

Haematological, biochemical and histological characteristics of broiler chickens fed graded levels of dry distilled cassava with soluble

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Abstract

This study was carried out to determine the haematological, biochemical and histological characteristics of broiler chickens fed graded levels of Dry Distilled Cassava with Soluble (DDCS). In a 28-day trial, one hundred and forty four (144) 5-week old, mixed Arbor Acre strain broiler chicks (average weight of 1.09 ± 0.02 kg) were randomly allocated to four dietary treatments of (0% DDCS, 4% DDCS, 8% DDCS and 12% DDCS) in completely randomized design. Each treatment had 2 replicates of 18 birds each. Feed and water were supplied ad libitum. Four birds from each treatment were randomly selected and blood sample collected via jugular puncture for haematological and biochemical evaluation at the termination of experiment. The analysis of blood components, red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), Mean cell volume (MCV), Mean corpuscular haemoglobin concentration (MCHC) and Mean cell haemoglobin (MCH) showed no significant ($P > 0.05$) differences among the broilers fed with experimental diets. There were no significant ($P > 0.05$) differences in the biochemical analysis of the broilers fed different levels of DDCS. Three birds were randomly selected from each treatment group and slaughtered for carcass evaluation and histological analysis of small intestine, kidney, spleen, liver and large intestine. It was concluded that DDCS can be included in the diets of broilers up to 12% without any deleterious effect on the haematological and serum biochemical characteristics. The feeding of DDCS to poultry at 4, 8, and 12% resulted in liver, kidney, small intestine and large intestine changes.

Keywords: Haematological, biochemical, histology, dry distilled cassava with soluble, broiler chicks

Des Caractéristiques hématologiques, biochimiques et histologiques des poulets à griller nourris à des niveaux classés de manioc distillé sec avec soluble (DDCS)



Résumé

Cette étude a été réalisée pour déterminer les caractéristiques hématologiques, biochimiques et histologiques des poulets de grillon nourris à des niveaux classés de manioc distillé sec avec soluble (DDCS). Au cours d'un essai de 28 jours, cent quarante-quatre (144) poussins mixtes de poulets à griller arbor acre (poids moyen de $1,09 \pm 0,02$ kg) ont été répartis au hasard à quatre traitements diététiques de (0% DDCS, 4% DDCS, 8% DDCS et 12% DDCS) dans un design complètement randomisé. Chaque traitement avait 2 répliques de 18 oiseaux chacun. L'alimentation et l'eau ont été fournies ad libitum. Quatre oiseaux de chaque traitement ont été choisis au hasard et un échantillon de sang prélevé par perforation jugulaire pour une évaluation hématologique et biochimique à la fin de l'expérience. L'analyse des composants sanguins, des globules rouges (RBC), des globules blancs (WBC),

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du volume cellulaire emballé (PCV), du volume moyen des cellules (MCV), de la concentration moyenne de corpusclar haemoglobine (MCHC) et de l'hémoglobine à cellules moyennes (HME) n'a montré aucune différence significative ($P>0,05$) entre les poulets de grillage nourris avec des régimes expérimentaux. Il n'y avait aucune différence significative ($P>0,05$) dans l'analyse biochimique des poulets de grillage nourris à différents niveaux de DDSCS. Trois oiseaux ont été choisis au hasard dans chaque groupe de traitement et abattus pour l'évaluation des carcasses et l'analyse histologique de l'intestin grêle, du rein, de la rate, du foie et du gros intestin. Il a été conclu que le DDSCS peut être inclus dans les régimes des poulets de grillage jusqu'à 12% sans aucun effet délétère sur les caractéristiques biochimiques hématologiques et sériques. L'alimentation du DDSCS aux volailles à 4, 8 et 12 % a entraîné des changements dans le foie, les reins, l'intestin grêle et les gros intestins.

Mots-clés: Hématologique, biochimique, histologie, manioc distillé sec avec soluble, poussins de poulets de grillage

Introduction

Despite the enormous improvement that has been achieved in the area of poultry production globally, it is still exigent to constantly push the boundary in sustainable poultry production as global population continues to grow exponentially. Increased human population and activity has not only put pressure on global food resources but also on the environment, which includes waste production and management. Therefore, research for alternative animal feed sources that is non-competitive with human feed, cost effective and environmentally sustainable for livestock production is a continuum. In recent times as Nigeria advocates the use of alternative and sustainable fuel source, it is important to proactively research into the potential use of the waste product that will result from this industry. Hence the need to research into alternative and sustainable poultry feed for ultimate performance, improved productivity and general carcass quality; it is essential to also consider the effects these unconventional feeds such as DDSCS – a waste product from the fermentation and distillation process of cassava in ethanol production will exert on the blood and serum characteristics of the animals (Mahmud *et al.*, 2016). Dry distilled product with soluble is obtained from the fermentation process of alcohol distillation,

from raw materials rich in carbohydrates and sugars such as molasses or juices of sugarcane, maize, beet, among others, and with the usage of yeasts (Trautwein *et al.*, 2008). Due to the fermentation process, the by-product such as vinasse is said to be high in organic acid which is reported to be a better substitute for antibiotics in improving growth performance and intestinal health morphology of birds (Mohamed *et al.*, 2014) this makes DDSCS a promising component of animal and poultry feed. Adenkola *et al.* (2011) reported that dietary constituents have measurable effects on blood components and blood constituents are widely used in nutritional evaluation and survey of animals. Blood biochemical analysis can assist the monitoring of poultry health, the diagnosis and treatment of diseases, and assess their health status (Schmidt *et al.*, 2007). This study therefore seeks to evaluate the effect of graded levels of DDSCS on haematology, biochemical and histological characteristics of broilers.

Materials and methods

Study area

The experiment was conducted at the poultry section of the Bioresources Development Centre (BIODEC), Ogbomoso. It lasted for four weeks (28 days). The DDSCS used in this study was obtained from Bio-ethanol Production plant

of National Biotechnology Development Agency (NABDA), located in BIODC, Onipanu, Ogbomoso, Nigeria.

Processing of DDCS

The samples were collected from production plant stored in tank using the outlet faucet (hose) of the storage tank. There were collected into jute bags; the water was allowed to drain out then sundried for two days. The processed samples were stored in a sack at room

temperature prior to proximate analysis by standard methods (AOAC, 1990).

Experimental diets

Four dietary treatments were formulated with 0, 4, 8 and 12% DDCS inclusion (Table 1). One hundred and forty-four (144), 5-weeks un-sexed *Arbor acre* strain broiler chicks were used. The birds were randomly allotted to four dietary treatments in a completely randomized design. Each treatment had 2 replicates of 18 birds each. The mean initial weight was 1.09g.

Table 1: Composition of experimental diets

Ingredients	0% DDCS	4% DDCS	8% DDCS	12% DDCS
Maize	54.75	54.75	54.75	54.75
Soybean meal	28.00	28.00	28.00	28.00
Wheat offal	12.00	8.00	4.00	-
DDCS	-	4.00	8.00	12.00
Bone Meal	3.00	3.00	3.00	3.00
Oyster shell	1.50	1.50	1.50	1.50
Salt	0.20	0.20	0.20	0.20
Vitamin Premix	0.25	0.25	0.25	0.25
Lysine	0.15	0.15	0.15	0.15
Methionine	0.15	0.15	0.15	0.15
Total	100.00	100.00	100.00	100.00
Calculated % CP	19.65	19.71	19.41	19.10
Metabolizable energy (Kcal/Kg)	2,874.22	2,789.42	2,714.62	2,639.82
Crude fibre (%)	3.73	4.97	6.17	7.38

Haematological and biochemical characteristics

At the end of the feeding trial, the birds were fasted for twelve (12) hours and the birds weighed and slaughtered by severing the jugular vein of four birds randomly selected from each treatment group to assess blood profile of the birds. The blood was then allowed to flow freely into labeled bijou bottles one of which contained a speck of EDTA while the others were without EDTA. The blood in the EDTA-containing bijou bottles was processed for haematology while those in bottles without EDTA were processed for biochemical analysis. The blood samples immediately after collection were taken to the laboratory in the Department of Chemical Pathology,

Ladoke Akintola University of Technology (LAUTECH) Teaching Hospital Ogbomoso for the determination of haematological and biochemical characteristics. Packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC) counts, Mean cell volume (MCV), Mean corpuscular haemoglobin concentration (MCHC) and Mean cell haemoglobin (MCH) were determined (Baker and Silverton, 1976). Blood samples for serum analysis after clotting, was centrifuged at 3,500 rpm in the laboratory using Gallenkamp laboratory centrifuge in order to obtain the serum. The centrifugation was carried out according to Mitruka and Rawnsley (1977) procedure. The serum obtained was used in

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determining creatinine, total protein, glucose, bilirubin, alkaline phosphatase, cholesterol, albumin and urea according to Randox procedure of chemical analysis (2010).

Histology

At the end of the 28 day experimental period the birds were fasted for a period of 12 hours, 3 birds each were randomly selected from each treatment group. The birds were de-feathered and eviscerated, and a section of representative tissue samples from small intestine, kidney, spleen, liver and large intestine were fixed in neutral – buffered 10% formalin and processed using standard paraffin – embedding histopathology techniques as described by Bancroft and Gamble (2008). The formalinized tissues were dehydrated in 4 grades of alcohol, cleared in 3 xylene grades and embedded in molten wax. The embedded tissues were then trimmed into blocks and 5µm tissue samples were sectioned from the blocks using a microtome. The sections were floated in a water bath and incubated at 60°C for 30 minutes. Staining was carried out by first clearing the section in 3 grades of xylene and rehydrating in 2 grades of alcohol. The sections were put into water and stained with hematoxylin for 15 minutes. Bluing was done with Ammonium chloride; differentiation in 1% acid alcohol was done before counterstaining with eosin. The stained slides were then mounted on glass slides using DPX (Distrene, Plasticiser and Xylene) a non-aqueous

permanent mountant.

Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) using SAS Statistical Package, SAS 2008. The means were separated using Duncan's multiple range test.

Results and discussion

The composition and chemical composition of experimental diets with different inclusion levels of DDGS are as shown in Table 1 and 2, respectively. The crude protein and crude fibre content increased with increase in the inclusion level of DDGS. The crude protein range was 25.81-26.25%. The results of the haematological parameters of broiler chickens fed different levels of DDGS are presented in Table 3. The analysis of haemological parameters showed that there were no significant ($P>0.05$) differences among the broilers fed experimental diets.

Haemological parameters are usually related to health status and are of diagnostic importance in clinical evaluation of the state of health. It serves as indicator of physiological, pathological and nutritional status of an animal (Okoruwa and Ikhimiya, 2014). It has been established that certain haematological parameters are associated with certain production traits in chickens, for example, high packed cell volume (PCV) and high haemoglobin (Hb) are indicators of high feed conversion ratio

Table 2: Chemical composition of diets fed to experimental broiler chickens

Parameter	0% DDGS	4% DDGS	8% DDGS	12% DDGS
Dry matter (DM)	93.18	92.94	93.49	93.33
<u>Analysis % of DM</u>				
Organic matter	88.85	92.61	91.04	89.56
Crude protein	25.81	26.25	26.25	26.25
Crude fibre	2.49	3.29	3.93	4.70
Ether extract	3.72	4.29	4.27	3.43
Ash	13.15	7.39	8.96	10.44
Nitrogen free extract	48.01	51.72	50.08	48.51

Table 3: Haematological parameters of broiler chickens fed graded levels of dry distilled cassava with soluble

Parameter	0% DDCCS	4% DDCCS	8% DDCCS	12% DDCCS	SEM	PROB
WBC (x 10 ⁹ /L)	109.98	106.23	105.98	101.50	2.44	0.74
RBC (x 10 ¹² /L)	2.33	2.14	2.11	2.23	0.04	0.14
PCV (%)	30.25	30.33	29.75	31.33	0.71	0.92
MCV (fl)	119.38	121.03	121.93	122.93	0.65	0.26
MCHC (g/dL)	7.28	7.40	7.98	7.03	0.28	0.71
MCH (pg)	8.95	8.00	10.64	8.80	0.43	0.14

^{a, b, c, d}: Means within each row with different superscript are significantly different ($p < 0.05$)

SEM = Standard error of mean, PROB = Probability

while high serum protein is an indicator of good feathering ability and tissue growth in chickens (Nyaulingo, 2013). Authors (Aletor *et al.*, 1989; Aletor and Egberongbe (1992) previous studies showed that blood variables that are most commonly affected by dietary treatments include packed cell volume (PCV) and red blood cell (RBC). Oyelola *et al.*, 2004 also reported that decreased RBC is usually associated with low quality feed and protein deficiency.

The WBC counts (x10⁹/L) from this study were 101.50, 105.98, 106.23 and 109.98 for 12% DDCCS, 8% DDCCS, 4% DDCCS and 0% DDCCS respectively. High WBC count is usually associated with microbial infection or the presence of a foreign body or antigen in the circulating system. The values obtained for WBC were within normal range for broilers reported by (Ahiwe *et al.*, 2014). The result showed that RBC counts range was 2.11-2.23 (x10¹²/L). The RBC range was in line with range 1.8-2.4 obtained by Zanu *et al.*, 2012 but lower than range of 3.08-3.20 reported by Owosibo *et al.*, 2013. The PCV range from this study is in line with PCV range of 25.67-31.00 and 20.00-32.00 reported by Ari and Ayanwale (2014) and Iyayi *et al.*, 2005 respectively. In this experiment, all the blood parameters fell within the normal range reported by Pampori (2003). The MCV (fl) obtained was lower than the range (128.0-135.3) reported by Adeyemo and Sani (2013) but in line with 92.44-122.09 reported by Akinola and

Ethuk (2015). The non-significant differences ($P > 0.05$) in the values of the haematological parameters compare to the values observed for the control shows that there is no deleterious effect of the inclusion of DDCCS at different levels on the serum parameters. Serum biochemistry is a liable biochemical system which can reflect the condition of the organism and the changes happening to it under influence of internal and external factors (Toghyani *et al.*, 2010). The results of the serum metabolites of broiler chicken fed graded levels of DDCCS are shown in Table 4.

There were no significant ($P > 0.05$) differences in the serum metabolites of broiler chickens fed different levels of DDCCS ($P > 0.05$). Serum proteins are mainly synthesized in the liver and plays important functions such as maintain blood volume through the colloidal osmotic effect, buffer blood pH, transport hormones and drugs, participate in cell coagulation and in the body defense against foreign agents, catalyze chemical reactions (enzymes) and regulate the metabolism (hormones) (Melillo, 2013). According to Harr *et al.* (2002), serum total protein values of broilers tend to be lower than those of mammals, ranging from 25.00 to 45.00 g/L. Melillo, 2013 reported that the main functions of albumin are the transport of several molecules and the maintenance of blood oncotic pressure. The values of albumin from this study was within the

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Table 4 : Serum metabolites of broiler chickens fed graded levels of dry distilled cassava with soluble

Parameter	0% DDCS	4% DDCS	8% DDCS	12% DDCS	SEM	PROB
Total protein (g/l)	34.50	38.33	33.25	30.00	1.31	0.28
Albumin (g/l)	9.28	10.70	10.93	13.13	0.65	0.24
Globulin (g/l)	25.23	27.63	22.33	16.90	1.43	0.10
Creatinine (umol/l)	46.25	71.33	61.00	51.00	5.39	0.42
AST (IU/L)	87.25	87.00	92.75	84.33	2.25	0.65
ALT (IU/L)	1.83	2.57	1.88	2.73	0.37	0.80
Cholesterol (mmol/L)	2.40	2.63	2.30	2.83	0.09	0.15
Triglyceride (mmol/L)	0.43	0.27	0.43	0.23	0.04	0.26
HDL (mmol/L)	1.80	1.87	1.75	2.10	0.06	0.23
LDL (mmol/L)	0.41	0.67	0.35	0.63	0.07	0.24
Uric acid (mmol/L)	0.48	0.38	0.46	0.35	0.03	0.47

^{a, b, c, d}: Means within each row with different superscript are significantly different (p<0.05)

SEM = Standard error of mean, PROB = Probability

range values of 11.80 to 15.60 g/L for female broilers and 8.70 to 12.50 g/L for male broilers reported by Rezende *et al.* (2017) and normal albumin values for broilers reported by Meluzzi *et al.* (1992). AST values were between 84.33 and 92.75 IU/L. The values of AST were greater than the range of 13.72-15.65 reported by Akinola and Etuk, 2015. The non-significant differences (P>0.05) in the values of the serum parameters compare to the values observed for the control shows that there is no deleterious effect of the different inclusion of DDCS at different levels on the serum parameters. The study of different enzyme activities such as ALT, AST and total protein have been found to be of great value in the assessment of clinical and experimental liver damage (Olawale, 2019). High levels of ALT indicates liver injury while the rise in the AST is usually accompanied by an elevation in the levels of ALT, which plays a vital role in the conversion of amino acids to keto acids (Hoffbrand and Pettit, 1997; Dash *et al.*, 2007). The uric acid values of 0.38-0.46 mmol/L for broilers obtained from this study is in line with the range between 0.12 and 0.89 mmol/L reported by Benez (2004).

The cholesterol range of 2.30-2.83 mmol/L from this study was within the cholesterol values of 2.63-3.48 mmol/L reported by Abdulazeez *et al.*, 2016 for broilers. Triglycerides are synthesized in the intestinal mucosa and in the liver from the digestion of dietary components and the absorption of fatty acid (Rezende *et al.*, 2017). Triglyceride range (0.23-0.43 mmol/L) from this study was less than 1.54-1.88 mmol/L reported by Evans *et al.* (1977) for broilers fed diet with around 3,000 Kcal metabolizable energy/kg but lower than 0.23-0.28 mmol/L obtained by Osorio *et al.* (2012) for broilers fed diet containing 18% crude protein and 3150 Kcal metabolizable energy/kg. Cholesterol travels in the blood as particles called lipoprotein is an important constituent of cell membranes modulating their fluidity; it is a precursor of steroid hormones (Kasprzak and Hetmański, 2004). Total Cholesterol is made up of low-density Lipoproteins (LDL), high-density Lipoprotein (HDL) and very low density Lipoprotein (VLDL). There are two known facts that Cholesterol must be in the rancid form to cause the arterial plaques that lead to partial blockage of the blood vessels while some forms of

Cholesterol are beneficial to man. High density Lipoprotein (HDL) has been established to protect against heart disease by mopping up circulating Cholesterol units in the system (Narahari, 2003). The culprit responsible for this oxidized form that narrows or “hardens” the arteries is the low-density Lipoprotein Cholesterol (LDL) and low levels of the HDL fraction (Kasprzak and Hetmański, 2004). Table 5 shows the histopathological changes observed in selected organs of broiler chickens fed with graded levels of DDGS. There were no changes observed in the spleen of the experimental birds. The result shows that

there were histological changes in the small intestine, liver, kidney and large intestine of birds fed 4%, 8% and 12% DDGS. This is probably caused by the inclusion of DDGS in the experimental diet. The histopathological changes observed indicate that incorporation of DDGS in broiler feed could cause adverse effects associated with changes in the small intestine, kidney, liver and large intestine. Contrary to report by Mohamed *et al.* (2014) that organic acids improved the intestinal morphology of birds, the high organic acid content in DDGS did not improve the intestinal morphology of the experimental birds in this study.

Table 5: Histopathological changes in selected organs of broiler chickens feed with graded levels of DDGS

Tissue	0% DDGS	4% DDGS	8% DDGS	12% DDGS
<i>Small intestine</i>	No visible lesion	Matting, loss of villi and hyperplasia of the mucosal gland in the lamina propria	Matting, clubbing and sloughing off of the villi	Loss of villi and lymphoid hyperplasia in the lamina propria
<i>Kidney</i>	No visible lesion	Tubular epithelia cell swelling and degeneration	Tubular epithelia cell swelling and degeneration	Renal congestion and tubular degeneration,
<i>Spleen</i>	No visible lesion	No visible lesion	No visible lesion	No visible lesion
<i>Liver</i>	No visible lesion	Hepatic congestion and degeneration	Hepatic congestion and degeneration	Hepatic congestion and degeneration
<i>Large intestine</i>	No visible lesion	Goblet cell hyperplasia	Goblet cell hyperplasia and edema in the submucosa	Loss of crypt and sloughing off of epithelia cells

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Plates 1-4 below shows the histopathologic changes in the small intestine, kidney, liver and large intestine.

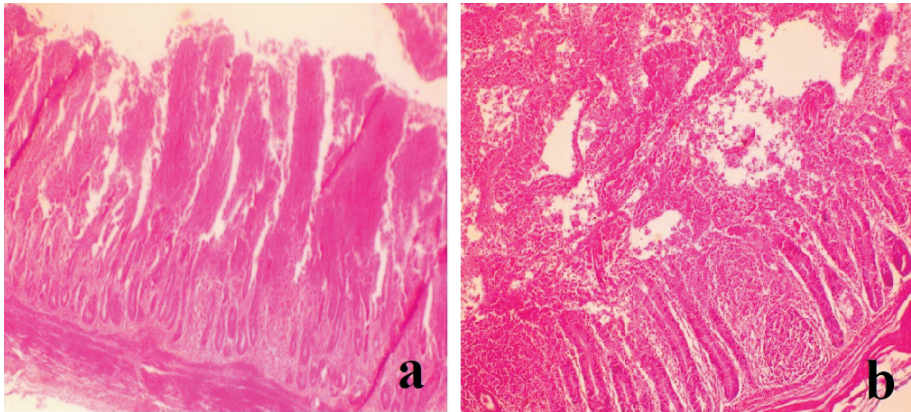


Plate 1a and b: Matting, loss of villi and hyperplasia of the mucosal gland in small intestine of birds fed 4 to 12% DDCS

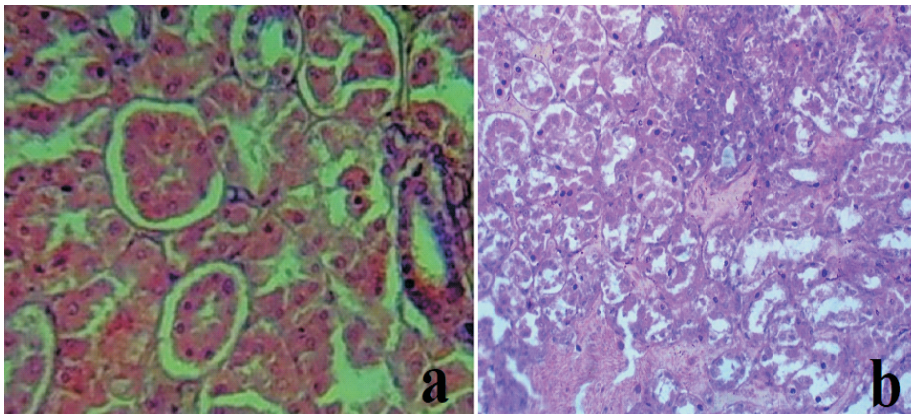


Plate 2a and b: Epithelial cell swelling and congestion of the renal tubules in birds fed 4 to 12% DDCS

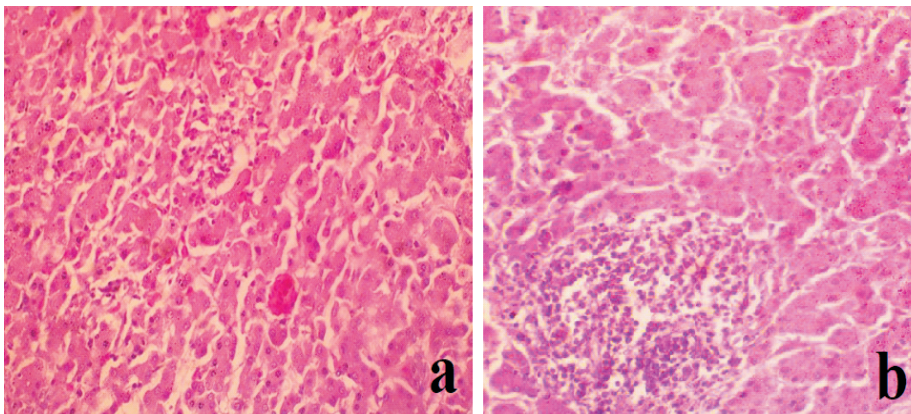


Plate 3a and b: Hepatic degeneration and necrosis in birds fed 4 to 12% DDCS

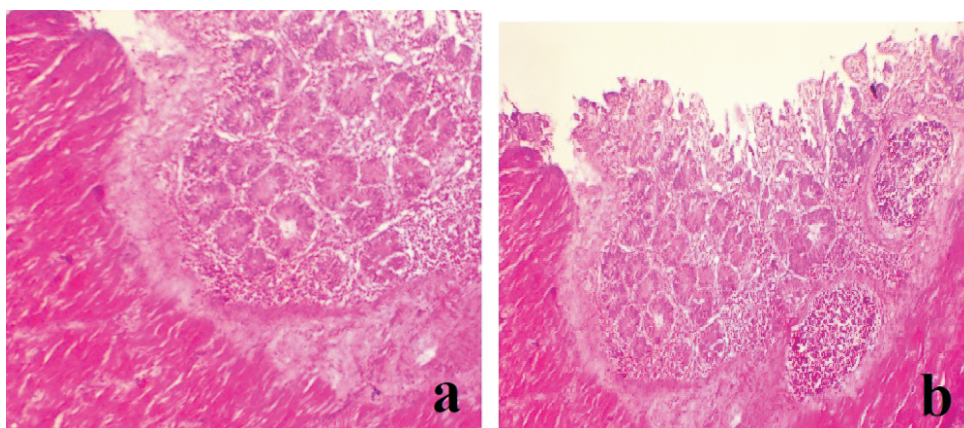


Plate 4a and b: Hyperplasia of goblet cell mucosal gland and loss of crypt in large intestine of birds fed 4 to 12% DDCCS

Conclusion

From the results it can be concluded that DDCCS can be included in the diet of broiler chickens up to 12% without any deleterious effect on the haematological and serum biochemical characteristics. Inclusion of DDCCS in the diet of broiler chickens up to 4% can result in histological changes in the liver, kidney, small intestine and large intestine of the broilers which can be due to increase in crude fibre level in the diets as the inclusion of DDCCS increases. Further study is however, required to ascertain the mechanism by which increased DDCCS in feed causes the lesions as observed in the histology of the broilers.

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