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THE EFFECTS OF REPLACEMENT LEVELS OF BOILED AND FERMENTED CASTOR SEED MEAL ON THE HAEMATOLOGY, SERUM BIOCHEMISTRY AND HISTOPATHOLOGY OF BROILER CHICKENS

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ABSTRACT
The objective of the study is to determine the effects of boiled and fermented castor seed meal on the haematology, serum biochemistry and histopathology of broilers chickens. A total of two hundred and twenty-five (225) day-old mixed sexed broilers were fed *ad libitum* with starter (0- 4 weeks) and finisher (5-9weeks) diets containing graded levels of boiled and fermented castor seed meal, which replaced 0 (control), 5, 10, 15 and 20 % of groundnut cake and soya bean meal in the respective diets. At the end of the experiments, blood samples were collected from 3 birds per treatment for the determination of haematological and serum biochemical parameters. Samples of the liver, lungs small intestine, kidney, heart and the spleen were collected from the slaughtered birds and immediately preserved in 10 % formalin, labelled and prepared for histopathological presentation. The packed cell volume (PCV) of the birds on the control (0) and 5% BFCSM were significantly ($P< 0.05$) higher than those on 10, 15 and 20% BFCSM. Alanine Amino transferase (ALAT) level of the birds fed on 10% BFCSM diet was significantly ($P < 0.05$) higher than the birds fed the control (0%) and 5 % BFCSM diets, but did not differ from the birds fed 15 and 20% BFCSM diets. Histopathological findings also revealed that there were adverse effect of the BFCSM on the liver, lungs, intestine, kidney, intestine, heart and spleen and the intensity of these effects increased with increase in the replacement levels of BFCSM from 5 to 20 % in the diets. It was concluded that boiled and fermented castor seed meal has adverse effect on the haematology, serum biochemistry and histopathology of broilers chickens.

KEYWORDS: Haematology, serum biochemistry, histopathology, boiling and fermentation castor seed meal.

INTRODUCTION

Poultry nutritionist have tried to harness and utilize non-conventional feeds that are not directly use by man because high cost of conventional feeds is one of the major factors militating against increase poultry/ livestock production in Nigeria and most developing countries. Some studies aimed at finding alternative energy sources in poultry diets include the use of guinea corn (Ali *et al*., 1975), cassava tubers (Igwebuike, 1988), maize offal and wheat offal (Nuhu *et al*.,2008), *Faiherbia albida* pods, (Garba *et al.,* 2008) and mango kernel (Diarra *et al.,* 2008). Some items used as sources of protein include *Acacia sieberiana* seeds (Mustapha and Oguntona, 1990), Velvet beans(*Mucunaspp*) (Carew and Gernat, 2005), cotton seed cake (Choct, 2006), Palm kernel extraction (Fafiolu *et al*., 2008) and Sorrel seeds(Kwari *et al.,* 2008) amongst others. Another such protein non-conventional alternative feed is castor seed meal. Literature available revealed that attempts have been made for the use of castor seed meal as an alternative source of protein for livestock and poultry, but a major limitation identified is the presence of anti-nutritional factors in the meal. The meal is obtained from the seed after the extraction of the oil from the seed. Castor seed grows in the wild all over Nigeria from the coastal area to the northern fringes (Raw Materials Research and Development Council (RMRDC),1996). Due to the economic importance of castor seed, the crop is gaining popularity and now it is an emerging crop.

The objectives of the study is to determine the effects of replacement levels of boiled and fermented castor seed meal on the haematology, serum biochemistry and histopathology of broilers chickens. Church *et al.* (1984) revealed that evaluation of blood profile of animals may give some insight as to the potentials of a dietary treatment to meet the metabolic needs of the animals. Similarly, Oyawuye *et al.* (1998) revealed that haematological indices like packed cell volume (PCV) haemoglobin concentration, leucocytes count and mean corpuscular volume are valuable in monitoring feed toxicity especially with feed constituents that affects the formation of blood. The physiological effects of dietary influence on animals are best judge by chemical constituent of the feed, performance of the animal and more closely by the cellular changes in the organs (Akande *et al.,* 2012).

MATERIALS & METHODS

Sources and processing of castor seed meal (The same and adopted from Mustapha *et al.,* 2015)

Large seeded castor seeds for the experiment were locally sourced in Damaturu main market, Yobe State, Nigeria. These seeds were multiplied for the research. The castor seeds were boiled at the temperature of 100^{o}C for 30 minutes and later sun-dried for three (3) days which was

aimed at detoxifying the meal. The meals were obtained after the extraction of the oil manually from the seeds.

Experimental birds and their management (The same and adopted from Mustapha *et al.,* 2015)

A total of two hundred and twenty-five (225) day-old mixed sexed Anak breed of broilers purchased from ECWA Farm, Jos, were used for the study. From day-old to 4 weeks of age, all the birds were brooded using kerosene lantern and stove to provide necessary brooding temperature. During the brooding period (0-4 weeks), birds were fed with broiler starter containing graded levels of boiled and fermented castor seed meal, which replaced 0 (control), 5, 10, 15 and 20 % of groundnut cake and soya bean meal in the respective diets. Water and the experimental diets were provided *ad libitum*. All the birds were vaccinated against Gumboro disease at 2 weeks and 5 weeks of age, while the vaccination against New castle disease was at 3 and 6 weeks of age. From 5 to 9 weeks of age the feeding continued with broiler finisher diets containing the respective graded levels of the BFCSM. Daily washing and cleaning of drinkers was undertaken throughout the experiment. There were five (5) experimental groups/treatments. Each group consisted of 45 birds with three (3) replicates of 15 chickens per replicate.

Experimental diets (The same and adopted from Mustapha *et al.,* 2015)

Five (5) experimental diets were formulated consisting of the test material – (boiled and fermented castor seed meal), which replaced 0 (control), 5, 10, 15 and 20 % of groundnut cake and soya bean meal in the diets. All the diets have similar crude protein (23 - 24 % for starter's and 19 - 20 % for finishers) and energy (3000 Kcal/kg) levels which conformed to the recommended levels for normal growth of broilers at both starter and finisher phases. The calculated composition of the broiler starter and finisher diets are presented in Tables 1 and 2.

Table 1: Ingredients composition (%) and calculated analysis of broiler starter diets (The same and adopted from Mustapha *et al.*,2015)

Levels of replacement of boiled and fermented castor seed meal (%)					
Ingredients	0	5	10	15	20
Maize	53.50	53.50	53.50	53.50	53.50
Wheat offal	9.00	9.00	9.00	9.00	9.00
Castor seed mea	l0.00	5.00	10.00	15.00	20.00
Groundnut cake	13.00	10.85	9.85	10.00	5.00
Soya bean mel	12.85	10.00	6.00	0.85	0.85
Fish meal	7.00	7.00	7.00	7.00	7.00
Blood meal	2.00	2.00	2.00	2.00	2.00
Bone meal	2.00	2.00	2.00	2.00	2.00
Salt(NaCl)	0.20	0.20	0.20	0.20	0.20
*Premix	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00	100.00
Calculated Analysis					
ME (Kcal/kg)	2933.77	2914.96	2894.31	2870.46	2856.21
Crude protein (%)	24.05	23.97	23.61	23.44	23.11
Crude fibre (%)	3.22	3.98	4.74	5.49	6.25
Calcium (%)	1.25	1.27	1.29	1.31	1.33
Phosphorus (%)	0.69	0.71	0.72	0.72	0.76
Lysine (%)	1.22	1.26	1.27	1.28	1.34
Methionine (%)	0.40	0.42	0.44	0.45	0.47

** Bio-mix starter, manufactured by Bio- organics Nutrients System Ltd, Lagos, supplied/kg: Vit A = 10,000,000.00 IU; Vit D_3 = 2,000,000.00 IU; Vit E = 23,000.00mg; Vit K_3 = 2,000.00mg; Vit B_1 = =1,800.00mg; Vit B_2 5,000.00mg; Niacin = 27,500mg; Pantothenic Acid = 7,500.00mg; Vit B_6 = 3,000.00mg; Vit B_{12} = 15.00mg; Folic Acid = 750.00mg; Biotin H_2 = 60.00mg; Choline Chloride = 300,000.00mg; Cobalt = 200.00mg; Copper = 3,000.00mg; Iodine = 1,000.00mg; Iron = 20,000.00mg; Manganese = 40,000.00mg; Selenium = 200.00mg; Zinc = 30,000.00mg; Antioxidant = 1,250.00mg.ME = Meatabolizable energy*.

Table 2: Ingredients composition (%) and calculated analysis of the experimental broiler finisher diets (The same and adopted from Mustapha *et al.*,2015)

Levels of replacement of boiled and fermented castor seed meal (%)					
Ingredients	0	5	10	15	20
Maize	61.00	61.00	61.00	61.00	61.00
Wheat offal	10.00	10.00	10.00	10.00	10.00
Castor seed meal	l0.00	5.00	10.00	15.00	20.00
Groundnut cake	10.85	9.85	9.85	5.85	1.00
Soya bean meal	11.00	7.00	2.00	1.00	0.85
Fish meal	2.50	2.50	2.50	2.50	2.50
Blood meal	2.00	2.00	2.00	2.00	2.00
Bone meal	2.00	2.00	2.00	2.00	2.00
Salt(NaCl)	0.20	0.20	0.20	0.20	0.20
*Premix	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00	100.00
Calculated Analysis					

ME (Kcal/kg)	2976.61	2955.93	2933.71	2917.86	2902.01
Crude protein (%)	20.30	20.11	19.93	19.64	19.31
Crude fibre (%)	3.21	3.97	4.73	7.09	6.24
Calcium (%)	0.95	0.96	0.99	0.89	1.04
Phosphorus (%)	0.53	0.51	0.55	0.65	0.59
Lysine (%)	0.95	0.85	0.91	0.92	1.02
Methionine(%)	0.32	0.33	0.35	0.44	0.39

** Bio-mix finisher, manufactured by Bio- organics Nutrients System Ltd, Lagos, supplied/kg: Vit A = 8,500,000.00 IU; Vit D_3 = 1,500,000.00 IU; Vit E = 10,000.00mg; Vit K_3 = 1,500.00mg; Vit B_1 = =1,600.00mg; Vit B_2 4,000.00mg; Niacin = 20,000mg; Pantothenic Acid = 5,000.00mg; Vit B_6 = 1,500.00mg; Vit B_{12} = 10.00mg; Folic Acid = 500.00mg; Biotin H_2 = 750.00mg; Choline Chloride = 175,000.00mg; Cobalt = 200.00mg; Copper = 3,000.00mg; Iodine = 1,000.00mg; Iron = 20,000.00mg; Manganese = 40,000.00mg; Selenium = 200.00mg; Zinc = 30,000.00mg; Antioxidant = 1,250.00mg. ME = Mtabolizable energy*.

Determination of blood parameters

(i) Haematological indices

At the end of the experiments, blood samples were collected from 3 birds per treatment for the determination of haematological and serum biochemical parameters. The birds were randomly picked, fasted overnight and bled early in the morning (7.00am) to avoid excessive bleeding. Fasting the birds is to avoid temporary elevation of blood metabolites by feeding as observed by Bush (1975). The blood samples were collected from the brachial vein using disposable syringes and needle (21 gauges). The blood samples were poured into sample bottle containing an anticoagulant, ethylene diamine tetra acetic acid (EDTA).

The pack cell volume (PCV) was determined using Wintrobe's micro- haematocrit method .The haemoglobin (Hb) concentration was calculated by haemoglobin-colorimeter reading of standard (Drabkin's solution) with unit in grammes per decilitre (g/dl). Neubauer counting chamber was used for both Red blood corpuscle (RBC) and white blood corpuscle (WBC) counts in accordance with Brown (1976).Mean corpuscular volume (MCV) and Mean corpuscular haemogloblin(MCH) were calculated by the formula given by Swenson (1970). The Mean corpuscular haemoglobin concentration (MCHC) was calculated as:

$$\text{MCHC} = \frac{\text{Haemoglobin}}{\text{PCV \%}} x\ 100$$

(ii) **Serum biochemical indices**

The blood serum glucose was estimated by orthololuidine method as described in WHO (1980). The total serum protein and serum albumin was determined by Buiret reactions (Bush, 1975). The total serum protein was first estimated and then performing fractionation on further volume of the sample to precipitate and remove globulin and this leaves only albumin in solution. The serum urea estimation was carried out by the diacetylmonoxine method (WHO, 1980).Serum calcium was determined by the use of Eagle Diagnostic calcium kit (Cat. No 2400) which included calcium colour reagent, calcium base reagent and calcium calibrator.

(iii) **Histopathological examination**

At the end of the experiment, three (3) chickens were slaughtered from each treatment. Samples of the liver, lungs, small intestine, kidney, heart and the spleen were collected from the slaughtered birds and immediately preserved in 10 % formalin and labelled properly. They were later prepared for histopathological presentation using routine histopathological methods and haemataxylin -eosin (Hand E) staining techniques as described by Junqueira and Carneiro (1980).

RESULTS

Haematological indices

The haematological indices of broilers chicken fed graded levels of boiled and fermented castor seed meal (BFCSM) are presented in Table 3.The packed cell volume(PCV) of the birds on the control (0) and 5% BFCSM were significantly($P < 0.05$) higher than those on 10, 15 and 20% BFCSM. The RBC count and lymphocytes % of the birds on 10% BFCSM diets were significantly ($P < 0.05$) higher than those on 15 and 20% BFCSM diet with those on 20% BFCSM diet recording the lowest values, but did not differ from those fed on 0 and 5 % BFCSM diets. For haemoglobin (Hb) concentration, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBC) count, basophils, eosinophils and monocytes, the birds on 20% BFCSM showed significantly ($P < 0.05$) higher values than those on 0 and 5% BFCSM diets. The birds on 20% BFCSM diet also recorded a significantly ($P < 0.05$) higher value of heterophils than the other treatment groups.

Serum biochemical indices

The serum biochemical indices of broiler chicken fed graded levels of boiled and fermented castor seed meal (BFCSM) are presented in Table 4. The total protein values of the birds on 20 % BFCSM diet was significantly ($P < 0.05$) higher than those on 5 and 15 % BFCSM but, not significantly ($P > 0.05$) different from those on 0 and 10 % BFCSM. The values of the total protein ranged 21.67–35.67 g/dl. The total cholesterol also showed significant ($P>0.05$) differences among the various treatment groups. The birds on the control (0%) and 20% BFCSM diet were significantly ($P<0.05$) higher than the birds on 5, 10 and 15% BFCSM diets. The glucose level of the birds fed 10% BFCSM diet was significantly ($P<0.05$) higher than those on control (0%) and 5% BFCSM, but did not differ from the birds on 15 and 20% BFCSM diets. The birds fed on 15% BFCSM diet showed significantly ($P<0.05$) higher bicarbonate levels than those on control (0) and 5% BFCSM, but did not differ from the birds on 10 and 20% BFCSM diets. The sodium level of the birds fed 20% BFCSM diet was significantly ($P < 0.05$) higher than the birds on control, but did not differ from the birds fed 5, 10 and 15% BFCSM diets. Alanine Amino transferase (ALAT) level of the birds fed on 10% BFCSM diet was significantly ($P < 0.05$) higher than the birds fed the control (0%) and 5 % BFCSM diets, but did not differ from the birds fed 15 and 20% BFCSM diets.

Table 3: Haematological indices of broilers fed graded levels of boiled and fermented castor seed meals

	Replacement levels of boiled and fermented castor seed meal (%)					
Parameters	0	5	10	15	20	SEM
Packed Cell Volume (PCV) %	29.00^{a}	29.67^{a}	25.33^{b}	25.67^{b}	25.33^{b}	0.77^{*}
Red Blood Cells (RBC) Count $x10^{6}/mm^{3}$	3.60^{ab}	3.58^{ab}	3.68^{a}	3.38^{bc}	3.13^{c}	0.12^{*}
Haemoglobin (Hb) Concentration (g/dl)	9.60^{b}	9.50^{b}	8.60^{c}	8.80^{c}	10.17^{a}	0.13^{*}
White blood Cells (WBC) Count $10^{3}/mm^{3}$	4.28^{c}	4.25^{c}	4.55^{b}	4.70^{a}	4.82^{a}	0.07^{*}
Mean Corpuscular Volume (MCV) (fl)	8.06^{ab}	7.96^{a}	6.88^{b}	7.59^{a}	8.09^{a}	0.24^{*}
Mean Corpuscular HaemoglobinMCH)(Pg)	26.70^{b}	25.52^{b}	23.3^{c}	26.06^{b}	32.46^{a}	0.80^{*}
Mean Corpuscular Haemoglobin Concentration (MCHC) (%)	33.13^{b}	32.03^{c}	34.00^{b}	34.30^{b}	40.16^{a}	0.78^{*}
Differential Counts						
Heterophils (%)	33.33^{bc}	34.67^{ab}	31.33^{d}	32.00^{cd}	36.33^{a}	0.84^{*}
Monocytes (%)	8.33^{a}	8.33^{b}	8.35^{b}	9.69^{a}	10.00^{c}	0.62^{*}
Eosinophils(%)	6.67^{b}	6.67^{b}	6.67^{b}	8.67^{a}	9.00^{a}	0.65^{*}
Basophils(%)	0.00^{b}	0.33^{b}	0.33^{b}	0.67^{b}	1.67^{a}	0.30^{*}
Lymphocytes (%)	51.67^{ab}	50.00^{ab}	53.33^{a}	49.00^{b}	43.00^{c}	1.59^{*}

* = Significant (P < 0.05): SEM = Standard Error of Mean; a, b, c, d = Means within the same row bearing different superscripts differ significantly (P < 0.0

Table 4: Serum biochemical indices of broilers fed graded levels of boiled and fermented castor seed meal

	Replacement levels of boiled and fermented castor seed meal (%)					
Parameters	0	5	10	15	20	SEM
Total Protein (g/dl)	32.00^{ab}	29.33^{b}	33.33^{ab}	21.67^{c}	35.67^{a}	1.74^{*}
Albumin (g/dl)	14.33	12.67	15.00	13.67	11.23	2.98^{NS}
Globulin (g/dl)	17.67^{a}	16.67^{a}	18.33^{a}	8.00^{b}	19.33^{a}	1.70^{*}
Glucose (g/dl)	11.57^{c}	14.27^{b}	15.97^{a}	14.80^{ab}	13.80^{b}	0.58^{*}
Creatinine (mmol/L)	58.33	58.00	58.00	59.33	67.33	6.58^{NS}
Cholesterol (mmol/L)	2.63^{a}	2.27^{b}	2.20^{b}	2.23^{b}	2.73^{a}	0.14^{*}
Urea (mmol/L)	4.46	4.36	4.43	4.43	4.56	0.11^{NS}
Calcium (mmol/L)	2.40	2.30	2.37	2.43	2.43	0.08^{NS}
Phosphate ($P0_4$)(mmol/L)	1.67^{b}	1.70^{b}	1.77^{b}	1.46^{c}	1.97^{a}	0.06^{*}
Sodium(Na) (mmol/L)	146.33^{b}	148.00^{ab}	150.07^{ab}	148.67^{ab}	154.33^{a}	3.25^{*}
Potassium (K) (mmol/L)	4.47	3.83	4.50	4.67	4.37	0.37^{NS}
Chloride(C l) (mmol/L)	118.67	122.00	128.00	124.67	126.33	4.14^{NS}
Bicarbonate ($HC0_3$) (mmol/L)	20.33^{b}	21.00^{b}	22.00^{ab}	24.33^{a}	22.33^{ab}	1.04^{*}
Alkaline Phosphate(IU/L)	182.33	189.67	189.33	183.33	181.00	6.27^{NS}
Aspartate Amino Transferase (ASAT) (IU)	189.33	176.33	188.69	192.00	184.07	7.52^{NS}
Alanine Amino Transferase (ALAT) mmol/L	9.00^{c}	11.33^{bc}	15.67^{a}	14.00^{ab}	14.00^{ab}	1.37^{*}

a, b, c = Means within the same row bearing different superscripts differ significantly(P < 0.05); * = Significant (P < 0.05); NS = Not significant (P > 0.0 5); SEM = Standard Error of Mean.

Histopathological findings

(a) Gross pathological lesions

The summary of gross pathological lesions in the liver, intestine, heart and spleen of broiler chickens fed graded levels of boiled and fermented castor seed meal (BFCSM) is presented in Table 5. The adverse effect of the BFCSM on these organs increased with increase in the replacement levels of the BFCSM from 5 to 20 % in the diets. For instance, the pale and friable appearance of the liver in the birds fed 20 % BFCSM was abnormal.

(b) Histopathological examination

The results of the histopathological examination of the liver, lungs, intestine, kidney, heart and spleen of broilers fed graded levels of BFCSM are presented in Plates 1 – 6. The summary of the histopathological lesions in these organs is also presented in Table 6.

The cross section of the liver of the birds fed control (0 % BFCSM) was normal. There was mild congestion of the central vein in the liver of the birds fed 5 % BFCSM. However, the degree of the congestion of the central vein with mononuclear infiltration in the sinusoid and necrosis of the hepatocytes increased with increase in the replacement levels of BFCSM in the diet (Plate 1).

The lungs of the birds fed the control (0 % BFCSM) diet were normal. The birds fed on 5 % BFCSM also show congestion of the lungs with mild mononuclear infiltration. The intensity of this degeneration in morphology increased in birds fed 15 and 20 % BFCSM. This led to collapse of para- bronchus with sloughing of epithelium of the air vesicle and mononuclear infiltration (Plate 2). The cross-section of the intestine of the birds fed on the control (0 % BFCSM) diet showed normal arrangement of the villi. However, the cross-section of the intestine of the birds fed on 5 % BFCSM showed disorientation of the villi with mild mononuclear infiltration in the muscularis mucosa. The intensity of these effects increased in the birds with increase in the replacement levels of the BFCSM in the diets (Plate 3).Sloughing of the villi with necrosis of Brunner's gland were noticed on the birds fed on 20 % BFCSM.

The cross- section of the kidney of birds fed the control diet (0 % BFCSM) was normal showing glomeruli and tubules. The cross- section of the birds fed 5 % BFCSM diet showed occluded Bowman's space. This transformed into tubular degeneration, necrosis with heterophilic infiltration in the renal interstitium in birds fed on 15 % BFCSM. The kidney of the birds fed on 20 % BFCSM

showed marked tubular necrosis with heterophilic infiltration (Plate 4).

The cross- section of the heart of the birds fed on the control diet (0 % BFCSM) showed normal orientation of fibre. The birds fed on 5 % BFCSM showed multi-focal areas of haemorrhages within the fibre and those on 10 % BFCSM showed mild necrosis of myocytes. The intensity of these effects increased from moderate necrosis of myocytes with heterophil infiltration to severe necrosis of myocytes with marked heterophil infiltration in birds fed 15 and 20 % BFCSM, respectively (Plate 5). The spleens of the birds fed on the control diet (0 % BFCSM) were normal. The birds fed on 10 % BFCSM showed mild lymphocytes depletion in the white pulp. The degree of this effect transformed from white pulp hypoplasia to splenic follicles undergoing possible regeneration of the white pulp (Plate 6).

Table 5: Summary of gross pathological lesions in selected organs of chicken fed graded levels of boiled and fermented castor seed meal

Experimental diets	Gross pathological lesions
T1(0 % BFCSM)	The liver showed normal. The intestine also showed normal arrangement of the villi; the heart showed normal arrangement of fibre; the spleen was normal.
T2 (5% BFCSM)	The liver had pale borders. There were multifocal petechiae on the jejunum and ileum; multifocal ecchymotichaemorrhages were seen in the endocardium; the spleen was slightly enlarged.
T3 (10% BFCSM)	The liver had pale borders. There were multifocal petechiae on the jejunum and ileum. Multifocal ecchymotichaemorrhages were seen in the endocardium.
T4 (15% BFCSM)	The liver was friable. There were multifocal petechiae on the duodenum, jejunum and ileum. Multifocal ecchymotichaemorrhages were seen in the endocardium. There were areas of ecchymotichaemorrhages on the thighs.
T5 (20% BFCSM)	The liver was pale and friable. There were multifocal petechiae along the whole intestinal length. Multifocal ecchymotichaemorrhages were seen in the epicardium and endocardium. The spleen was contracted and small.

Table 6: Summary of histopathological lesions in selected organs of broiler chickens fed graded levels of boiled and fermented castor seed meal

Experimental Groups	Organs/ Lesions	Congestion	Hemorrhage	Mononuclear Infiltration	Polymorh Infiltration	Necrosis	Degeneration	Hypoplasia	Hyperplasia
T1 (0 % BFCSM)	Heart	-	-	-	-	-	-	-	-
	Liver	-	-	-	-	-	-	-	-
	Kidney	-	-	-	-	-	-	-	-
	Lungs	-	-	-	-	-	-	-	-
	Spleen	-	-	-	-	-	-	-	-
	Intestine	-	-	-	-	-	-	-	-
T2 (5% BFCSM)	Heart	+	+	-	-	-	+	-	-
	Liver	+	-	++	-	+	+	-	-
	Kidney	+	-	-	-	-	-	-	-
	Lung	++	-	+	-	-	+	-	-
	Spleen	++	-	+	-	-	+	+	-
	Intestine	+	-	+	-	-	+	-	-
T3 (10 BFCSM	Heart	+	++	-	-	+	+	-	-
	Liver	++	-	++	-	+	+	-	-
	Kidney	+	-	+	+	+	+	-	-
	Lung	++	+	+	-	-	+	-	-
	Spleen	++	-	+	-	-	+	++	-
	Intestine	+	-	+	+	+	+	-	-
T4 (15% BFCSM	Heart	+	+	+	+	+	++	-	-
	Liver	++	-	++	+	++	++	-	-
	Kidney	+	-	++	+++	+++	+++	-	-
	Lung	++	-	+	-	-	+	-	-
	Spleen	+	-	+	-	+	+	++	-
	Intestine	+	-	++	++	++	++	-	-
T5 (20% BFCSM	Heart	+	+	++	+++	+++	+++	-	-
	Liver	++	-	++	+	++	++	-	-
	Kidney	++	-	++	+++	+++	+++	-	-
	Lung	++	+	++	+	+	++	-	-
	Spleen	++	-	+	-	+	+	+	++
	Intestine	+	-	++	++	++	++	-	-

=Negative, + Mild, ++ Moderate, +++ Severe

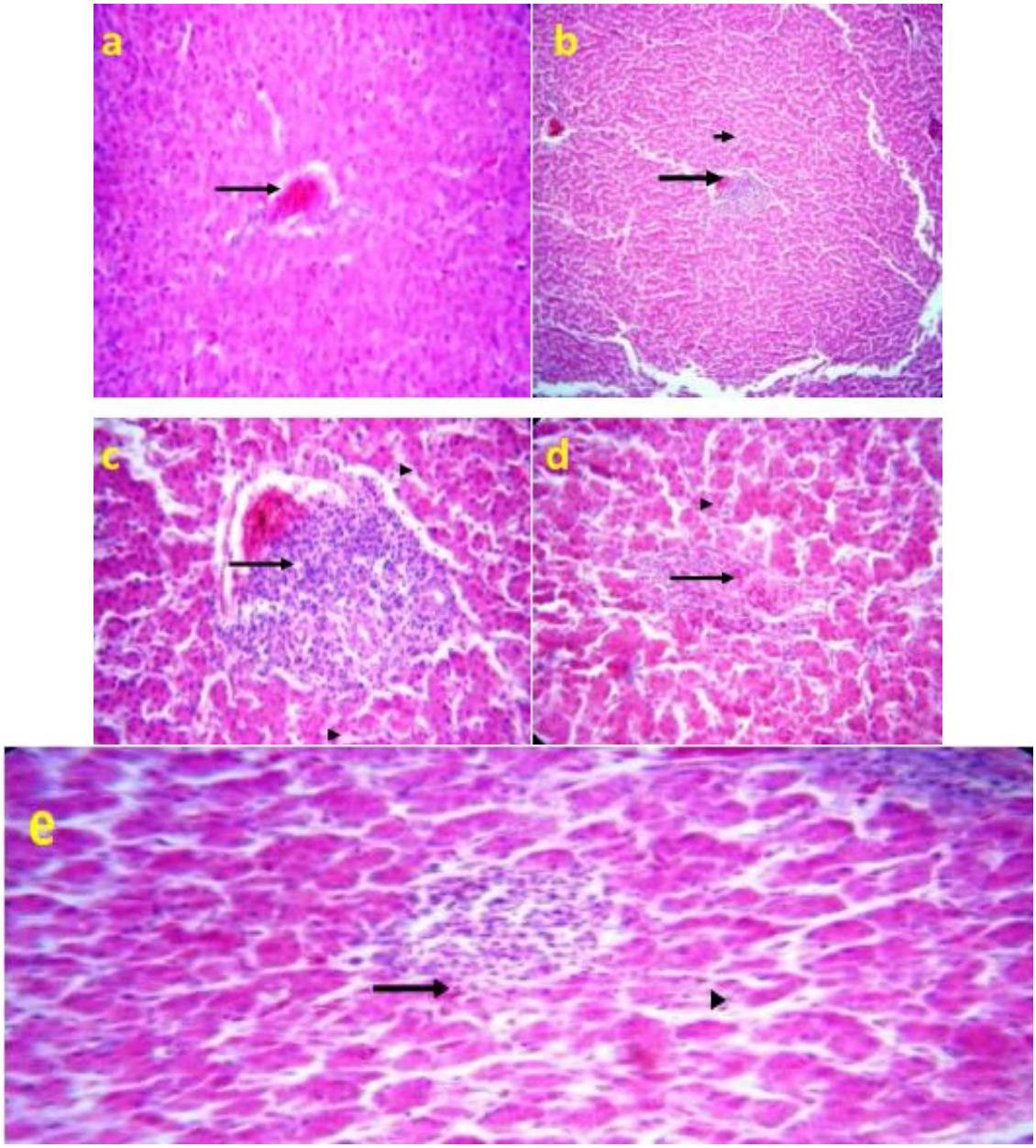
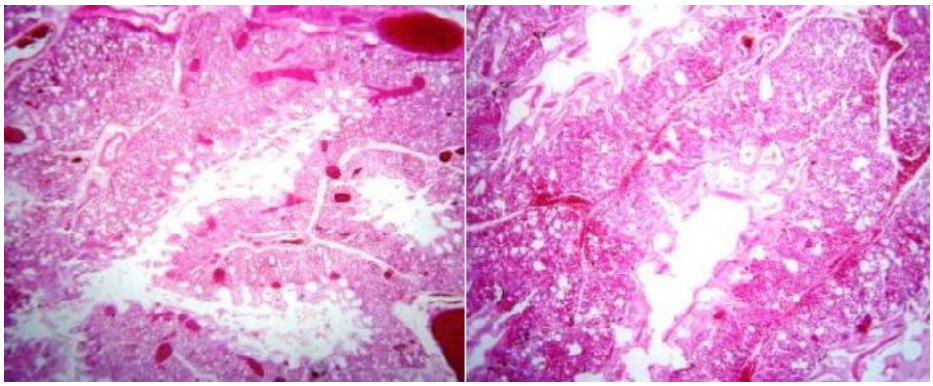


PLATE 1: Section of the liver **(a)** Control (0 % BFCSM) - the liver was normal **(b)** 5 % BFCSM congestion of central vein (long arrow) with mild necrosis (short arrow) **(c)** 10 % BFCSM showing severe congestion of central vein (arrow) with mononuclear infiltration in the sinusoids (arrow heads) **(d)** 15 % BFCSM showing congested central vein (arrow) and necrosis of hepatocytes (arrow head) **(e)** 20 % BFCSM showing congestion of the central vein (arrow) disorientation of the hepatic cords, necrosis of hepatocytes and mononuclear infiltration (arrow heads).



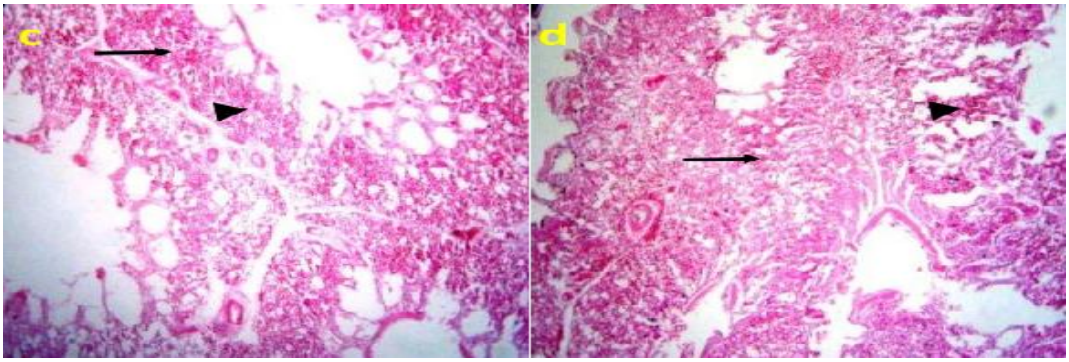


PLATE 2: Section of the lung **(a)** Control(0 % BFCSM) was normal **(b)** 5 % BFCSM congestion (long arrow) with mild mononuclear infiltration (short arrow) **(c)** 15 % BFCSM showing congestion (arrow) and mononuclear infiltration (arrow head) **(d)** 20 % BFCSM showing collapse of the parabronchus (arrow) with sloughing of the epithelium of the air vesicle and mononuclear infiltration (arrow heads).

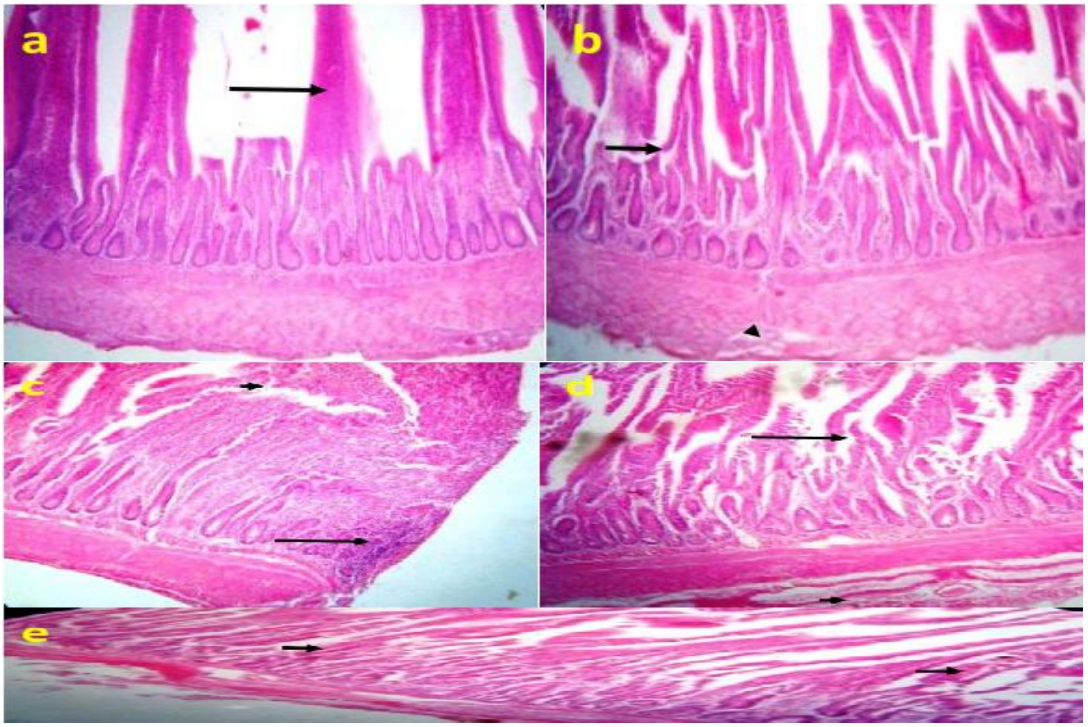
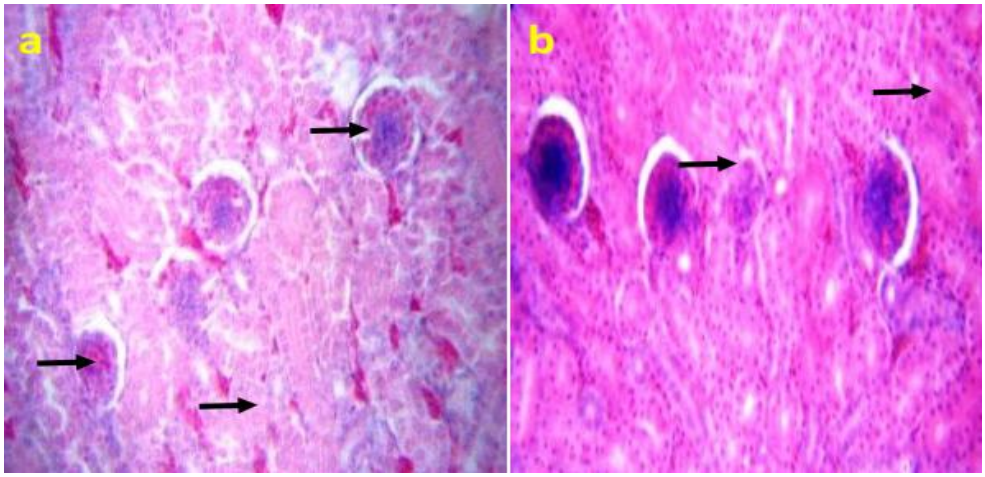


PLATE 3: Section of the intestine **(a)** Normal(0 % BFCSM) showing arrangement of villi (arrow) **(b)** 5 % BFCSM showing disorientation of the villi (long arrow) with mild mononuclear infiltration in the muscularis mucosa (short arrow) **(C)** 10 % BFCSM showing matting of the villi (short arrow) and mononuclear infiltration in the sub mucosa (long arrow) **(d)** 15 % BFCSM showing sloughing of the villi (long arrow) and disorientation of the muscularis mucosa due to oedema (short arrow) **(e)** 20 % BFCSM showing sloughing of the villi with necrosis of the Brunner's glands (arrow).



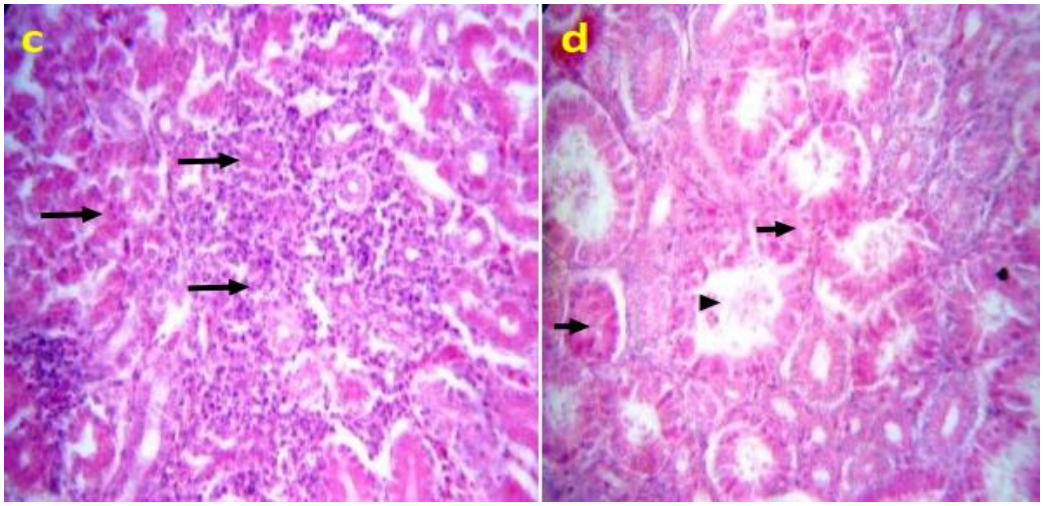


PLATE 4: Section of the kidney **(a)** Normal control (0 % BFCSM) showing glomeruli and tubules (arrows) **(b)** 5 % BFCSM showing occluded Bowman's space (long arrows) **(c)** 15 % BFCSM showing tubular degeneration, necrosis with heterophilic infiltration in the renal interstitium (arrows) **(d)** 20 % BFCSM showing marked tubular necrosis (short arrow) with heterophilc infiltration (arrow head).

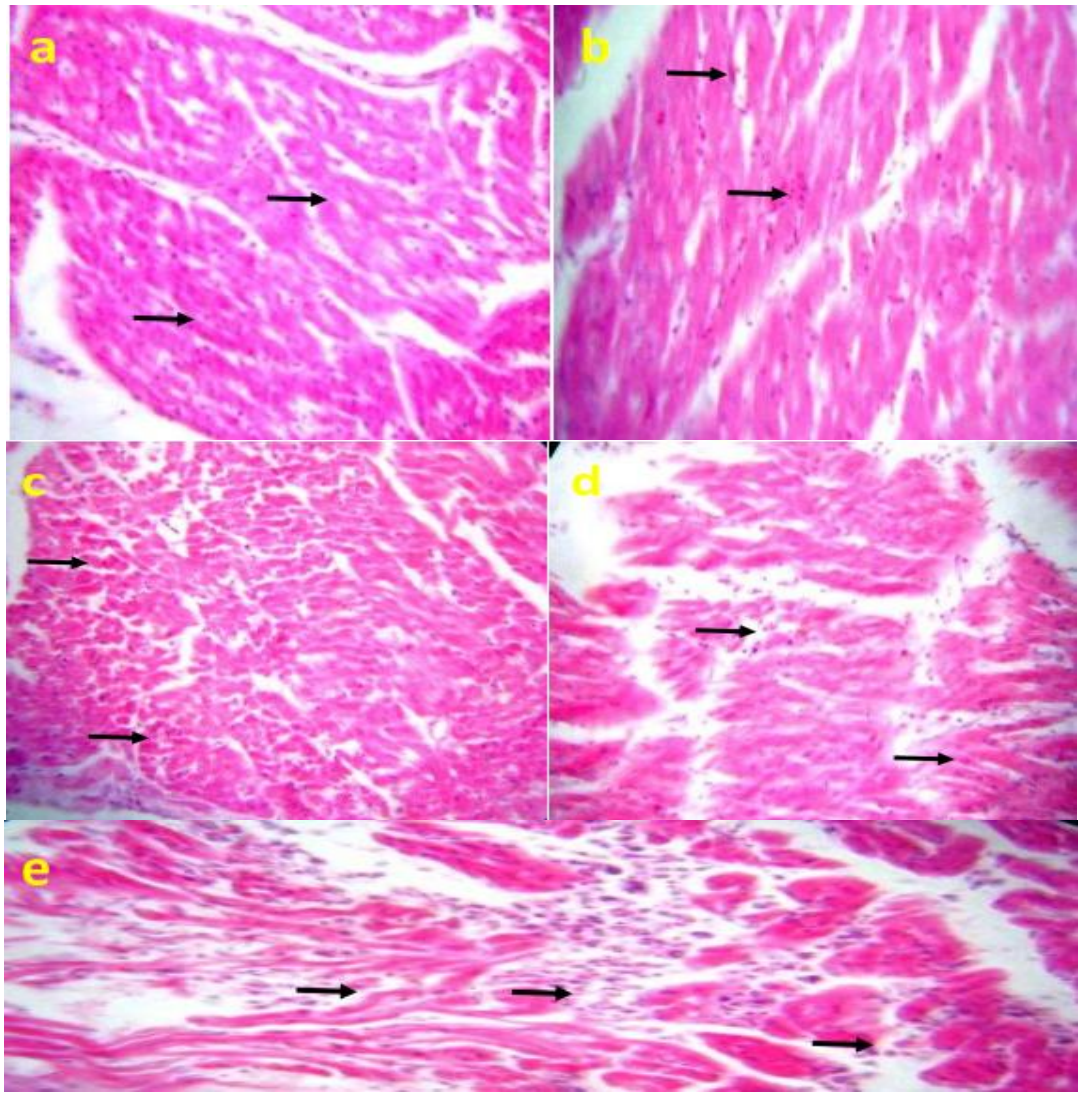


PLATE 5: Section of the heart **(a)** Normal (0 % BFCSM) showing orientation of fibers (arrows) **(b)** 5 % BFCSM showing multifocal areas of haemorrhages within the fibres (arrows) **(C)** 10 % BFCSM showing mild necrosis of the myocytes (arrows) **(d)** 15 % BFCSM showing moderate of myocytes with heterophil infiltration (arrows) **(e)** 20 % BFCSM showing severe necrosis of myocytes with marked heterophil infiltration (arrows).

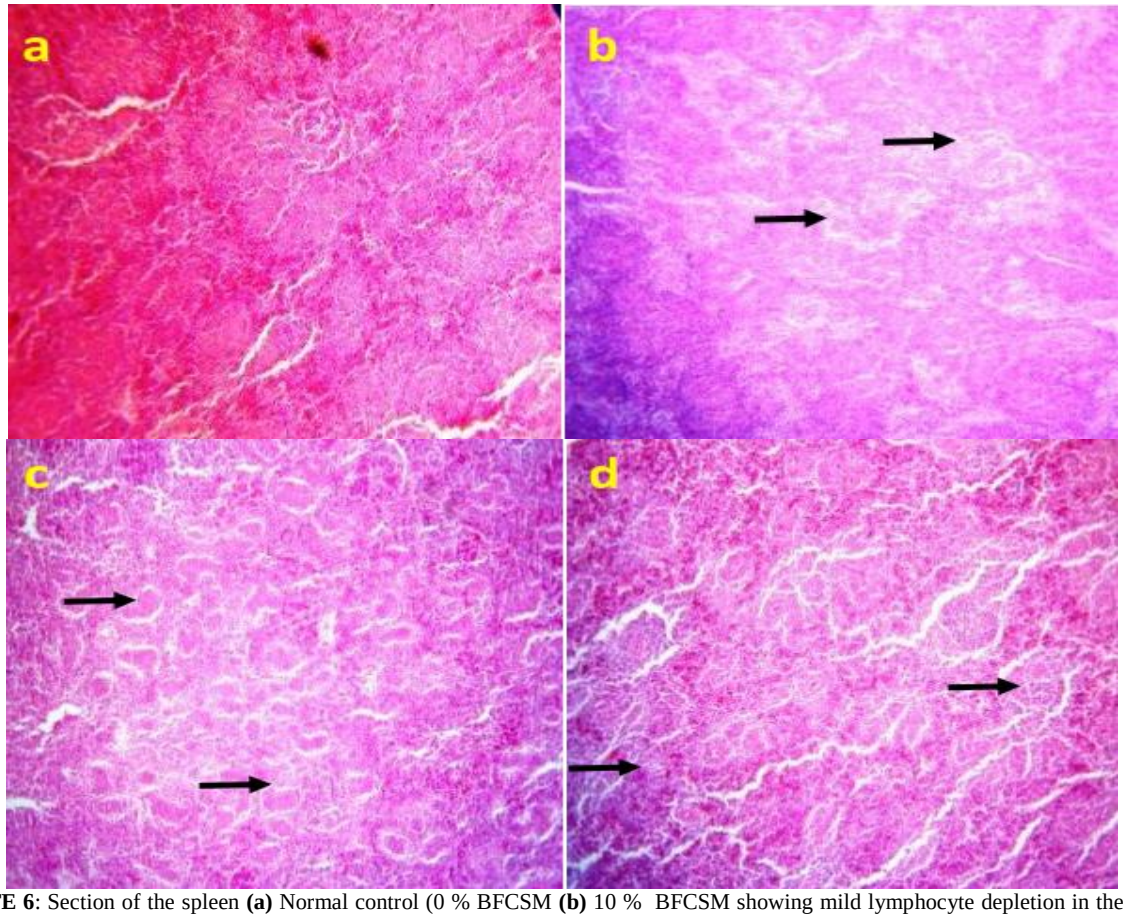


PLATE 6: Section of the spleen **(a)** Normal control (0 % BFCSM **(b)** 10 % BFCSM showing mild lymphocyte depletion in the white pulp (arrows) **(c)** 15 % BFCSM showing white pulp hypoplasia (arrows) **(d)** 20 % BFCSM showing a splenic follicles undergoing possible re-generation of the white pulp (arrows).

DISCUSSIONS

Haematological indices

The PCV values which ranged from 25.33 to 29.67 % obtained in this study were slightly lower than the normal range of PCV (30.00 to 35 %) reported by Swenson (1970) and Campbell *et al.* (2003) for domestic chickens. The RBC which ranged from 3.13 to $3.60x10^6 mm^3$ is however, within the normal range (2.88 to $4.12x10^6$ mm^3) reported by Swenson (1970) and Campbell *et al*. (2003) for domestic chickens. The significant ($P<0.05$) decrease in PCV and RBC and the significant ($P < 0.05$) increase in MCH and MCHC in the birds fed 20 % BFCSM are indication of anaemia. This is in line with the report of Bush (1975) who revealed that decrease in PCV and RBC and increase in MCH and MCHC in animals are signs of anaemia. This result is also similar to the findings of Ojediran *et al*. (2012) who reported an increase in MCH and MCHC as the cause of anaemia in broilers fed differently processed *Jatropha* kernel meal.

An increase in the WBC count is indicative of toxic condition (Bush, 1975). The observed increase in WBC count especially in the birds fed 15 and 20 % BFCSM may be interpreted as defensive action against the possible presence of residual ricin or infection in the birds. William (1995) reported that castor seed are used for the treatment of AIDS patients because it increases their WBC count. Similarly, the observed increase in heterophils in birds fed 20 % BFCSM could also be possibly due to the presence of residual toxin or infection in the birds. This is in agreement with Harmon (1998) who reported that heterophils form the first line of cellular defence against invading microbial pathogen/foreign materials in the lungs and air sac where resident macrophages are lacking.

Serum biochemical indices

The total protein value obtained in this study were similar to the range of 21.50 – 27.25 g/l obtained by Ojediran *et al.* (2012) when they fed processed *Jatropha curcas* kernel to broiler chickens. *Jatroph acurcas* is another member of the family Euphorbiaceae to which castor seed (*Ricinus cummunis*) also belongs and is known to contain toxic and anti-nutritional factors (EFSA, 2008). The *Jatropha curcas* seed contain curcin a toxic glycolprotein with a 54 % homology with the ricin A chain and with similar mode of action with ricin. The values (2.20 – 2.73 mmol/L) for total cholesterol obtained in this study were lower than the 3.44 mmol/L and 3.94 – 4.02 mmol/L reported by Bowes *et al*.(1989) and Nazifi *et al.* (2011), respectively. As such it's likely that birds were not predispose to heart diseases due to the cholesterol levels. The glucose level values obtained in this study all fall within the normal range (10.33 – 12.20 mmol/L) and (6.20 – 19.40mmol/L) for broilers reported by Hazelwood (1986) and Nazifi *et al.* (2011) respectively. The values of the sodium obtained in this study were within the normal range of 145 – 155

mmol/L as reported by Bowes *et al*. (1989). The birds on 0 and 5 % BFCSM diets recorded ALAT values which were similar to the value of 11.7.IU/L reported by Daramola *et al.* (2005), while the birds on 10, 15 and 20 % BFCSM diets recorded values which were above this value. The slightly higher values of ALAT observed in birds fed 10, 15 and 20% BFCSM diet could possibly be due to the effect of residual ricin in the diets at the higher replacement levels. Ojediran *et al.* (2012) reported that the appearance of abnormal amounts of certain enzymes of intercellular origin in the blood reflects damage to an organ or tissue. The liver is rich in some enzymes such as ALAT and Aspartate Amino Transferase (ASAT) and its damage often results in releasing these enzymes into the blood (Kaplan *et al.,* 2003). The ASAT, albumin, potassium and urea levels in the birds fed on the different levels of BFCSM were not affected by the replacement levels of castor seed meal. This indicates that up to 20 % replacement level of BFCSM has no adverse effect on these parameters

Histopathological findings

(a) Gross pathological lesions

The adverse effect of the BFCSM on these organs increased with increase in the replacement levels of the BFCSM from 5 to 20 % in the diets. The details of the adverse effects on these organs are presented in the histopathological examinations below.

(b) Histopathological examinations

Liver

The pale and friable appearance of the liver in the birds fed 20 % BFCSM was abnormal and could likely be due to the increase in metabolic activities in the liver. This increase in metabolic activities could probably be due to the detoxification of the anti-nutritional factors particularly ricin by the liver. This was in line with the findings of Robert (1976) who revealed that the liver cells detoxify many poisonous substances by absorbing them and then changing them chemically. Akande *et al*. (2012) has reported similar histopathological changes in the liver and kidney of broilers fed untreated castor bean cake and/or BFCSM at 10 % replacement level. The authors also explained that the severity of the histopathological effect was dependent on the level of residual anti-nutritional factors present in the untreated and treated castor bean cake as well as to the duration of exposure to the castor bean toxin. They further inferred that despite the detoxification achieved with processing methods, the residual toxin particularly in fermented and boiled castor seed may pose serious health challenges to birds with prolonged exposure. Similarly, Jensen and Allen (1981) reported that in poultry, the commonest lesion were severe fatty changes in the liver, widely distributed internal petechial haemorrhages or ecchymoses and catarrhal enteritis.

Lungs

The increase in degeneration in morphology observed in the lungs may result in dyspnea which may be the cause of the death and highest mortality of 35.55 % recorded for the birds fed on 20 % BFCSM. These could be linked to the presence of the residual anti- nutritional factor – ricin toxin in the diet. Similar to the above result, EFSA (2008) reported that in experimental animals, inhalation of ricin resulted in inflammation of bronchial tree and alveoli and lung oedema caused by injured endothelium cells.

Intestine

The appearances of multi- focal petechiae along the whole intestinal length are signs of irritation and alterations of the intestinal wall, possibly caused by residual ricin toxic substances in the diet. Sell *et al.* (1985) observed that such changes are capable of reducing the absorptive capacity of the small intestine. Various workers have reported similar effects of castor seed toxin- ricin on the intestine and other internal organs of animals exposed to ricin. For example, EFSA (2008) reported that at autopsy the mucous membrane of the intestine was congested and a thick mucous exudates was seen within the lumen of most of the ricin exposed animals. Aslani *et al.* (2007) reported that pathology of dead animals previously fed castor seed meal also revealed gastroenteritis, necrosis and haemorrhage in the heart and kidney. Carew *et al.*(2003) observed loss of mucosal lining of the small intestine in chicks fed raw velvet bean (Mucuna seed) which brought about decreased villi length with consequent reduction in the surface area for absorption in the small intestine.

Kidney

The observed transformation of the kidney into tubular degeneration, marked tubular necrosis with heterophilic infiltration in the renal interstitium in birds fed on 15 and 20 % BFCSM, were possibly caused by the presence of residual ricin in the diets. Similarly, EFSA (2008) autopsy report revealed that the kidney of ricin exposed animal was congested with frequent haemorrhages and by microscopy, an acute tubular necrosis was also observed not only in the kidney, but also in the liver, intestine, spleen and lymph nodes.

Heart

The multi- focal ecchymotichaemorrhages noticed in the epicardium and endocardium were signs of deterioration in the heart. These could cause cardiac arrest and the subsequent highest mortality (35.55 %) recorded especially in birds fed 20 % BFCSM. This finding was similar to the report of Aslani *et al*. (2007) and EFSA (2008) who stated that the autopsies of animals exposed to ricin showed haemorrhages and necrosis in the hearts.

Spleen

The birds fed on 10 % BFCSM showed mild lymphocytes depletion in the white pulp. The degree of this effect increased in the birds fed 15 and 20 % BFCSM in the diets and obviously affects the functions of the spleen. The spleen is the largest lymphatic organ in the body and is an important in the immune system, producing white blood cells that fight infection and synthesis antibodies. It also acts as a filter for purifying blood, removing microbes, worn out or damage red blood cells,

The above histopathological changes observed in the liver, lungs, intestine, kidney, heart and spleen with the increase in the replacement levels of BFCSM could also be related to the results obtained in the serum biochemistry of the birds. Of particular interest was the increase in the level of ALAT at higher replacement levels of the BFCSM. Kaplan *et al.* (2003) reported that the liver is rich in some enzymes such as ALAT and aspartate amino transferase (ASAT) and its damage often results in releasing these enzymes into the blood.

CONCLUSION

It was concluded that the replacement of BFCSM especially at higher levels (15 and 20 %) has adverse effect on the haematology, serum biochemistry and histopathology of broilers chickens.

RECOMMENDATION

In an earlier paper which was the first aspect of this study i.e the effect of replacement levels of boiled and fermented castor seed *(Ricinus cummunis*) meal on the productive performance, nutrient digestibility, carcass characteristics and cost effectiveness in broilers, the author recommended for the need for further research on the detoxification process for castor seed meal so that more economic benefit could be drive from its utilization. Consequently, with the results obtained which shows increase in WBC and lower cholesterol levels in birds fed 15 and 20 % BFCSM are of interest and also require further investigations. Increase in WBC in the body increases the body defense system.

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