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Full Length Research Paper

Physiological response of rabbit bucks to prolonged feeding of cotton seed cake-based diets supplemented with vitamin E

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Sixty-four (64) weanling rabbit bucks, 5 to 6 weeks old, were involved in a 2 x 4 factorial experiment to evaluate the effects of prolonged feeding of cottonseed cake (CSC) – based diets with or without vitamin E supplementation on the physiological response of the bucks. There were eight treatment combinations comprising four CSC (0, 5, 10 and 15%) levels and two vitamin E supplementation levels (0 and 30 mg/kg diet). Bucks were fed treatment diets for a period of twenty weeks after which blood was collected for haematological and serum analyses. The haematological parameters assessed include: Packed cell volume (PCV), red blood cell (RBC), haemoglobin concentration (Hb), white blood cell (WBC) and the differential counts. The serum parameters include the serum glucose, urea, creatinine, alanine transaminase (ALT), aspartate transaminase (AST), total protein, albumin, globulin and total cholesterol. The PCV, RBC and neutrophils declined ($P < 0.05$) with increase in CSC level; while the WBC and lymphocytes increased ($P < 0.05$) with increasing level of CSC. Other haematological parameters were not significantly ($P > 0.05$) affected by CSC level. Bucks that were fed 0, 5, 10 and 15% CSC supplemented with vitamin E recorded higher values for PCV, RBC and Hb than those that were not supplemented. At 15% CSC level, supplementing the diet with vitamin E significantly ($P < 0.05$) reduced WBC and lymphocyte counts. Serum urea, total protein and albumin were significantly ($P < 0.05$) lowered by the increasing level of CSC. Significant ($P < 0.05$) increase was observed in ALT and AST with increase in CSC level. Glucose and total cholesterol were not affected by CSC level. Supplementing the diet with vitamin E at all CSC levels reduced creatinine, ALT and AST levels significantly ($P < 0.05$). It was concluded that prolonged feeding of CSC – based diets to rabbit bucks had adverse effects on blood constituents of the bucks. However, supplementing such diets with 30 mg/kg diet of vitamin E ameliorated the adverse effects.

Key words: Rabbit bucks, haematology, serum chemistry, cottonseed cake, vitamin E.

INTRODUCTION

Over the years, groundnut cake (GNC) and soyabean (SBM) meal have remained the major protein sources in the diets of non-ruminant animals. These ingredients are also highly consumed by man, and as such, there is stiff competition between man and livestock for their consumption. Hence their prices are becoming more and more prohibitive. A possible alternative that is not popular for feeding non-ruminants is cottonseed cake (CSC). Cottonseed cake is a by-product from the extraction of oil

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from whole cottonseed. The CSC is an excellent source of protein, energy and fibre for a variety of livestock species (NCPA, 1995).

The CSC is one of the industrial by-products not consumed directly by human beings and as such can reduce livestock feed cost (Bamigbose, 1998). The nutritional potential of CSC has been documented. It is rich in crude protein (35 to 46%) and contains over 10% phosphorus and 70 to 80% TDN (Coppock et al., 1987; NCPA, 1995). However, it has not been fully exploited for monogastric feeding. This is because it contains gossypol, a polyphenolic compound that is very toxic to the monogastric animals.

Gossypol is a natural toxin present in the cotton plant to protect it from insects. Non-ruminant animals such as pigs, poultry and rabbits cannot tolerate much gossypol before toxicity signs develop (Meyer and McDowell, 2003). Deleterious effects of gossypol-containing diets on various physiological processes in animal's body have been reported. Blood is an important index of physiological and pathological changes in an animal (Mitruka and Rawnsley, 1977). Cottonseed cake has been reported to impact negatively on blood constituents in cattle (Lindsery et al., 1980; Gray et al., 1993; Velasquez-Pereira et al., 1998).

Chase et al. (1994) reported that gossypol resulted in anaemia with consequent reduction in red blood cells and increased red blood cell fragility. According to Nikokyris et al. (1991), plasma glucose and albumin were reduced by cottonseed cake in growing lambs. Adeyemo (2008) reported that replacing 60% of soyabean meal (SBM) with CSC in diets of egg type chicken resulted in significantly lower packed cell volume (PCV) and red blood cell (RBC) during the first four weeks of feeding. But after eight weeks of feeding, the values for the PCV and RBC were no longer significantly influenced. Gossypol has been reported to cause adverse effect on biological membranes by generating oxygen free radicals which are damaging metabolites in the body. Vitamin E (Tocopherol) is an antioxidant that protects cells from the effects of the free radicals which are potentially harmful by-products of metabolism (ODS, 2007). According to Weler and Sies (1988) vitamin E is the major scavenger for reactive oxygen species inside membranes of body tissues. The effects of dietary gossypol from cottonseed meal on performance of rabbits have been documented (Taha et al., 2006; Shaaban et al., 2008). However, there is dearth of information on the use of physiological parameters to assess the adverse effects of cottonseed-containing diets on rabbits, particularly with the introduction of vitamin E to partially counteract the adverse effects. Efforts towards detoxifying cottonseed cake for non-ruminant feeding have not reached satisfactory conclusion. Therefore, there is the need to address the nutritional bottleneck caused by gossypol in the CSC. This study was therefore conducted to evaluate the effects of prolonged feeding of CSC to rabbit bucks on the hematological and serum biochemical characteristics of the bucks. The possibility of using vitamin E to counteract any adverse effect by the CSC on the blood parameters of the bucks was also investigated.

MATERIALS AND METHODS

Site of experiment

The experiment was conducted at the rabbitry unit of Teaching and Research Farm of Ladoke Akintola University of Technology, Ogbomoso, Oyo State Nigeria. The climate attributes of Ogbomoso has been described by Oguntoyinbo (1978). Ogbomoso is located on latitude 8°151N and Longitude 4°151E. The mean annual rainfall is 1247 mm and the relative humidity between 75 and 95%. The mean annual temperature is about 26.2°C. The city is situated at the altitude of between 300 and 600 m above sea level. It is within the derived savanna zone of Nigeria.

Animals and management

Sixty-four (64) weaned crossbred (New Zealand White X Chinchilla) rabbit bucks, aged 5 to 6 weeks, and with an average weight of 511.98 g were balanced for weight and allotted 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, denoted as minus (-) vitamin E and plus (+) vitamin E respectively. Animals were allowed an acclimatization period of one week during which they were fed with the control diet composed of treated against endo – and ecto-parasites. After the acclimatization period, the animals were first subjected to eight weeks feeding trial after which the diets were adjusted to that of growers' rabbit. The rabbits were then fed the diets for another twelve (12) weeks. The bucks were housed individually in wooden cages equipped with screened floors and raised to a height of 45 cm from the concrete floor. Feeds and clean, cool drinking water were supplied *ad libitum*. Feeding was done twice per day (0800 and 1600 h). Eight (8) bucks were allocated to each treatment in a 2 x 4 factorial arrangement with each animal serving as a replicate. The gross compositions of the experimental diets both at pre-pubertal and post-pubertal phases are shown in Tables 1 and 2.

Blood collection and analysis

At the end of 20th week of feeding, blood was collected from the ear vein of 3 rabbits per treatment using needle and syringe (23"G). The blood that was meant for haematological analysis was collected into plastic bottles coated with anticoagulant, ethylene di-amine acetic acid (EDTA). The blood that was meant for serum biochemical analysis was collected into bottles that did not contain anticoagulant.The haematological parameters determined were packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell (RBC) count, white blood cell (WBC) count and the differential leukocyte counts (lymphocytes, neutrophils, eosinophils and monocytes). The haematological constants, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from the PCV, RBC and Hb using the formula described by Jain (1986). The serum parameters analyzed were glucose, urea, creatinine, aspartate aminotransferase (AST), alanine amino-transferase (ALT), alkaline phosphatase (ALP) total protein, albumin, globulin and total cholesterol. The haematological parameters were analyzed as follows. The PCV was determined by centrifuging the micro haematocrit capillary tubes containing blood samples in a haematocrit centrifuge for 5 min and read with haematocrit reader. The RBC, WBC and the differential counts

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

Ingredient	CSC							
	- Vitamin				- Vitamin			
	T_1 (0%)	T_1 (5%)	T_1 (10%)	T_1 (15%)	T_1 (0%)	T_1 (5%)	T_1 (10%)	T_1 (15%)
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 D_3, 2500 IU; vitamin E, 50.00 mg; vitamin K_3, 2.50 mg; vitamin B_1, 3.00 mg; vitamin B_2, 6.00 mg; vitamin B_6, 6.00 mg; niacin, 40 mg; calcium pantothenate, 10 mg; biotin, 0.08 mg; vitamin B_{12}, 0.25 mg; folic acid, 1.00 mg; chlorine chloride,300 mg; manganese, 100 mg; iron, 50 mg; zinc, 45 mg; copper, 2.00 mg; iodine, 1.55 mg; cobalt, 0.25 mg; selenium, 0.10 mg; antioxidant, 200 mg.

were determined using improved Neubauer haemocytometer, while the Hb was determined using cyanmethaemoglobin method of Jain (1986).The blood samples for serum analysis were allowed to clot. The samples were then centrifuged for separation of serum. The sera obtained were stored in the refrigerator until needed for analysis. The total protein was determined using Biuret method (Tietz, 1995), while albumin level was determined by the Bromocresol green method (Toro and Ackermann, 1975; Tietz, 1995). Globulin was obtained by calculating the difference between total protein and albumin values. The ALT and AST activities were determined according to the method of Hoder and Rej (1983) and Rej and Hoder (1983). The ALP activities were determined by the method of McComb et al. (1983).

Statistical analysis

Data generated were subjected to analysis of variance (ANOVA), using the General Linear Model (GLM) of SAS (1999) for a 2 x 4 factorial within a completely randomized design. Means were separated by Duncan's option of the same statistical package.

RESULTS

Long-term effects of dietary cottonseed cake on the haematological parameters of rabbit bucks

The long-term effect of dietary cottonseed cake on the haematological parameters of rabbit bucks is shown in Table 3. Haemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), eosinophils and monocytes were not significantly ($P > 0.05$) affected by CSC levels in the diets. However, the PCV declined ($P < 0.05$) from 39.17% in rabbits that were fed the control diet to 33.67% in those that were fed 15% CSC. Rabbit bucks fed 5, 10 and 15% CSC had lower RBC values than those that fed the control diet.

White blood cell count increased significantly ($P < 0.05$) with increased CSC level in the diet. The MCHC value for the control group (30.97%) was significantly ($P < 0.05$)

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

Ingredient	- Vitamin				+ Vitamin E			
	T_1 (0%)	T_2 (5%)	T_3 (10%)	T_4 (15%)	T_5 (0%)	T_6 (5%)	T_7 (10%)	T8 (15%)
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
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
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 D_3, 2500 IU; vitamin E, 50.00 mg; vitamin K_3, 2.50 mg; vitamin B_1, 3.00 mg; vitamin B2, 6.00 mg; vitamin B6, 6.00 mg; niacin, 40 mg; calcium pantothenate, 10 mg; biotin, 0.08 mg; vitamin B_{12}, 0.25 mg; folic acid, 1.00 mg; chlorine chloride, 300 mg; manganese, 100 mg; iron, 50 mg; zinc, 45 mg; copper, 2.00 mg; iodine, 1.55 mg; cobalt, 0.25 mg; selenium, 0.10 mg; antioxidant, 200 mg.

Table 3. Long-term effect of dietary cottonseed cake (CSC) on the haematological parameters of rabbit bucks.

Parameter	T_1 (0% CSC)	T_2 (5% CSC)	T_3 (10% CSC)	T_4 (15% CSC)	SEM
Hb (g/100ml)	12.07	12.23	12.23	11.28	0.50
PCV (%)	39.17^{a}	35.17^{ab}	36.50^{ab}	33.67^{b}	1.43
RBC (x10^6/lμ)	5.85^{a}	5.45^{ab}	4.98^{b}	4.88^{b}	0.24
WBC (x10^3/μl)	5.43^{c}	5.71^{c}	6.67^{b}	7.22^{a}	0.16
MCV (fl)	67.99	64.89	73.95	69.68	3.77
MCH (μg)	20.92	22.53	24.84	23.45	1.27
MCHC (%)	30.97^{b}	34.76^{a}	33.60^{ab}	33.67^{ab}	1.13
Lymphocytes (%)	44.87^{c}	49.64^{b}	55.33^{a}	59.72^{a}	1.50
Neutrophils (%)	41.88^{a}	38.98^{a}	33.08^{b}	29.46^{b}	1.71
Eosinophils (%)	2.86	3.06	3.20	2.80	0.16
Monocytes (%)	8.87	8.33	8.40	8.03	0.40

$^{a,b:}$ Means along the same row with different superscripts differ significantly (P < 0.05).
Hb = Haemoglobin concentration; PCV = packed cell volume; RBC = red blood cell; WBC = white blood cell; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration.

lower than that of the 5% CSC group (34.76%), whereas MCHC for the bucks that fed on 10 and 15% CSC were not significantly different from those of bucks fed the control and 5% CSC diets. Cottonseed cake level increased lymphocyte counts significantly (P < 0.05). However, neutrophil count decreased (P < 0.05) with increased CSC level.

Interaction effect of prolonged feeding of cottonseed cake and vitamin E supplementation on the haematological parameters of rabbit bucks

The interaction effect of prolonged feeding of CSC and vitamin E supplementation on the haematological parameters of rabbit bucks is presented in Table 4.

Table 4. Interaction effect of long-term feeding of cottonseed cake and vitamin E supplementation on the haematological parameters of rabbit bucks.

Parameter	Vitamin E	CSC Level				SEM
		0%	5%	10%	15%	
Hb (g/100ml)	- vitamin	11.30	11.63	12.17	10.63	0.41
	+ vitamin	12.83	12.83	12.30	11.93	0.22
	SEM	0.59	0.68	0.35	0.35	
PCV (%)	- vitamin	37.33^{a}	33.67^{ab}	34.67^{ab}	31.67^{b}	1.25
	+ vitamin	41.00^{a}	36.67^{c}	38.33^{b}	35.67^{c}	0.82
	SEM	1.54	1.83	1.06	1.52	
RBC (x103/µl)	- vitamin	5.47	5.13	4.43	4.50	0.23
	+ vitamin	6.23	5.77	5.53	5.27	0.14
	SEM	0.30	0.27	0.27	0.31	
WBC (x103/µl)	- vitamin	5.43^{c}	5.83^{c}	6.53^{b}	8.18^{ax}	0.33
	+ vitamin	5.42^{b}	5.59^{b}	6.81^{a}	6.27^{aby}	0.20
	SEM	0.09	0.21	0.13	0.45	
MCV (fl)	- vitamin	69.86	66.07	73.37	70.93	2.87
	+ vitamin	66.13	63.71	69.52	68.43	2.09
	SEM	4.68	3.10	3.04	3.16	
MCH (µg)	- vitamin	21.09	22.78	27.43	24.07	1.15
	+ vitamin	20.76	22.27	22.26	22.83	0.63
	SEM	1.56	0.98	1.29	1.22	
MCHC (%)	- vitamin	30.54	34.49	35.05	33.81	0.92
	+ vitamin	31.39	35.03	32.14	33.54	0.73
	SEM	1.56	0.42	1.01	0.92	
Lymphocytes (%)	- vitamin	45.13^{c}	50.60^{bc}	57.47^{ab}	64.07^{ax}	2.42
	+ vitamin	44.61^{c}	48.67^{bc}	53.19^{ab}	55.36^{ay}	1.39
	SEM	0.79	0.90	2.16	2.45	
Neutrophils (%)	- vitamin	43.77^{a}	36.91^{ab}	30.94^{bc}	24.36^{cy}	2.42
	+ vitamin	39.99	41.04	35.22	34.56^{x}	1.31
	SEM	2.06	1.33	2.07	2.61	
Eosinophils (%)	- vitamin	2.79	3.13	3.14	3.01	0.13
	+ vitamin	2.93^{ab}	2.99^{a}	3.25^{a}	2.59^{b}	0.09
	SEM	0.12	0.16	0.15	0.18	
Monocytes (%)	- vitamin	8.44	9.36	8.45	8.56	0.29
	+ vitamin	9.29	7.29	8.34	7.50	0.32
	SEM	0.38	0.60	0.37	0.42	

^{a,b,c}: Means along the same row with different superscripts differ significantly (P < 0.05).
^{x,y}: Means within the same column with different superscripts for a variable differ significantly (p < 0.05).
Hb = Haemoglobin concentration; PCV = packed cell volume; RBC = red blood cell; WBC = white blood cell; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration.

Table 5. Long-term effect of dietary cottonseed cake on the serum parameters of rabbit bucks.

Parameter	T_1 (0% CSC)	T_2 5% CSC)	T_3 (10% CSC)	T_4 (15% CSC)	SEM
Glucose (mmol/*L)*	2.80	2.63	2.72	2.53	0.13
Urea (mmol/*L)*	6.95^{a}	6.20^{b}	6.27^{b}	5.87^{b}	0.14
Creatinine (µmol/*L)*	66.50^{a}	62.17^{b}	67.00^{a}	63.67^{ab}	1.15
ALT (IU/L)	31.67^{b}	40.67^{a}	40.83^{a}	45.17^{a}	2.02
AST (IU/L)	49.00^{b}	52.33^{ab}	55.17^{a}	55.17^{a}	1.79
ALP (IU/L)	136.33	131.17	133.83	134.00	1.96
Albumin (mg/L)	35.17^{a}	26.17^{b}	26.33^{b}	25.33^{b}	1.15
Globulin (mg/L)	30.67	33.17	31.83	30.50	1.27
Total Protein (mg/L)	66.00^{a}	59.33^{b}	58.17^{b}	55.83^{b}	1.51
Total cholesterol (mmol/L)	28.17	28.33	27.00	29.50	1.11
Albumin/Globulin	1.16^{a}	0.80^{b}	0.84^{b}	0.84^{b}	0.05

$^{a,b,c:}$ Means along the same row with different superscripts differ significantly (P < 0.05).
AST = Aspartate transaminase; ALT = alanine transaminase; ALP = alkaline phosphatase.

Haemoglobin concentration was not significantly (P>0.05) influenced by the interaction effect. However, at all levels of CSC, vitamin E supplementation showed a non-significant improvement in haemoglobin concentration of rabbit bucks.The PCV values across the CSC levels without vitamin E supplementation were significantly (P < 0.05) different from one another. Corresponding values of PCV for the bucks that were supplemented with vitamin E were significantly (P < 0.05) different from each other. Although supplemented bucks had higher PCV values than the non-supplemented bucks at all CSC levels, the differences were not significant (P > 0.05).The interaction between CSC and vitamin E supplementation did not significantly (P > 0.05) affect the RBC of the bucks. However, at all CSC levels, vitamin E supplementation tended to increase the RBC. White Blood Cell count was significantly (P < 0.05) influenced by the interaction of CSC and vitamin E. Variations between the bucks that were fed 0, 5, 10 and 15% CSC with and without vitamin E supplementation were significant (P < 0.05) for WBC. At 15% CSC, vitamin E supplementation reduced the WBC value significantly (P < 0.05).The MCV values for bucks fed varying levels of CSC with or without vitamin E supplementation were similar to one another. Variations between the CSC levels supplemented and non-supplemented with vitamin E for MCH and MCHC were not significant (P > 0.05). Lymphocyte count increased with increased CSC level, with and without vitamin E supplementation. However, vitamin E supplementation lowered lymphocyte count at all CSC levels investigated, the reductions being significant (P < 0.05) only at 15% level.On the contrary, neutrophils decreased significantly (P < 0.05) with increase in CSC level without vitamin E supplementation. At 15% CSC level, vitamin E supplementation recorded a significantly (P < 0.05) higher neutrophil count than that of the non-supplemented counterparts. Eosinophils was similar for the non-supplemented CSC groups but was significantly (P < 0.05) different among the supplemented CSC groups.

Long-term effect of dietary cottonseed cake on the serum parameters of rabbit bucks

The long-term effect of dietary cottonseed cake on the serum parameters of rabbit bucks is presented in Table 5. Serum glucose, alkaline phosphatase and total cholesterol were not significantly (P > 0.05) affected by CSC level. Serum urea was significantly (P < 0.05) depressed from 6.95 mmol/L for the control group to 5.87 mmol/L in the 15% CSC group. Creatinine was significantly (P < 0.05) affected by CSC with a sinusoidal trend. The control and 10% CSC groups had similar values which were significantly (P < 0.05) different from those of 5 and 15% groups.

The ALT and AST were significantly (P < 0.05) influenced by CSC level. The ALT value for 5, 10, and 15% groups were significantly (P < 0.05) higher than that of the control group but were statistically similar to one another. The AST value for the control group was significantly (P < 0.05) lower than the value for 5, 10, and 15% groups. While the albumin and total protein values were significantly (P < 0.05) lowered by CSC level, serum globulin was not significantly affected. Albumin/globulin ratio for 0% group (1.16) was significantly (P < 0.05) higher than for the other groups which were not significantly (P < 0.05) different from one another.

Interaction effects of prolonged feeding of cottonseed cake-based diet and vitamin E supplementation on the serum parameters of rabbit bucks

The interaction effect of long-term feeding of cottonseed cake and vitamin E supplementation on the serum parameters of rabbit bucks is presented in Table 6. Rabbit bucks fed 0, 5, 10 and 15% CSC without vitamin E had similar ($P > 0.05$) glucose values. Vitamin E supplementation at different CSC levels did not have significant ($P > 0.05$) effect on serum glucose except at 15% level ($P < 0.05$). Serum urea values were significantly ($P < 0.05$) different between rabbit bucks fed different levels of CSC with and without vitamin E supplementation. The corresponding values of serum urea for the vitamin–supplemented bucks were not significantly ($P > 0.05$) different from those of non-supplemented animals.

Creatinine values across CSC levels without vitamin E supplementation were not significantly ($P > 0.05$) different but those with vitamin E supplementation had significant ($P < 0.05$) variations with no definite pattern across the CSC levels. Except at 10% CSC level, vitamin E supplementation lowered the creatinine level in the serum of the treated bucks.

Variations between the bucks exposed to different levels of CSC with and without vitamin E were significant ($P < 0.05$) for ALT. Bucks fed 0% CSC with vitamin E supplementation had significantly ($P < 0.05$) lower ALT than those fed 5, 10 and 15%. At all levels of CSC, vitamin E supplementation tended to lower the AST ($P<0.05$). Increased CSC level without vitamin E supplementation significantly ($P < 0.05$) lowered AST. The ALP values across the CSC levels without vitamin E supplementation were comparable ($P > 0.05$). Although at all CSC levels, vitamin E supplementation increased the ALP, the increase was significant ($P < 0.05$) only at 10%.

Total protein and albumin for rabbit bucks fed 0% CSC without vitamin E supplementation was significantly ($P<0.05$) higher than those fed 5, 10 and 15%, which were not different ($P>0.05$) from one another. At all levels, vitamin E supplementation did not increase ($P > 0.05$) serum total protein. At all levels, vitamin E supplementation did not boost serum albumin. Serum globulin and total cholesterol were not significantly ($P > 0.05$) affected by the interaction of CSC and vitamin E supplementation. Albumin/Globulin ratio for bucks that were fed control diet with or without vitamin E supplementation was significantly ($P < 0.05$) higher than for those fed 5, 10 and 15% CSC with or without vitamin E supplementation.

DISCUSSION

The long-term feeding of CSC from this study revealed a significant decline in the values of PCV and RBC. This depression in PCV and RBC values with increasing CSC level could be attributed to the presence of gossypol in the test ingredient. This result is consistent with the report of Velasquez-Pereira et al. (1998) but in contrast with the report of Amao et al. (2009) for pre-pubertal rabbit bucks. However, the PCV values were within the normal physiological range for mature rabbit bucks (Mitruka and Rawnsley, 1977). The value for RBC at 0% CSC (control) was within the normal reference range, while the value fell progressively below the normal reference range from 5% CSC to 15% CSC. This reduction in PCV and RBC also indicates low protein intake or availability and mild anaemia as reported by Maxwell et al. (1990). Similar observation has been reported for chickens (Adeyemo, 2008). The Hb concentrations obtained from this study were within the normal physiological range reported by Mitruka and Rawnsley (1977) for adult rabbits. The similarity in the Hb values among the treatments suggests that although the PCV and RBC were depressed by CSC, oxygen-carrying capacity of the blood was not adversely affected.

The WBC was significantly increased and was within the normal range (Mitruka and Rawnsley, 1977). This increment could be related to a response to increased gossypol in the diets of the bucks. The increased lymphocyte count with increasing CSC level was associated with the phagocytic property of the WBC. The decrease in neutrophil count suggests neutropenia in the cottonseed-fed animals. This decreased neutrophil counts in animals could be due to the presence of gossypol in the cottonseed-based diets compared with the control. Decreased neutrophil production by bone marrow resulting in neutropenia could be due to damage by toxins usually after repeated or prolonged exposure among other factors (Bush, 1991).

The observation in this study that vitamin E supplementation increased PCV was consistent with the report of Velasquez-Pereira et al. (1998). This increase in PCV value at corresponding CSC level by vitamin E could be due to the effect of vitamin E which counteracted the effect of gossypol in the CSC-based diets. Also, the reduced WBC values by vitamin E supplementation could be due to the effect of the vitamin E in reducing the toxic effect of gossypol in the diets; thereby limiting the WBC that were elicited.

The values obtained for serum glucose in this study were similar among dietary CSC levels. They were however, higher than the reported values for physiologically normal male rabbits (Mitruka and Rawnsley, 1977). The similarity in the glucose values could be due to the isocaloric nature of the experimental diets. The higher values in this study as against the reported values suggest that the diets were adequate in energy supply to the animals. The higher values of serum glucose for the groups of bucks that were supplemented with vitamin E over the non-supplemented groups indicate that vitamin E exerted an effect on the energy utilization by the bucks. This effect could be adduced to the antioxidant property of vitamin E to counter any negative effect of the gossypol in the CSC-based diets.

The observed decrease in serum urea with increasing CSC level with and without vitamin E supplementation

Table 6. Interaction effects of long-term feeding of cottonseed cake and vitamin E supplementation on the serum parameters of rabbit bucks.

Parameter	Vitamin E	CSC Level				SEM
		0%	5%	10%	15%	
	- vitamin	2.90	2.50	2.40	2.27^{y}	0.10
Glucose (mmol/L)	+ vitamin	2.70	2.77	3.03	2.80^{x}	0.09
	SEM	0.10	0.15	0.20	0.15	
	- vitamin	6.93^{a}	6.13b	6.20^{b}	5.80^{b}	0.15
Urea (mmol/L)	+ vitamin	6.97^{a}	6.27b	6.33^{ab}	5.93^{b}	0.14
	SEM	0.10	0.16	0.14	0.07	
	- vitamin	70.33^{x}	68.33^{x}	68.33	67.33^{x}	0.66
Creatinine (µmol/L)	+ vitamin	62.67^{ay}	56.00^{by}	65.67^{a}	60.00^{aby}	1.33
	SEM	1.84	3.12	1.03	1.89	
	- vitamin	35.33^{bx}	43.33^{ab}	44.33^{abx}	48.67^{ax}	1.88
ALT (IU/L)	+ vitamin	28.00^{by}	38.00^{a}	37.33^{aby}	41.67^{ay}	1.96
	SEM	1.84	2.82	2.32	2.30	
	- vitamin	51.67^{bx}	55.33^{abx}	59.00^{ax}	59.67^{ax}	1.64
AST (IU/L)	+ vitamin	46.33^{by}	49.33^{ay}	51.33^{ay}	50.67^{ay}	0.96
	SEM	1.97	2.16	2.55	2.34	
	- vitamin	133.67	128.67	129.00^{y}	131.67	1.29
Alkaline phosphatase (IU/L)	+ vitamin	139.00	133.67	138.67^{x}	136.33	1.39
	SEM	2.91	1.60	2.48	1.88	
	- vitamin	65.67^{a}	59.33^{ab}	58.00^{b}	55.33^{b}	1.47
Total protein (mg/L)	+ vitamin	66.33^{a}	59.33^{b}	58.33^{b}	56.33^{b}	1.45
	SEM	1.61	1.36	1.62	0.54	
	- vitamin	34.33^{a}	27.00^{b}	26.33^{b}	25.00^{b}	1.15
Albumin (mg/L)	+ vitamin	36.00^{a}	25.33^{b}	26.33^{b}	25.67^{b}	1.42
	SEM	0.75	0.70	0.42	0.76	
	- vitamin	31.33	32.33	31.67	30.33	0.91
Globulin (mg/L)	+ vitamin	30.00	34.00	32.00	30.67	1.04
	SEM	1.50	1.45	1.56	0.85	
	- vitamin	27.67	28.00	27.33	30.00	0.69
Total cholesterol (mmol/L)	+ vitamin	28.67	28.67	26.67	29.00	0.77
	SEM	1.60	0.88	0.58	0.62	
	- vitamin	1.11^{a}	0.84^{b}	0.84^{b}	0.84^{b}	0.05
Albumin/Globulin Ratio	+ vitamin	1.22^{a}	0.75^{b}	0.84^{b}	0.84^{b}	0.06
	SEM	0.07	0.05	0.04	0.05	

$^{a,b,c:}$ Means along the same row with different superscripts differ significantly ($P < 0.05$). $^{x,y:}$ Means within column with different superscripts for a variable differ significantly ($P<0.05$).
AST = Aspartate transaminase; ALT = alanine transaminase; ALP = alkaline phosphatase.

could be attributed to increasing gossypol level accompanying the CSC levels. It has been reported (Iyayi and Tewe, 1998) that the nature of dietary protein influenced blood urea levels of normal subjects as the varying levels of urea observed among treatments could be due to the nature of the amino acids in the protein thereby varying the quality of proteins in the experimental diets.

The reduced level of total protein below the normal standard values of 6.00 to 8.30 g/dl (60.00 to 83.00 mg/L) as reported by Mitruka and Rawnsley (1977) is an indication that the bucks exposed to CSC suffered significantly from inadequate synthesis and concentration of serum total protein and albumin which suggests hypoproteinemia and hypoalbuminemia conditions in the animals. This implies that there was an alteration in protein metabolism of the animals, since protein synthesis is related to the amount of available protein (Iyayi and Tewe, 1998) in the diet. The gossypol in CSC probably inhibited the metabolism of protein in the diets. The observation that the groups of animals that received vitamin E supplementation had comparable values for total protein and albumin indicates that vitamin E supplementation did not enhance the protein metabolism for synthesis of total protein and albumin.

The significant increase in ALT and AST with increasing level of CSC, although within the reported range (Mitruka and Rawnsley, 1977) is an indication that the rabbits suffered some tissue damage in the heart, kidney and/or liver due to cellular destruction by gossypol toxicity. The corresponding decrease in the values of ALT and AST by vitamin E supplementation suggests the capability of vitamin E to prevent tissue damage in the vital organs (heart, kidney and liver) of the body when CSC is fed to rabbit bucks. Hence it could be deduced that vitamin E exhibited the potential to ameliorate the adverse effect of gossypol in the CSC.

The non-significant variation in ALP values for different CSC levels with or without vitamin E suggests that the CSC had no significant influence on the serum alkaline phosphatase. Alkaline phosphatase is a membrane-bound enzyme that is used to diagnose bone and liver disorders as well as exposure to drug. Production of ALP is increased in response to primary or secondary hepatocellular disorders. The observation from this study contradicts the report of Gawai et al. (1995) that gossypol depressed liver microsomal enzymes of rats that were treated with 5 mg of gossypol for 5 days. It also disagrees with the report of Velasquez-Pereira et al. (1998) who observed that CSC with and without vitamin E reduced alkaline phosphatase. The observed significant variation in the values of creatinine for the animals fed both supplemented and non-supplemented CSC indicates that the animals suffered from muscle wastage or disorders. Gossypol could cause oedema, hypertrophy and dilation of heart and cardiac muscle degeneration in several species (Abou-Donia, 1989). At corresponding levels in this study, vitamin E significantly reduced the creatinine values. This suggests the potential of vitamin E in inhibiting the action of gossypol to cause muscle wastage. This observation disagrees with the report of Velasquez-Pereira et al. (1998) who reported non-significant variations for creatine kinase among bulls fed cottonseed meal with and without vitamin E supplementation.

It was concluded that prolonged feeding of CSC – based diets to rabbit bucks had adverse effects on blood constituents of the bucks. However, supplementing such diets with 30 mg/kg diet of vitamin E ameliorated the adverse effects.

Abbreviations

CSC, Cottonseed cake; **PCV,** packed cell volume; **RBC,** red blood cell; **Hb,** haemoglobin concentration; **WBC,** white blood cell; **ALT,** alanine transaminase; **AST,** aspartate transaminase, **ME,** Metabolizable energy.

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