.73	13.94	13.68	11.20	11.75	12.14	11.78	13.72	9.40	0.47
Thigh	7.94	7.43	6.70	5.73	7.48	7.68	8.59	8.61	2.11	0.81
Drumstick	8.47	8.23	7.14	6.13	6.29	6.90	7.02	8.03	1.96	0.69
*Organs (%)										
Heart	0.66	0.63	0.53	0.48	0.54	0.63	0.51	0.62	0.16	0.83
Kidney	0.47	0.63	0.37	0.54	0.31	0.35	0.52	0.42	0.16	0.50
Liver	3.50	3.08	2.39	2.19	2.42	2.86	3.16	2.23	0.72	0.57
Spleen	0.92	0.22	0.20	0.16	0.15	0.22	0.21	0.13	0.31	0.42
Proventriculus	0.84	0.71	0.48	0.50	0.66	0.62	0.73	0.61	0.16	0.45
Gizzard	3.82	3.67	2.86	2.85	3.62	3.37	3.50	3.70	0.91	0.86

[abcd] Means on the same row having the different superscripts are significantly (P<0.05) different

*Expressed as the percentage of live weight

4.4.0 Effect of cyromazine and litter depth on histopathology of Broilers Organs

The effect of cyromazine residue on the histopathology examination of kidney of broilers is shown in 1, 2, 3 and 4. Plates 1 shows the kidney of birds on the control diet , plate 2 shows birds on 0.25 g/ kg of cyromazine. This shows disseminated congestion (blue arrow), the glomeruli shows no significant lesion (green arrows), focal area of intertitial infiltration by inflammatory cells (black arrows) and tubular necrosis and desquamation (slender arrows). Plates 3 shows the histopathology of birds fed 0.50 g/kg at week 4. There is focal area of calcification, and focal area of interstitial infiltration by in flammatory cells, tubular necrosis.Plates 4 show disseminated congestion (blue arrows) and area of extensive hemorrhagic lesion (breaking arrow), disseminated tubular necrosis (green arrows), focal area of interstitial infiltration by inflammatory cells (black arrows). The glomeruli appear congested for birds fed 0.75 g/kg cyromazine at week 8.

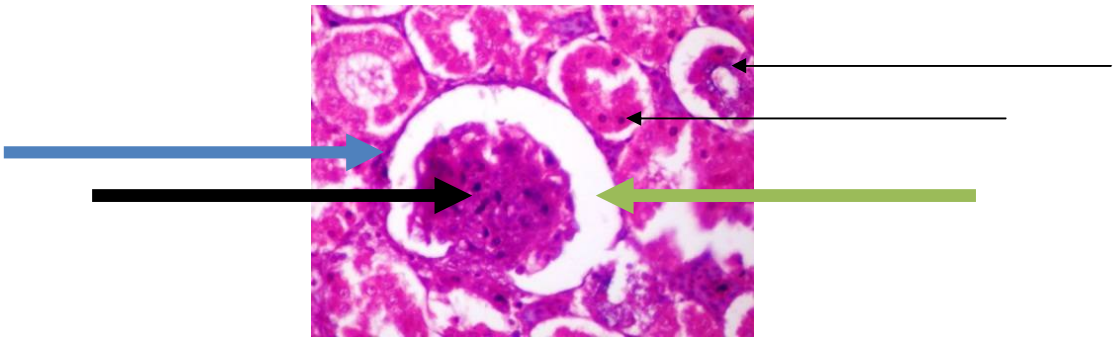
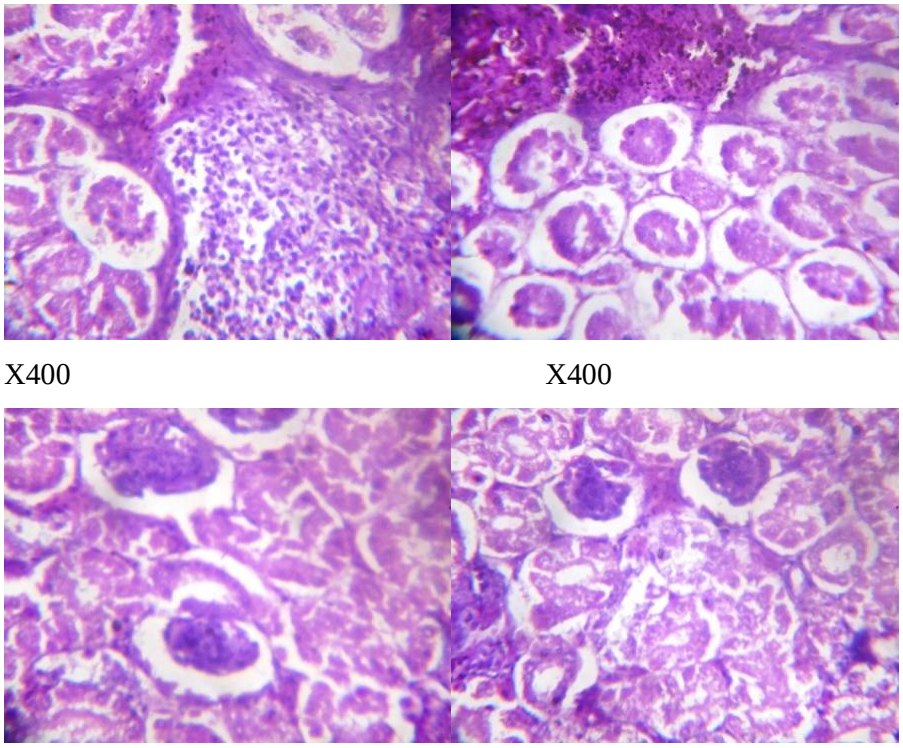


Plate 1
Treatment 1
The black arrow showed points to the glomeruli while the slender arrows are the convoluted tubules of the renal cortex well defined. This part is essentially normal. The glomerular is well postioned with sufficient bowman's space (green arrows) and the bowmans capsule is well defined (blue arrow).

Plate 1: histopathology of kidney of control



X400 X400

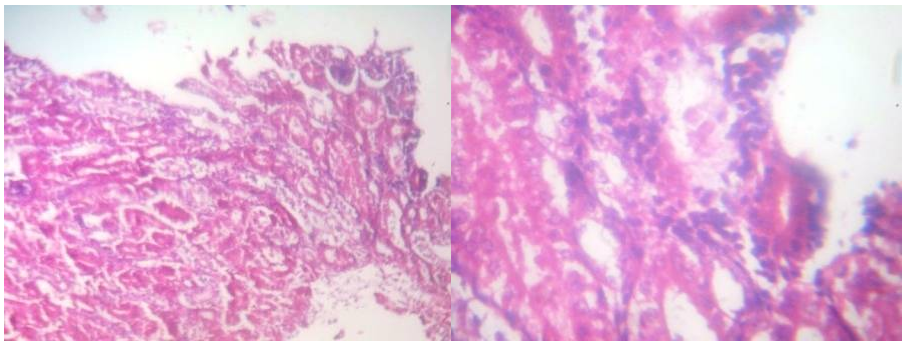
X400 X400

Plate 2

Treatment 2

Plate 2 showed disseminated congestion (blue arrow), the glomeruli shows no significant lesion (green arrows), focal area of intertitial infiltrastion by inflammatory cells (black arrows) and tubular necrosis and desquamation (slender arrows).

Plate 2: histopathology of kidney of treatment 2



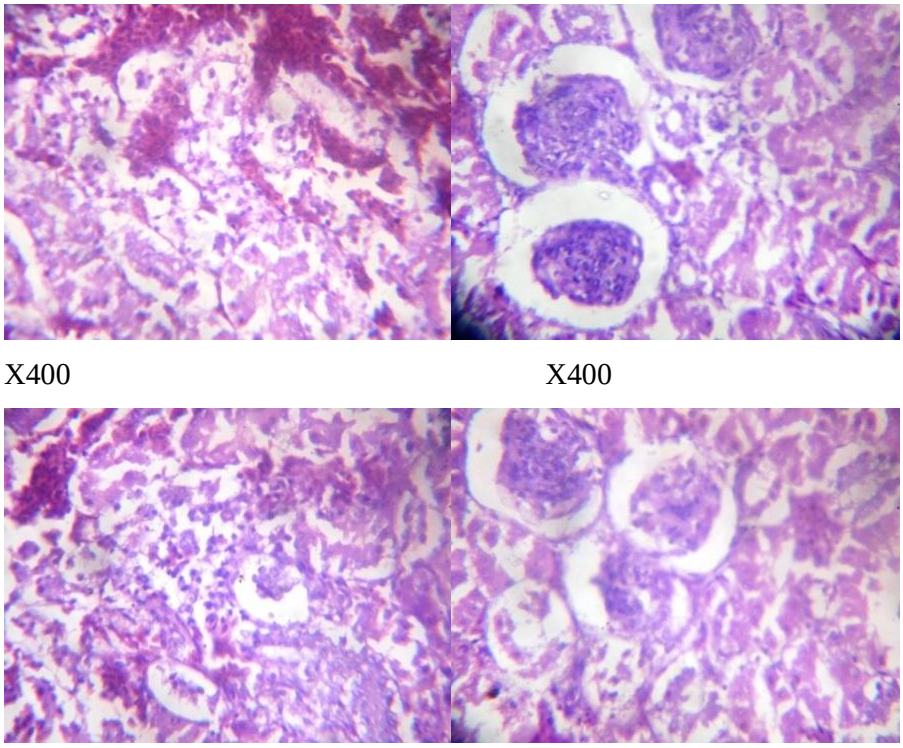
X400

Plate 3

Treatment 3.

Plates 3 showed focal area of calcification (green arrow), and focal area of interstitial infiltration by in flammatory cells (slender arrow), tubular necrosis (green arrow).

Plate 3: histopathology of kidney of treatment 3



X400 X400

X400 X400

Plate 4

Treatment 4

Plates 4 showed disseminated congestion (green arrows) and area of extensive hemorrhagic lesion (breaking arrow), disseminated tubular necrosis (green arrows), focal area of interstitial infiltration by inflammatory cells (black arrows). The glomeruli appear congested (slender arrows)

Plate 4: histopathology of kidney of treatment 4

4.4.1 Effect of cyromazine and litter depth on histopathological examination of broiler liver.

The effect of cyromazine residue on the histhological examination of liver is shown in plates 5, 6, 7 and 8. Plate 5 showed the liver of birds that had no cyromazine (control). There is no leison on hepatocyte, centrilobular vein.Plate 6 showed the liver of birds fed 0.5g/kg cyromazine inclusion showed congestion involving veins and sinusoid (blue arrows), focal area of lymphoid aggregate (breaking arrow) disseminated vacuolation of the parenchyma and mild disseminated periportal infiltration by inflammatory cells. Treatment 3 plates shows congestion involving veins and sinusoid, focal area of lymphoid aggregate disseminated micro vesicular steatosis and disseminated periportal infiltration by inflammatory cells.

Treatment 4 also showed congestion involving veins and sinusoid, focal area of lymphoid aggregate with disseminated necrosis of the hepatocytes and disseminated periportal infiltration by inflammatory cells.

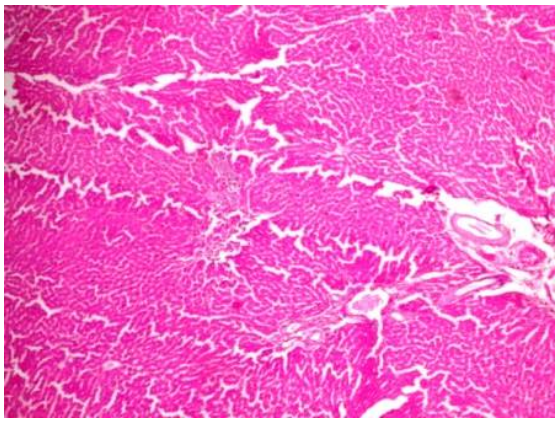
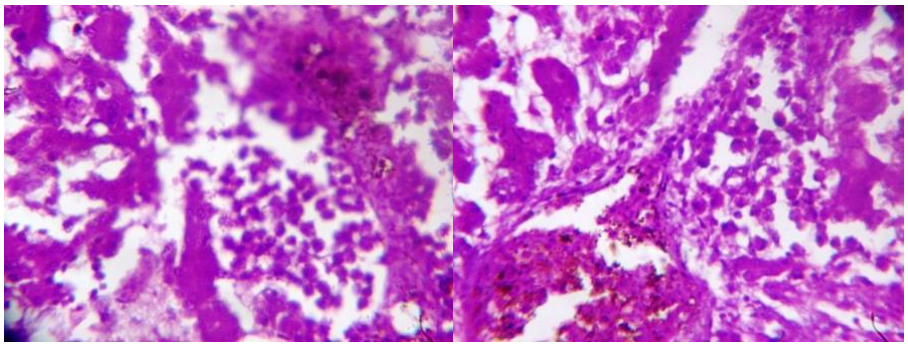


Plate 5

Plates 5 showed no significant lesion. The hepatocytes appear normal the centrilobular vein and the portal tract appear normal .

Plate 5: histopathology of liver of the control



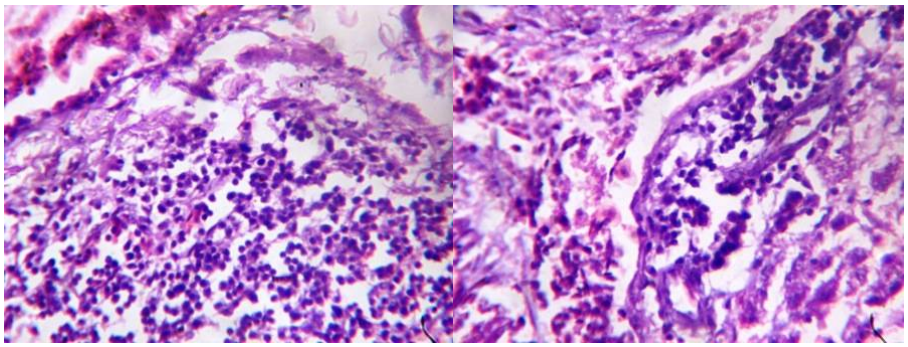
X400 X400

Plate 6

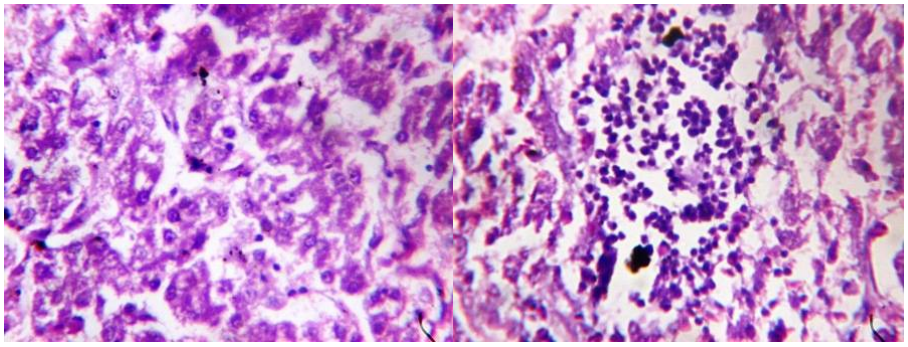
Treatment 2

Plates 6 showed congestion involving veins and sinusoid (blue arrows), focal area of lymphoid aggregate (breaking arrow) disseminated vacuolation of the parenchyma (green arrows) and mild disseminated periportal infiltration by inflammatory cells (black arrows).

Plate 6: histopathology of the liver of treatment 2



X400 X400



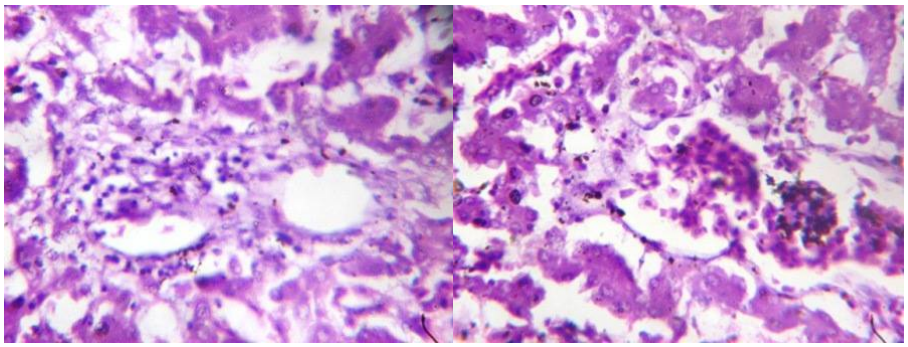
X400 X400

Plate 7

Treatment 3

Plates 7 showed congestion involving veins and sinusoid (blue arrows), focal area of lymphoid aggregate (breaking arrow) disseminated microvesicular steatosis (green arrows) and disseminated periportal infiltration by inflammatory cells (black arrows).

Plate 7: histology of liver of treatment 3



X400 X400

Plate 8

Treatment 4

Plates 8 showed congestion involving veins and sinusoid (blue arrows), focal area of lymphoid aggregate (breaking arrow) disseminated necrosis of the hepatocytes (green arrows) and disseminated periportal infiltration by inflammatory cells (black arrows).

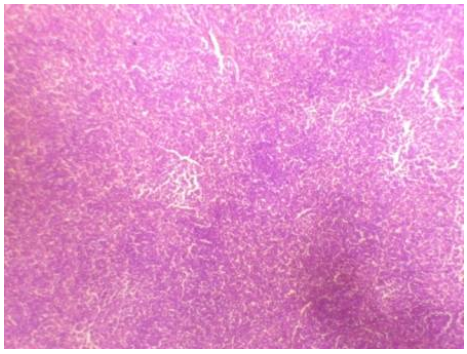
Plate 8: histopathology of liver of treatment 4

4.4.2 Effect of cyromazine and litter depth on histopathology of Broilers Organs

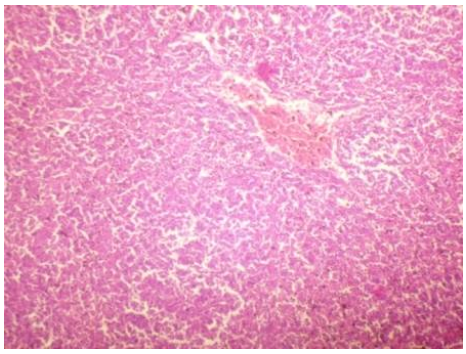
The effect of cyromazine residue on the histhopathology examination of spleen of broilers is shown in plate 9, 10 and 11. Plate 9 showed the spleen of the control diet. There is congestion of sinuses (blue arrows), the white and red pulps are admixed they showed no delineation which is essentially normal.

Plate 10 showed the result of birds fed diet containing 0.25 g/kg. There is congestion of sinuses, trabecular artery shows very mild hyperemia, the white and red pulps are admixed they show no delineation.

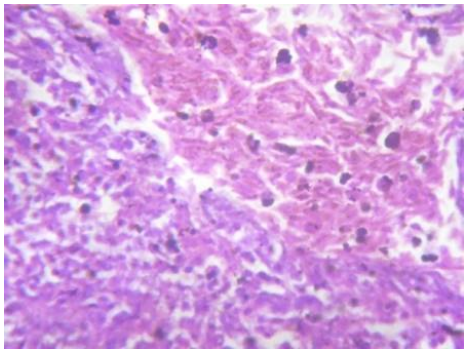
Plates 11 show the result of birds fed 0.50 g/kg cyromazine. There is congestion of sinuses, trabecular arteries with hyperemia are also seen, there is sclerosis of the wall of the trabecular arteries and the white and red pulps show no delineation.



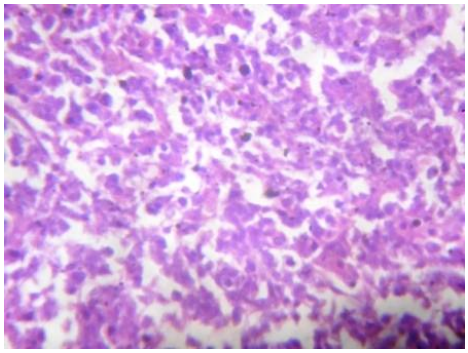
X40



X100



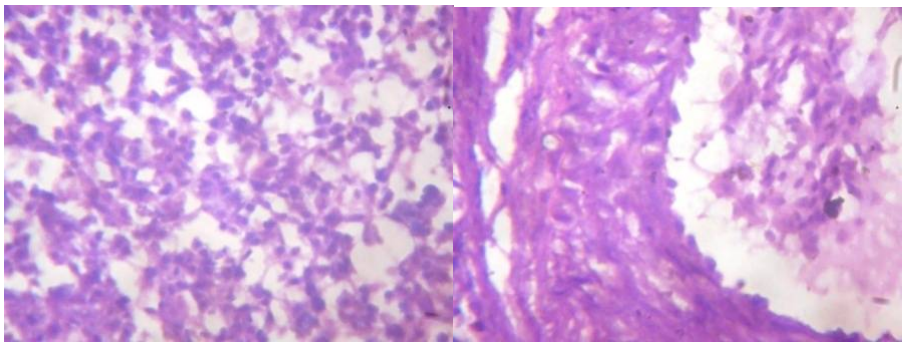
X400



X400

Spleen 9.
Treatment 1
Plates showed congestion of sinuses (blue arrows), the white and red pulps are admixed they show no delineation. This is essentially normal.

Plate 9: histopathology of spleen of the control



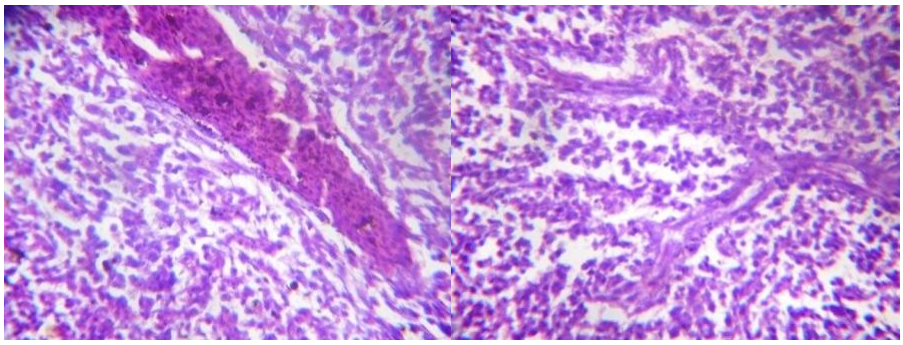
X400 X400

Plate 10

Treatment 2

Plates 10 show congestion of sinuses (broken arrows), trabecular artery shows very mild hyperemia (slender arrows) the white and red pulps are admixed they show no delineation.

Plate 10: histopathology of spleen of treatment 2



X400 X400

Plate 11

Treatment 3

Plates 11 show congestion of sinuses (blue arrows), trabecular arteries show hyperemia (breaking arrow) are seen, there is sclerosis of the wall of the trabecular arteries (slender arrow) and the white and red pulps show no delineation.

Plate 11: histopathology of spleen of treatment 3

4.4.3 Effect of Cyromazine residue in meat of broiler chickens

The effect of cyromazine inclusion on residue in drumstick and thigh tissues of broiler chicken is presented in table 26. Birds on 0.25 g/kg and 0.5 g/kg of cyromazine were significantly ($P<0.05$) different from 0.75 g/kg and the control (0 g/kg). Birds on 0.25 g/kg and 0.5 g/kg showed higher values (0.31 and 0.28) respectively. At week 4, birds on 0.75 g/kg showed the highest value (0.28) while birds on 0.25 and 0.5 g/kg had similar value (0.21) and the least value (0.004) in the 0 g/kg. At week 6, birds fed 0.75 g/kg showed highest value (0.35) followed by 0.5 g/kg (0.32) and least value (0.002) in 0 g/kg. The residue level increased with inclusion level. At week 9, birds fed 0.75 had the highest value (0.20) followed by 0.5 g/kg (0.12) and the least in the control 0 g/kg. Week 10 follows the same trend as week 9 with the highest value of 0.16 and least value (0.01).

The cyromazine residue in thigh is statistically different ($P<0.05$) at week 4 with birds on 0.75 g/kg having the highest value (0.57) while birds on 0.25 g/kg and 0.50 g/kg having similar values (0.29 and 0.28) respectively and the control (0 g/kg) having the least value. Birds on 0.75 g/kg inclusion level are also statistically different from others with the highest value (0.28) and a least value in the control (0.002). Birds on 0.50 g/kg and 0.25 g/kg are statistically different from others at week 9 with both having higher values (0.18 and 0.16) and the least value in 0 g/kg (0.013). At week 10, birds on 6.50 g/kg had the highest residue value (0.18) and the least in the control

Table 26 Main effect of cyromazine residue on broiler chicken

	DRUM						THIGH					
Week	0.00g/kg	0.25g/kg	0.50g/kg	0.75g/kg	SEM	Pvalue	0.00g/kg	0.25g/kg	0.50g/kg	0.75g/kg	SEM	Pvalue
2	0.001^{c}	0.31^{a}	0.28^{a}	0.22^{ab}	0.01	0.0003	0.001	0.24	0.28	0.31	0.05	0.25
4	0.004^{c}	0.21^{b}	0.21^{b}	0.28^{a}	0.02	0.004	0.009^{c}	0.28^{b}	0.29^{b}	0.57^{a}	0.03	0.008
6	0.002^{c}	0.29^{ab}	0.32^{b}	0.35^{a}	0.16	0.003	0.002^{b}	0.29^{c}	0.32^{b}	0.76^{a}	0.08	0.04
7	0.003	0.16	0.16	0.26	0.10	0.56	0.001	0.15	0.22	0.35	0.70	0.44
8	0.006	0.15	0.16	0.23	0.11	0.16	0.002^{c}	0.16^{b}	0.21^{ab}	0.28^{a}	0.02	0.0001
9	0.014^{c}	0.15^{ab}	0.12^{b}	0.20^{a}	0.41	0.07	0.013^{d}	0.16^{a}	0.18^{a}	0.12^{b}	0.02	0.0001
10	0.010^{c}	0.11^{ab}	0.08^{b}	0.16^{a}	0.01	0.002	0.011	0.13^{b}	0.18^{a}	0.11^{b}	0.01	0.0001

^{abcd} Means on the same row having the different superscripts are significantly ($P < 0.05$) different

4.4.4 Effect of Melamine inclusion on residue in meat of broiler chickens

The effect of melamine residue on drumstick and thigh tissues of broiler chicken is presented in table 27. The drumstick shows significant ($P<0.05$) difference at week 2, 7, 8, 9 and 10. At week 2, birds fed 0.50 g/kg showed a higher value (0.40) than other inclusion levels. At week 7 (7 days withdrawal), birds fed 0.75 showed the highest value 0.28. Week 8 and 9 followed the same trend as week 7. At week 10, birds on 0.25 g/kg and 0.75 g/kg showed a higher residue value (0.23) than 0.5 g/kg and 0 g/kg. In all the weeks, birds on 0 g/kg inclusion level showed the least residue values.

The melamine in thigh showed significant difference ($P<0.05$) at week 6 and 10. At week 6, bird fed 0.75 g/kg and 0.50 g/kg cyromazine inclusion levels showed a higher value (0.46 and 0.47) with the least value (0.01) in the control group (0 g/kg). At week 10 (28 days withdrawal), birds on 0.5 g/kg and 0.75 g/kg were statistically ($P<0.05$) different from birds on 0.25 g/kg and 0 g/kg.

Table 27 Melamine residue in the meat of broiler chickens fed diets containing cyromazine broiler chickens

	DRUM						TIGH					
Weeks	0.00g/kg	0.25g/kg	0.50g/kg	0.75g/kg	SEM	P value	0.00g/kg	0.25g/kg	0.50g/kg	0.75	SEM	P value
2	0.001^{c}	0.40^{a}	0.24^{b}	0.20^{b}	0.04	0.0001	0.023	0.19	0.11	0.29	0.06	0.17
4	0.005	0.19	0.23	0.18	0.06	0.17	0.001	0.31	0.32	0.42	0.05	0.11
6	0.003	0.38	0.41	0.45	0.22	0.33	0.011^{c}	0.35^{ab}	0.46^{a}	0.47^{a}	0.06	0.0001
7	0.19^{c}	0.23^{ab}	0.27^{b}	0.38^{a}	0.06	0.0004	0.022	0.23	0.29	0.31	0.19	0.43
8	0.08^{d}	0.17^{c}	0.29^{b}	0.35^{ab}	0.09	0.02	0.017	0.21	0.23	0.25	0.14	0.30
9	0.09^{c}	0.24^{a}	0.13^{b}	0.29^{a}	0.07	0.01	0.019	0.19	0.19	0.18	1.59	0.19
10	0.02^{c}	0.23^{a}	0.20^{b}	0.23^{a}	0.04	0.0001	0.019^{c}	0.12^{b}	0.16^{a}	0.14^{a}	0.04	0.04

^{abcd} Means on the same row having the different superscripts are significantly ($P < 0.05$) different

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

Like other agrochemical, cyromazine is highly effective in pesticide control but it is also toxic to human and the environment. In addition, melamine (chemical name 1, 3, 5 triazine, 2, 4, 6 triazine), a potential degradation product or metabolite of cyromazine is a suspected carcinogen (Aldrich 1987 and Carbras *etal* 1990) which made it of public health concern. Results of the survey showed that 72.4% of respondents are using larvacide which indicated that many farmers are aware of its usage. Many farmers use it to get rid of flies, while some use it to boost the feed crude protein because of its high nitrogen without having the knowledge of its residual effect on the consumers. Response from many users of larvacide indicated that most farmers use larvacide to get rid of odour without bothering if it decomposes or not and not having the knowledge of its public health implication. 93.1% of farmers indicated that larvacide improves performance which might be as a result of high nitrogen value of cyromazine as reported by Ossborne *et al*., (2008) and Merck, 2001. This is also justified by report of Brake 1983 that cyromazine increased egg production. Wilson 1983, also reported high egg production and zero mortality when birds were fed 1000mg/kg cyromazine. 82.9% respondent dump litter in nearby land which can wash off and sip into nearby rivers and well. 89.7% respondents showed that larvicide reduces odour. This is why many use it in order to prevent their neighbours from complaining without having the knowledge of the residual effect and environmental impact.

In this research, the proximate composition of diets showed that with increase in inclusion level, the crude protein increased. The higher crude protein values in the diets could be due to the high nitrogen in melamine (a metabolite of cyromazine) which agrees

with previous study of Osborne *et al.*, (2008). Merck, 2001 also reported an exceptional high N content of melamine. The CP of the control diet was lower to the NRC (1994) level (22 - 25%) for starter, however it was boosted by cyromazine inclusion (24.5%) which justifies the report of Setiogi (2008) that melamine is purposely added to feedstuff as an adulterant to increase the protein levels of feeds. This was also confirmed from the result of true protein percentage which shows a wide difference to the percentage of crude protein in the study. The low true protein in the control and inclusion level shows that feedstuffs have low protein.

Crude Protein digestibility of nutrient increased as cyromazine inclusion level increased which is contrary to the findings of Kidd *et al*., (1996) who reported that an increase in dietary CP had lower utilization in birds fed with melamine inclusion. In this study, it was observed that CP digestibility was low which might give indication that the CP digestibility of diets containing cyromazine is low. Digestibility rating in this study ranged from 55.83% and 62.18% which is within the moderate rate in accordance with the rating of Alimuddin (2000) who rated digestibility percentage as good when above 70%; moderate at 40 and 60% and very low when below 40%. Although there is no difference in values of the parameters measured which implies that all birds in all treatments utilized effectively the additives present in their feed. This agrees with Brakes (1985) who reported that there is improved nutrient utilization with cyromazine inclusion**.**

The result of this study showed that there was significant increase in feed intake between 0, 0.25, 0.5 and 0.75g/kg cyromazine inclusion levels at 2weeks. This disagrees with related literature (Cecil *et al.*, 1981; Wilson *et al*., 1983; Brake *et al*., 1984; Brake *et al*., 1985 and Brake *et al*., 1989) which reported no difference in feed intake after inclusion

of 1000mg/kg larvadex (with cyromazine as active ingredient) in the diet of birds. Also, at 4 weeks, there was significant increase in feed intake for the treated group compared with the control group, which significantly increased the weight of birds on 0.50g/kg. This might be that the birds are used to the cyromazine diet at this stage. This was contrary to the findings of Brake *et al*., (1989) when he administered levels of 2000mg cyromazine to turkeys which decrease their feed intake and growth. However, higher feed intake which is evidence that they had a greater appetite which was also confirmed by the interaction between cyromazine inclusion and litter depth. This agrees with a similar report by Cruywagen , (2009) but in contrary to a previous study reported by Brand *et al*.(2009) who fed young poults graded levels of melamine from hatch to 21 days and indicated a reduced feed intake with inclusion level compared with control. According to the findings of Brake *et al*. (1989) who administered levels of 2000 mg/kg cyromazine or less are non-toxic to turkeys; however, a marked decrease in feed intake and growth were observed in turkeys receiving levels between 500-2000 mg/kg cyromazine. Despite the occurrence of suppressed body weights and poor reproductive performance, these production traits were reversible after cyromazine was withdrawn from the diets of broiler breeders (Brake *et al*., 1984), layers (Brake *et al*., 1985) and turkeys (Brake *et al.*, 1989). This disagrees with the findings from this study. At 8 weeks (2 weeks withdrawal), there was no reversible change in growth performance, as reported by Brake *et al*. (1984) where there was reversible production traits after it was withdrawn from broiler breeder. As the level of cyromazine in the diet increased, there was an increase in body weight gain. This disagrees with an earlier study by Ledoux *et al*. (2009) who fed graded levels of melamine to young broilers from day old to 14 days. Brake *et al*. (1985) also reported excellent feed conversion ratio (FCR) compared to the

control group which is contrary to the findings in this study where the control group showed a better feed conversion ratio.

High mortality observed at the earlier stage (2 weeks), was observed in birds fed higher levels of cyromazine compared with the control and those on lower levels. This finding shows that broilers cannot tolerate cyromazine up to 0.75g/kg at early age. It showed that it is toxic to the birds at this level. This agrees with findings of Ledoux *et al* (2009) who reported that mortality was observed in birds fed on 2.5g/kg and 3% melamine as early as day five. A similar result was observed by Brand *et al* (2009) on young turkey poults fed graded levels of melamine from hatch to 21 days. High mortality at the highest level of inclusion could be as a result of immunity being suppressed which was also similar to report of Brake *et al* (1984) when broiler breeder was fed high dose of cyromazine and concluded that it might be due to high sensitivity of broilers to cyromazine. High mortality might be due to high sensitivity of broilers to cyromazine as reported by Brake *et al* (1984, 1985, 1989) when he had to reduce his inclusion from 3g/kg to 1g/kg due to high mortality.

The results on litter depth of birds showed that birds on 3cm litter depth had better performance than 5cm litter depth during 0 to 6weeks. This contradicts the findings of Hague and Chowdhury (1994) that types and depth of litter have no effect on growth rate of broilers. However, at 2 weeks withdrawal period (week 8), birds on 5cm litter depth showed better performance in all inclusion levels than 3cm litter depth.

Furthermore, the carcass, dressing percentage, total carcass yield, cut parts and even the organs were not significantly influenced by the treatments and did not follow a specific pattern that can be attributed to treatment effect. This is similar with the result of Srisuda

et al (2010) when melamine or urea-formaldehyde was fed to broiler chicken. It is also related to report of Asaniyan *et al* (2007) that carcass had no significant influence on the organ growth, implying that identical organ growth could be attained by raising broilers at different litter depths on different litter types (Bilgili *et al.,2000*). However, this observation was contrary to the report of Yuchang *et al* (2010) that reported an increase in weight of liver and kidney when a high dose of melamine was fed to broilers.

Haematological values obtained for birds fed diet containing cyromazine in this study showed that initially (2 weeks) the control group had lower values for RBC, PCV and Hb compared to the inclusion group. This might be as a result of low crude protein in the diet. This is similar with earlier works by Suchy *et al* (2004). Decrease of RBC and Hb also agrees with Stakova *et al* (2014) who reported that lower values may be related to the impairment of liver function due to insufficient synthesis of coagulation factors or lower protein synthesis. Decrease of RBC could be related to the administration of feed containning cyromazine which is similar to the report by Strokova *et al* (2014) when feed was contaminated with melamine and cyanuric acid. He also stated that insoluble complexes of melamine- cyanurate will be formed which normally impairs the integrity of membrane of erythrocyte. Although, a long term administration level up to 6wks of melamine may have impact on the intrinsic haematopoiesis as a result of erythropoietin which is synthesized in the kidney. Lower WBC in this experiment is similar to Stokova *et al* (2014) who reported that lower leucocyte count was caused by the impairment of cell membrane integrity and also affected by possible tissue destruction. Lu *et al*. (2012) found that especially kidney tissue is damaged due to the formation of uroliths. It can also be related to liver toxins on bone marrow (Doubek *et al* 2010). The slight decrease in MCHC at 6 weeks is similar to report of Wang *et al*. (2010) which might be as a result of immature erythrocytes.

Higher glucose values observed in birds fed 0.5 and 0.75g/kg by cyromazine agrees with earlier work by Brand *et al*. (2012) when graded level of melamine was fed to broiler chicken. Low uric acid in the control group throughout the experiment agrees with report of Bowes *et al* (1989) that low protein diets are thought to initiate low uric acid levels. The high uric acid in the inclusion group at the early stage of the birds might be responsible for the increase in mortality. Consistent high uric acid levels also indicate that there is impairement in protein metabolism.

The activity of AST enzyme was lower in the treated group ($P<0.05$) compared with the control. This agrees with report of Doubek *et al*. (2010) that the activity of AST increases when erythrocytes are damaged. In this experiment, erythrocytes increased with inclusion level and as such the activities of AST decreases. It follows that most causes of liver cell injury are associated with an AST that is lower than the ALT (Moussavian *et al* 1985). Higher creatine level in birds fed cyromazine treated diets could be an indication of renal impairment and kidney malfunction.

Histhopathological examination of organs in this study shows crystals in the kidney of the treated group. This was similar to earlier work by Bai *et al* (2010) who observed crystals in kidney samples of laying hens fed with melamine for 34 days. Brake *et al* 1989 also observed renal injury after cyromazine was fed to turkey at 1000mg/kg and more. Reimschussel *et al* (2008) also reported that chicken fed melamine could develop crystals containing uric acid. Previous studies by Puschner *et al* 2007; Dobson *et al*, 2008; Reimschuessel *et al*. (2008) also shows that pigs, fish, cats and rat fed melamine developed renal crystals. Srisuda *et al.* (2010) also observed golden brown crystals in the liver, kidney and spleen tissues of broilers chicks and described a series of linear function used in predicting the dietary melamine dosage. Congestion of sinuses of the

spleen with no delineation observed in birds fed treated diets indicated that cyromazine had effect on the integrity of the spleen. Congestion of vessels of the liver with focal area of lymphoid, severe periportal infiltration by inflammatory cells and mild dissemination of bile deposition was similar to the findings of Wilson *et al.*(1983) who reported fatty liver from necropsy test. This could be as a result of lower RBC and Hb as reported by Stakova *et al*. (2014) that liver impairment and failure can occur.

Lam *et al*. (2009) and Ledoux *et al.* (2009) also reported crystal formation in bile and histopathologic lesions in kidneys which revealed crystals similar to those observed in renal tubules of cats with melamine associated renal failure. The interstitial crytals in this study could cause renal failure as a result of increased renal pressure which reduces renal blood flow (Reimschuessel 2008). The conjestion of the glomeruli in this study indicated that toxins removal from the kidney may be altered or delayed as reported by Sebastian (2009) that toxins are removed by the kidney via glomerular infiltration, tubular excreations through passive diffusion and active tubular secreation. Tubular necrosis, sclerosis, desquamation in this study indicated that there may be obstruction which may reduce the flow of blood to the tubules which is more susceptible to injuries. This destruction can lead to the formation of stones in the distal tubules and may also encourage crystal formation and ultimately causing acute renal failure (Bhalla *et al*., 2009). All these features of the kidney could be as a result of lower WBC as reported by Stakova *et al*. (2014) that there could be impairement of the cell membrane integrity and tissue destruction.

The appearance of calcification and inflammatory cells of the kidney at week 7 of the highest cyromazine inclusion level is similar to earlier work by Brand *et al.*(2009) when

turkey poult were fed melamine diet. Lesions in kidneys which reveals crystals is similar to renal tubules in cats with melamine associated renal failure Ledoux *et al* (2009)

Melamine residue in drumstick and thigh found in this study is above 2.5mg/kg of the maximum allowable concentration of human food (WHO 2008; Setiogi, 2008). It is below the legal limit of melamine that led to the death of six infants and over 50000 that were being hospitalised as a result of scandal in infant milk (McDonald 2002, Macartney 2008, WHO 2010). This indicates that the chicken raised with this inclusion level will not pass the test for melamine by BBC News (2008). The melamine residues in all the treatments in this study is above the 0.0007mg/kg found in breast muscle as reported by Caldas (2007) who stated that 7% of cyromazine supposed to be metabolised to melamine. At 28 days withdrawal (10 weeks), the residue level in 0.25g/kg 0.50g/kg and 0.75g/kg for Drumstick is higher than the 0.2mg per kg body mass of the tolerable daily intake (TDI) which the World Health Organizations food safety estimated as the amount a person could stand per day without incurring a bigger health risk (Endrezl 2008). However, the melamine residue for thigh is lower than than that of drumstick but is still above 0.2mg/kg which the European Union set as standard for acceptable human consumption as reported by Harrigton (2010). It is also higher than the 0.35mg by the Canadian Authority.

All the treatments showed higher value above 0.05mg/kg for both Drumstick and Thigh which is the maximum residue for cyromazine in poultry meat as stipulated by the US Code of Federal Regulations (1987). Although there was reduction in the residue value of thigh with increase in the withdrawal period but still higher than the US Code of Federal Regulations. Residue found in day 28 agrees with report by Brake *et al* (1991) that Cyromazine could still be found in tissues of birds after 13 weeks of withdrawal.

Caldas 2007 reported that 97% of ingested cyromazine is excreted within 24 hours. Although Caldas inclusion levels were 5mg/kg which might have relieved some pressure to manage cyromazine excretion compared to 25mg/kg of Brake *et al* (1991). The slight residue in the control might be that the raw ingredient used for compounding the feed might be from plant uptake as reported by Simoneaux and Marco (1983a) or have been adultrated with cyromazine as reported by Setiogi (2008)

Cyromazine residue in this study is higher than 0.007 and 0.010mg residue in hen fed cyromazine as reported by Cheung 1986. Also in Israel, when broiler chicken trial was conducted in 1987 (Altenburger, 1987) with 5mg/kg cyromazine, residue of 0.06mg in breast muscle was found after 4hours of withdrawal period. Boone and Cheung (1986) also reported 0.05mg when hens were fed 2.5mg/kg cyromazine for 27 days. In a study conducted in Japan in 2000 (Inoue, 2000) when cyromazine was fed at 5mg/kg, for 28days and 2 days withdrawal, 0.05mg was found in the muscle of hen. High residue of cyromazine found in tissue with high inclusion level agrees with Brake *et al* 1991 who reported that the higher the dose of cyromazine, the longer the residual toxicity duration persisted. Tortora (1991) also confirms this when 150mg/kg was administered to dairy goat.

Residue found after 28days withdrawal agrees with earlier work of Brake *et al* (1991) who reported that after feeding layers cyromazine the first 20 weeks of age, residue could still be found in the body tissue and droppings 13 weeks after cyromazine withdrawal. Caldas 2007 reported possible pathway for cyromazine metabolism in different animal species and concluded that in rat, goat, sheep and hen, cyromazine can be metabolised to melamine which was also confirmed from the result of this study. USEPA, 2007 also reported that 10% of ingested cyromazine will convert metabolically

to melamine. Chou *et al* 2003 tested chicken egg, beef, mutton and pork samples for any cyromazine and melamine residues, none of the samples revealed detectable melamine levels(>0.02mg/kg) and only beef samples contained 0.04mg/kg cyromazine. It is also higher than 0.03mg/kg found in loin cut part in ruminant as a result of cyromazine metabolism invivo. Boone *et al* 1985 reported a contrary report that there is a rapid withdrawal times of one to two days, no cyromazine was detected in meat and egg samples after feeding 0.5mg/kg cyromazine in the feed. Although 2.5mg/kg is the maximum limit of any vet drug that may be traced as a residue in animal tissue or products (Zhue *et al*, 2000) which all the residual in this study is above.

5.2 Conclusion

From the results of this study, it can be concluded that:

- Survey reveals that 72.4% of respondents are already using larvacide without the knowledge of its residue on meat and the environment
- Proximate analysis of diets showed a significant increase ($P < 0.05$) in crude protein with increasing levels of cyromazine.
- Growth performance was positively influenced by the inclusion level of cyromazine both at the starter and finisher phases
- Interraction of cyromazine inclusion and litter depth have significant effect on the performance of the birds
- Inclusion levels of cyromazine had no significant influence ($P > 0.05$) on DM and CP digestibility at starter phase. However, 0.75g/kg cyromazine significantly ($P < 0.05$) resulted in higher values of EE and ash digestibility at finisher phase.
- Carcass characteristics of broiler chickens were not significantly influenced by the inclusion of cyromazine.
- Haemathology and serum indices of birds fed cyromazine inclusion levels above 0.50g/kg showed an increase in erythrocyte and decrease in AST levels.
- High dose of cyromazine led to haemorrhagic lesions, necrosis and congested glomeruli of kidney. while the liver showed lymphoid aggregate, necrosis of hepatocyte and inflammatory cells and the spleen showed congested sinuses with sclerosis of the trabecular arteries
- Birds fed cyromazine supplemented diets showed a higher residue of both cyromazine and melamine than the recommended residue of 0.05mg/kg
- High residue of cyromazine and melamine was observed in thigh and drumstick of broiler chickens after 28 days withdrawal period

5.3 Recommendations

- The level of cyromazine in this study is not recommended for the safety of human consumption, therefore lower levels of inclusion should be used in further studies to ascertain a safe inclusion levels
- Other mode of application of cyromazine could be explored without having to include in the feed of birds e.g spraying of appropriate quantities and concentration on the litter.
- Broiler study cannot essentially give the estimate of withdrawal period because of short production cycle
- More accurate withdrawal rate should be estimated in order to guide the industry on cyromazine safety levels
- Histopathology results of this work shows that kidney appeared to be damaged more severely by cyromazine ingestion indicating that cyromazine should be used with caution as a feed additive in broilers
- None of the inclusion levels in this study can be recommended for inclusion in poultry diet based on the residues found in meat even after withdrawal of cyromazine
- Urgent and appropriate public health actions need to be initiated and educated on inclusion of larvacide on feed

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APPENDIX

FEDERAL UNIVERSITY OF AGRICULTURE, ABEOKUTA

Dear respondents, this questionnaire is designed to assess the perceived impact of lavacide usage in poultry production and the environment. Responses will be treated with utmost confidentiality.

1) Age……
2) Location ……..
3) Gender: Male ( ), Female ( )
4) Marital status. Single ( ), married ( ),Widowed ( ), Divorced ( ),
5) Household size……..
6) Educational level: No formal education ( ), Primary education ( ), Secondary education ( ), University ( ) Others……..
7) Religion: Christianity ( ), Islamic ( ) Traditionalist ( )
8) Ethnic group …….
9) What management system are you using on your farm? Deep litter ( ), cage system ( )
10) Number of birds…….
11) Types of birds……
12) Do you use lavacide? Yes ( ), No ( )
13) What age of birds do you introduce lavacide ……
14) How often do you use lavacide? Always ( ), Fortnightly( ), Quarterly ( ), specify
15) Once lavacide has been used, how often do you notice the flies again? After a month ( ), two months interval ( ), Quarterly ( ),Specify ( )
16) How often do you change your litter? Every month ( ), twice a year ( ), Specify ( )
17) Where do you dump your litter? Nearby river bank ( ), nearby land ( ), Pit ( ), Specify ( )
18) Do you notice the litter is decomposing? Yes ( ) No( )
19) Do you think that birds on lavacide perform better? Yes( ), No( )
20) If yes , in which of these; egg size ( ), shelf life of egg ( ),number of cracks ( ), weight of bird ( ), quantity of feed eaten/ day ( )
21) Do you believe lavacide truly reduces odour? Yes ( ), No ( )

Once lavacide is used, how long does it take to perceive odour in the environment again? Two months ( ), Four months ( ), Specify ( )