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Histological Changes in Liver and Kidney of Rabbit Buck Fed Diet Containing Cottonseed Cake Supplemented with Vitamin E.

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Abstract
Sixty-four (64) weanling rabbit bucks, aged 5-6 weeks with an average weight of 511.98g, were randomly allocated to eight treatment combinations comprising four levels (0, 5, 10 and 15%) of cottonseed cake (CSC) and two levels (0, 30mg / kg diet) of vitamin E supplementation with eight replicates in a 2x4 factorial experiment. Animals were fed the treatment diets for 20 weeks. At the end of the feeding trial, 3 rabbits per treatment were sacrificed and their internal organs including liver and kidneys were carefully dissected out. Samples of organs were fixed in 10% formaldehyde solution and later processed for histological assessment. It was observed that CSC induced various degrees of damage to the architecture of the liver and kidney of the rabbit bucks. Histological examination revealed a dose-dependent degeneration, cellular infiltration and necrosis of liver hepatocytes. At all levels of the CSC, vitamin E (30mg/kg diet) corrected the damages done to the liver. Mild degenerative changes were observed for the kidney structure at 5% and 10% CSC levels. The renal degenerative changes became severe at 15% CSC inclusion level. Vitamin E supplementation ameliorated these adverse effects. Thus, vitamin E conferred some protection on the liver and kidney against the adverse effects of the CSC. It was concluded that if cottonseed cake is included in diets of rabbits at any level, it should be adequately supplemented with vitamin E.
Keywords: Rabbit buck, Liver, Kidney, Histology, Cottonseed cake, Vitamin E.

Introduction

In the recent time, there appears to be a growing interest in the research involving utilization of cottonseed cake (CSC) by livestock especially the non-ruminants (Ojewola *et al*., 2006; Taha *et al*., 2006; Adeyemo and Longe, 2007; Pousga *et al*., 2007; Adeyemo, 2008 and Amao, 2009). This could be associated with the current prohibitive prices of the major protein ingredients, soyabean meal (SBM) and groundnut cake (GNC), for monogastric feeding.

Cottonseed cake (CSC) is known to contain gossypol, a toxic polyphenolic compound of significant physiological and biochemical implication. Toxic effects of gossypol include damages to reproductive organs of male animals (Randel *et al*., 1992; Risco *et al*., 1993; Taha *et al*., 2006; Amao *et al*., 2012a). According to Randel *et al*., (1992) the effect of gossypol in the male are dose-and time-dependent. Several biochemical substances of physiological importance including inert gases in insecticides, fluoride and nanosilver, have been reported to cause severe histological changes in liver and kidney of animals (Zhan *et al*., 2006; Garba *et al*., 2007; Akradi *et al*., 2012). Recently, Odetola *et al*. (2012) reported that replacing 40% (and above) of soyabean meal with whole kenaf seed meal in diets of rabbits caused hepatic degeneration and necrosis in the liver of the rabbits. Available recent data have not included information on histological changes in the liver and kidney of rabbits exposed to diets containing CSC.

Vitamin E has been reported to counter the adverse effects of gossypol on major physiological functions in bulls (Velasquez – Pereira *et al*., 1998) and rabbit bucks (Amao *et al*., 2012b). However, information is lacking on the effect of CSC and vitamin E supplementation on the histological structures of liver and kidney of rabbit. This study was therefore conducted to evaluate the histological changes in liver and kidney of rabbit bucks fed CSC-based diets. It also investigated the possibility of using vitamin E to counter or ameliorate the negative effect of gossypol in the CSC (if any) on the liver and kidney of the bucks.

Materials and methods

Site of experiment

The experiment was conducted at the rabbitry section of the Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso, Oyo State Nigeria. The climatic condition of Ogbomoso has been described by Oguntoyinbo (1978). Ogbomoso is situated on Latitude 8^{o} 15^{l}N and Longitude 4^{o} 15^{l}. The mean annual rainfall is 1247mm and the relative humidity is between 75 and 95%. The mean annual temperature is about 26.2^{o}C. The altitude is between 300m and 600m above sea level. It is in the derived savanna zone of Nigeria.

Animals and management

Sixty–four (64) weanling crossbred (New Zealand White X Chinchilla) rabbit bucks aged 5-6 weeks and averagely weighing 511.98g were balanced for weight and allocated to eight (8) treatment combinations of four dietary levels (0, 5, 10,and 15%) of cottonseed cake (CSC) and two (2) levels (0 and 30mg/kg diet) of vitamin E supplementation. Animals were acclimatized for a period of one week during which they were fed with the control diet (16% crude protein (CP) and about 2500 kcal Kg^{-1} ME). They were also treated against endo- and ecto-parasites. After the acclimatization period, the animals were subjected to eight weeks feeding trial during which their pre-pubertal performance were monitored. After this phase, the experimental diets were adjusted to that of a growing rabbit and the rabbits were further fed the diets for another twelve (12) weeks, making a total of twenty (20) weeks. The rabbits were housed individually in wooden cages. Feeding was done twice per day, 08:00hr and 16:00hr (i.e. 8:00am and 4:00pm). Eight (8) bucks were assigned to each treatment in a 2 x 4 factorial arrangement with each rabbit serving as a replicate. The gross composition of the experimental diets at both pre-pubertal and post – pubertal phases are presented in Tables 1 and 2, respectively.

Histological slide preparation and evaluation

At the end of the twenty – week feeding trial, three bucks of similar weight were selected from each treatment and sacrificed. The animals were cut open and the visceral organs carefully dissected out. The histological slide preparation followed a standard procedure. Samples of liver and kidney were cut and fixed in 10% formalin solution for a week. The samples were washed in 50%, 70% and 90% alcohol for 2 hours each to remove water (dehydration). They were then transferred into xylene solution to get rid of the alcohol (clearing) and then infiltrated with paraffin wax and embedding media in an enclosure called mould (Amao, 2009). The embedded tissues were left until hardened blocks were formed. Sectioning was done using a microtome (Rotary Kepee Model KD 202A), to cut only the original tissue at a preset thickness of 5µm. The sectioning was done serially and the products floated out in water bath. Satisfactory sections were then stained with haematoxylin and eosin on microscope slides. Finally, the slides were mounted on a microscope for histological examination.

The slides were read for histological indicators in order to observe possible degenerative changes on the liver and kidney structure using a microscope connected to a computer system. A photo-micrographic software - Phoenix Micro Image Analysis (2003) version 1.3B was used to project the slides on the computer for clear assessment. The slides were subsequently captured and printed for interpretation.

Results and discussion

The micrograph of the liver structure of bucks fed diets containing CSC with or without vitamin E is shown in Figure 1. No histological changes or alterations were observed in the liver of the control bucks (Figure 1A). There was normal arrangement of the hepatocytes with normal sinosoids and central vein. In Figure 1B, similar observations were made except that vitamin E supplementation tended to cause infiltration of lymphocytes in the central vein. Figure 1C is the liver structure of the bucks that were fed diets containing 5% CSC. The central vein was observed to be dilated with inflammatory cellular infiltration. There were degenerative changes and vacuolations in the hepatocytes and sinosoids. The liver was necrotic in appearance. In Figure 1D (5% CSC + Vitamin E) the degenerative changes and vacuolation of hepatocytes were corrected by vitamin E. At 10% CSC inclusion level (Figure 1E), the central vein was further dilated and there were severe degeneration and erosion of the hepatocytes. The liver cells were also necrotic and the sinosoids were less defined. When the 10% CSC diet was supplemented with vitamin E (Figure 1F), the negative effect of CSC on the liver was neutralized. At 15% CSC, severe erosion and degeneration of hepatic cells were evident. The central vein was highly dilated and the entire liver structure was remarkably disorganized (Figure G). The introduction of vitamin E to the diet at this inclusion level of CSC offered a great protective effect against the deleterious action of the CSC on the liver of rabbit bucks.

Figure 2 shows the kidney structure of rabbit buck fed CSC with or without vitamin E supplementation. In Figure 2A (control), there were no adverse histological changes in the renal structure of the bucks. At 5% and 10% inclusion levels of CSC in diets of rabbit buck (Figures 2C and 2E), only mild degenerative changes were observed. These changes were however corrected by vitamin E (Figures 2D and 2F). At 15% inclusion level (Figure 2G), the damaging effect of CSC was more pronounced on the renal histological structure with the glomeruli and tubules becoming extensively disorganized. However, with vitamin E supplementation (Figure 2H), the histological alterations caused by CSC diets, were greatly corrected. It was observed that vitamin E exhibited the potential to ameliorate the adverse effect of CSC on the kidney structure of the bucks.

The observation in this study that diets containing CSC, at various levels of inclusion, induced varying degrees of histological changes in the liver of rabbit buck is an indication of the hepatotoxic effect of the CSC occasioned by gossypol in it. This calls for serious intervention, especially in areas of CSC abundance, and for economic reason, where there may be need to incorporate CSC in diets of rabbits; as the liver which is the major organ of metabolism would be at risk of being damaged if adequate measure is not taken to safeguard it against the harmful effect of gossypol in the CSC. This observation is consistent with the report of Odetola *et al*. (2012). Any damage to the liver or kidney consequently leads to impairment of their functions. Findings from this study indicate that vitamin E could be very useful in this regard.

The mild alterations observed in kidney structures of bucks fed diets containing 5 and 10% CSC, and the severe disruption of the renal structure at 15% inclusion level, support the earlier report that the effects of gossypol on physiological processes in animal's body are both time- and dose- dependent (Randel *et al*., 1992). The hepato-protective effect demonstrated by vitamin E in this study could be related to its antioxidant property by which it destroys free radicals in the body. Free radicals are known to damage biological membranes in animal's body. It has been reported that one of the mechanisms of action of gossypol is by generating free radicals which are damaging by-product of body's metabolism (Barhoumi and Burghardt, 1996). Results from this study also revealed that vitamin E conferred some protection against the damaging effect of CSC on the kidney of rabbit bucks.

It was clear from this study that cottonseed cake-based diets showed adverse effect on the liver and kidney of rabbit bucks, while up to 30mg/ kg diet vitamin E supplementation exhibited the potential to safeguard these vital organs against the adverse effect. This implies that the high nutritional potential of CSC could be harnessed for rabbit feeding if pragmatic measures could be adopted in handling it. Therefore, CSC should not be fed to rabbit bucks unless adequately supplemented with vitamin E.

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Table 1: Gross composition and calculated nutrients of experimental diets for pre-pubertal rabbit bucks

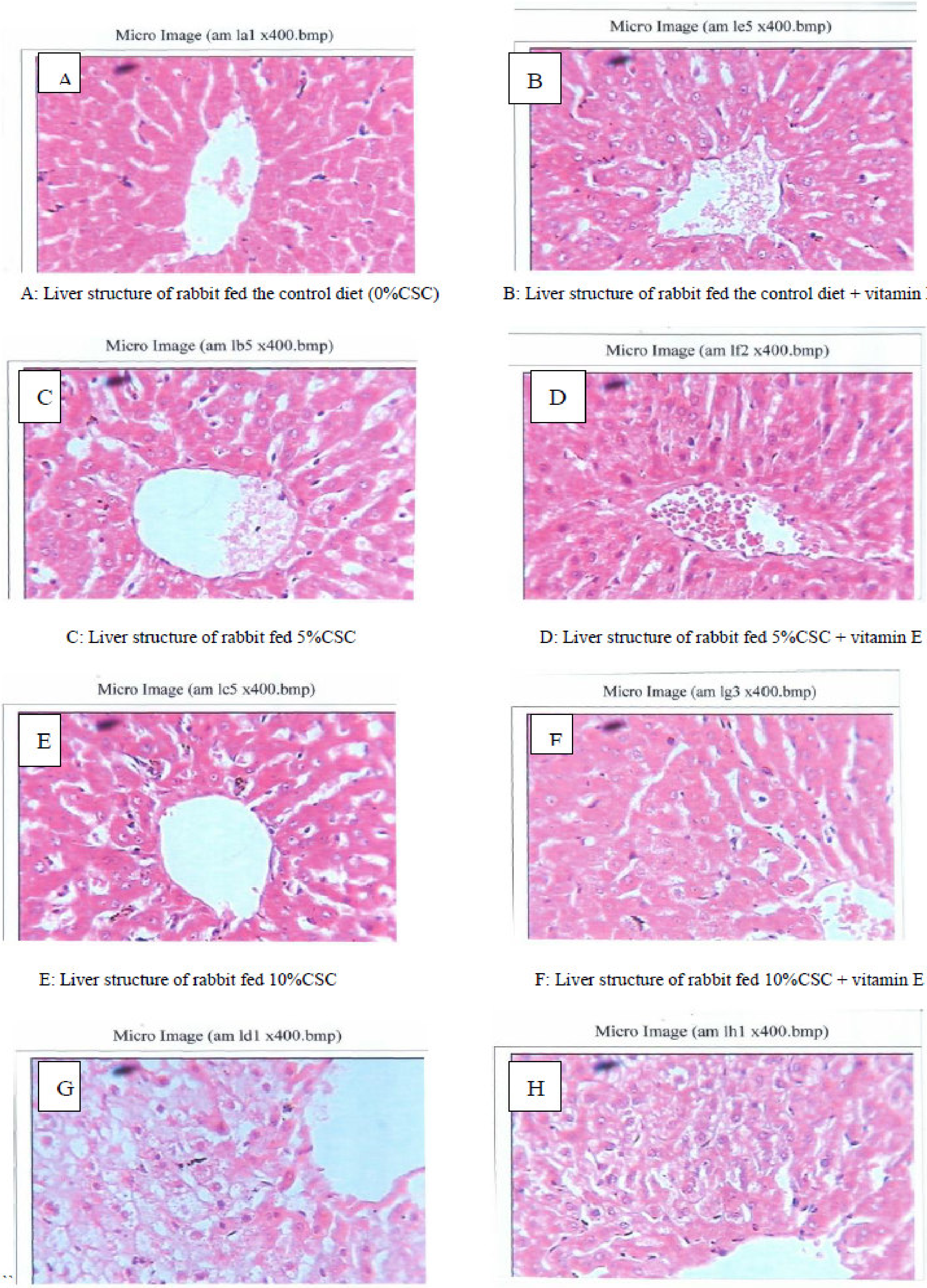
	-Vitamin				+Vitamin E			
	T1	T2	T3	T4	T5	T6	T7	T8
Ingredient	(0% CSC)	(5% CSC)	(10% CSC)	(15% CSC)	(0% CSC)	(5% CSC)	(10% CSC)	(15% CSC)
Maize	44.04	42.37	40.66	40.15	44.04	42.37	40.66	40.15
Groundnut Cake	20.21	17.90	15.59	13.10	20.21	17.90	15.59	13.10
Rice husk	30.00	29.00	28.00	26.00	30.00	29.00	28.00	26.00
Cottonseed Cake	0.00	5.00	10.00	15.00	0.00	5.00	10.00	15.00
Fishmeal	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Bone meal	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Oyster shell	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Vit/min Premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.5	0.50
Vitamin E	-	-	-	-	+	+	+	+
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated nutrients								
Crude Protein (%)	16.00	16.01	16.00	16.01	16.00	16.01	16.00	16.01
Metabolizable Energy (ME) (Kcal/kg diet)	2523.07	2515.75	2507.04	2520.70	2523.07	2515.75	2507.04	2520.70
Crude Fibre (%)	11.00	11.72	12.51	13.03	11.00	11.72	12.51	13.03
Lysine (%)	0.546	0.547	0.548	0.549	0.546	0.547	0.548	0.549
Methionine (%)	0.223	0.227	0.231	0.237	0.223	0.227	0.231	0.237

* Premix composition (per kg of diet): vitamin A, 12,500 IU; vitamin D3, 2500 IU; vitamin E, 50.00mg; vitamin K3, 2.50mg; vitamin B1, 3.00mg; vitamin B2, 6.00mg; vitamin B6, 6.00mg; niacin, 40mg; calcium pantothenate, 10mg; biotin, 0.08mg; vitamin B12, 0.25mg; folic acid, 1.00mg; chlorine chloride,300mg; manganese, 100mg; iron, 50mg; zinc, 45mg; copper, 2.00mg; iodine, 1.55mg; cobalt, 0.25mg; selenium, 0.10mg; antioxidant, 200mg

Table 2: Gross composition and calculated nutrients of experimental diets for post-pubertal rabbit bucks

	-Vitamin			+Vitamin E				
	T1	T2	T3	T4	T5	T6	T7	T8
Ingredient	(0% CSC)	(5% CSC)	(10% CSC)	(15% CSC)	(0% CSC)	(5% CSC)	(10% CSC)	(15% CSC)
Maize	44.94	45.55	46.14	47.93	44.94	45.55	46.14	47.93
Groundnut Cake	17.31	12.70	8.11	3.32	17.31	12.70	8.11	3.32
Rice husk	32.00	31.00	30.00	28.00	32.00	31.00	30.00	28.00
Cottonseed Cake	0.00	5.00	10.00	15.00	0.00	5.00	10.00	15.00
Fishmeal	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Bone meal	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Oyster shell	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Vit/min Premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.5	0.50
Vitamin E	-	-	-	-	+	+	+	+
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated nutrients								
Crude Protein (%)	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Metabolizable Energy (ME) (Kcal/kg diet)	2505.42	2495.77	2485.95	2484.07	2505.42	2495.77	2485.95	2484.07
Crude Fibre (%)	11.36	11.44	11.51	11.29	11.36	11.44	11.51	11.29
Lysine (%)	0.514	0.490	0.466	0.442	0.514	0.490	0.466	0.442
Methionine (%)	0.199	0.199	0.198	0.198	0.199	0.199	0.198	0.198

* Premix composition (per kg of diet): vitamin A, 12,500 IU; vitamin D3, 2500 IU; vitamin E, 50.00mg; vitamin K3, 2.50mg; vitamin B1, 3.00mg; vitamin B2, 6.00mg; vitamin B6, 6.00mg; niacin, 40mg; calcium pantothenate, 10mg; biotin, 0.08mg; vitamin B12, 0.25mg; folic acid, 1.00mg; chlorine chloride,300mg; manganese, 100mg; iron, 50mg; zinc, 45mg; copper, 2.00mg; iodine, 1.55mg; cobalt, 0.25mg; selenium, 0.10mg; antioxidant, 200mg



A: Liver structure of rabbit fed the control diet (0%CSC)

B: Liver structure of rabbit fed the control diet + vitamin E

C: Liver structure of rabbit fed 5%CSC

D: Liver structure of rabbit fed 5%CSC + vitamin E

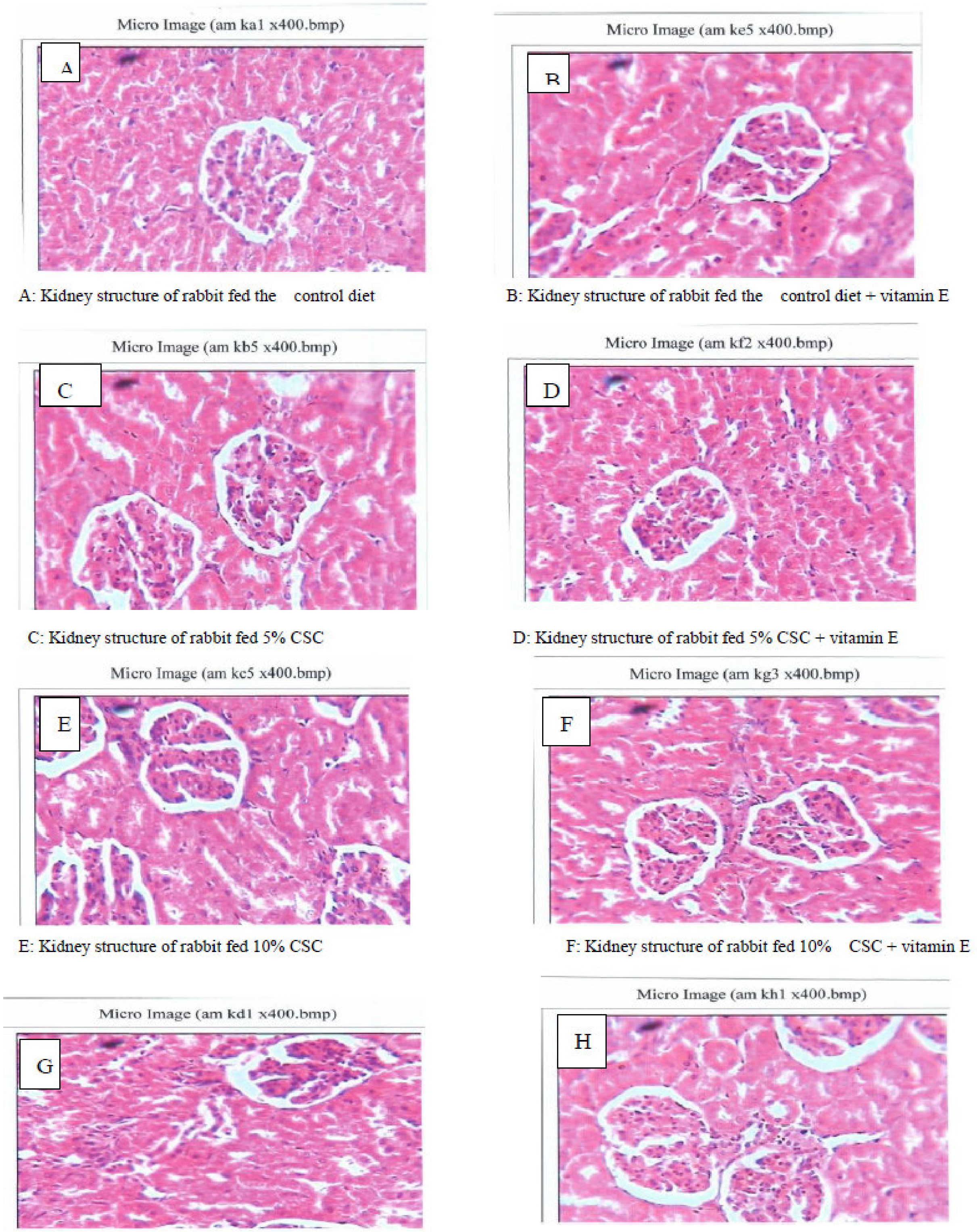
E: Liver structure of rabbit fed 10%CSC

F: Liver structure of rabbit fed 10%CSC + vitamin E

G: Liver structure of rabbit fed 15%CSC

H: Liver structure of rabbit fed 15%CSC + vitamin E

Figure 1. Liver structure of rabbit buck as affected by CSC level with or without vitamin E supplementation



A: Kidney structure of rabbit fed the control diet

B: Kidney structure of rabbit fed the control diet + vitamin E

C: Kidney structure of rabbit fed 5% CSC

D: Kidney structure of rabbit fed 5% CSC + vitamin E

E: Kidney structure of rabbit fed 10% CSC

F: Kidney structure of rabbit fed 10% CSC + vitamin E

G: Kidney structure of rabbit fed 15% CSC

H: Kidney structure of rabbit fed 15% CSC + vitamin E

Figure 2. Kidney structure of rabbit buck as affected by CSC level with or without vitamin E supplementation