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Haematological Response of Pre-pubertal Rabbit Bucks Fed Cottonseed Cake-based Diets Supplemented with Vitamin E

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Abstract: An investigation was conducted in a 2 x 4 factorial experiment to evaluate the effect of Cottonseed Cake (CSC) with or without vitamin-E supplementation on the haematological parameters of pre-pubertal rabbit bucks. The experiment involved 8 treatment combinations comprising four CSC levels (0, 5, 10 and 15%) and two vitamin-E supplementation levels (0 and 30 mg/kg diet) denoted as minus (-) vitamin-E and plus (+) vitamin-E. A feeding trial lasted for eight weeks after which blood was collected from the animals and analyzed for Haemoglobin concentration (Hb), Packed Cell Volume (PCV), Red Blood Cell Count (RBC) and White Blood Cell Count (WBC). Analysis of variance showed that CSC had significant ($p<0.05$) depressing effect on Hb, while WBC values increased ($p<0.05$) with increasing level of CSC. The PCV and RBC were not significantly affected by CSC. Vitamin-E supplementation did not have significant ($p>0.05$) effect on the haematological parameters of the rabbit bucks. Interaction effect of CSC and vitamin-E did not affect ($p>0.05$) HB, PCV and RBC while only WBC was significantly ($p<0.05$) affected by the interaction effect. It was concluded that CSC had adverse effect on some haematological parameters and that vitamin-E showed a tendency to correct the adverse effect. Probably a higher dose of vitamin-E is required to elicit a greater ameliorative affect.

Key words: Haematology, pre-pubertal, rabbit bucks, cottonseed cake, vitamin E

INTRODUCTION

Cottonseed Cake (CSC) is a potential source of protein, energy and fibre for a variety of livestock species (NCPA, 1995). However, it contains gossypol, a polyphenolic factor of great physiological and metabolic importance, which restricts its use to ruminant feeding. Nutrition has been recognized as one of the factors that can cause alterations in the biochemical and physiological processes that could compromise the stability and functions of blood constituents, with the consequent negative effects on the health of the subject. Thus, blood analysis is a useful diagnostic tool for assessing the health status of an animal, for clinical evaluation, for survey (Hail, 1979) and physiological/pathological conditions (Cole, 1986). According to Mitruka and Rawnsley (1977), blood is an important index of physiology and pathological changes in the organism. Cottonseed cake has been reported to impact negatively on blood parameters in cattle (Lindsey *et al*., 1980; Gray *et al*., 1993; Velasquez-Pereira *et al*., 1998). This was attributed to the presence of gossypol. Diets containing gossypol have been reported to result in lower haemoglobin concentration and high erythrocyte osmotic fragility. Chase *et al*. (1994) reported that haematological effect of gossypol included anaemia with reduced number of red blood cells and increased red blood cell fragility. The implication of this is severe reduction in oxygen-carrying and-releasing capacity of the blood.

Vitamin-E is an antioxidant that has been reported to improve antioxidative status of live pigs (Lauridsen *et al*., 1999). Since one of the major functions of vitamin-E is to protect and preserve the integrity of cell membrane, probably vitamin E could ameliorate or completely counter the adverse effect of gossypol contained in cottonseed cake. The objective of this study therefore, was to evaluate the effect of CSC with or without vitamin-E supplementation on the haematological characteristics of pre-pubertal rabbit bucks.

MATERIALS AND METHODS

Sixty-four (64) weanling crossbred (New Zealand White x Chinchilla) rabbit bucks, aged 5-6 weeks, were balanced for weight and allocated to eight treatment combinations comprising four levels of cottonseed Cake (CSC) (0, 5, 10 and 15%) and 2 levels of vitamin-E supplementation (0 and 30 mg/kg diet) denoted as

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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
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, 50.00 mg; vitamin K3, 2.50 mg; vitamin B1, 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 B12, 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. ME = Metabolizable Energy

minus (-) vitamin-E and plus (+) vitamin-E respectively. After a period of one week of acclimatization, rabbits were subjected to an eight week feeding trial. Table 1 shows the gross composition of the experimental diets. At the end of the eight week trial, three (3) animals from each treatment were bled for haematological assessment. Blood was collected from the ear vein of the bucks using a needle and syringe. The blood was collected into plastic bottles containing anticoagulant-Ethylene Diamine Tetra Acetic acid (EDTA) and was immediately cooled in an ice-pack and taken to the laboratory for analysis.

The haematological parameters determined wee Packed Cell Volume (PCV), Haemoglobin concentration (Hb), Red Blood Cell Count (RBC) and White Blood Cell Count (WBC). The PCV was determined using Wintrobe microhaematocrit centrifugation method of Jain (1986) and Wintrobe (1988). The Hb was measured by the cyanmethaemoglobin (PYE Unicom, England). The RBC and WBC counts were determined using the Neubauer haemocytometer method as described by Jain (1986).

Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) which are derived variables were calculated from PCV, Hb and RBC using appropriate formulae as described by Jain (1986).

Data obtained were subjected to Analysis of Variance (ANOVA) using the General Linear Model (GLM) for 2 x 4 factorial in a completely randomized design (SAS, 1998). Means were separated by Duncan's multiple range option of the same software.

RESULTS AND DISCUSSION

The results of this investigation are presented in Table 2-4. The main effect of cottonseed cake on haematological parameters of pre-pubertal rabbit bucks is shown in Table 2. Cottonseed cake level had significant ($p<0.05$) effect on Hb and WBC. Haemoglobin concentration was significantly ($p<0.05$) lower for bucks that were fed 5% CSC, but non-significantly ($p>0.05$) for those that were fed 10% and 15% CSC than those that were fact the control diet. The value of WBC increased significantly ($p<0.05$) from $6.41x10^3/\mu$ for the control to $9.69x10^3/\mu$ for 15% CSC. The lower Hb value for the bucks that were fed with CSC is an indication that CSC inclusion in the diet has a tendency to reduce oxygen-carrying and-releasing capacity of blood. The significant increase in WBC value with increasing CSC level indicates the presence of toxin, to which the body reacted by increasing the number of white blood cells to counter the effect of the toxin. The PCV and RBC were not significantly ($p>0.05$) affected by CSC level. This shows that erythropoiesis was not impaired in rabbit bucks. Platelet counts for rabbits that were fed 5% CSC (242.00) was significantly ($p<0.05$) lower than that of the control (343.83) but increased non-significantly with increased level of CSC. The blood constants namely MCV, MCH and MCHC were significantly affected by CSC level. Rabbits that were fed 5%, 10% and 15% CSC had significantly ($p<0.05$) higher MCV values than that of the control but they were not significantly ($p>0.05$) different from one another. The value of MCH increased $p<0.05$) with increasing level of CSC. Rabbits fed 10% and 15%

Table 2: Effect of cottonseed cake level on the haematological parameters of pre-pubertal rabbit bucks

Parameter	T_1 (0% CSC)	T_2 (5% CSC)	T_3 (10% CSC)	T_4 (15% CSC)	SEM
Hb (g/100 ml)	12.82^{a}	10.68^{c}	11.57^{ab}	11.57^{ab}	0.51
PCV (%)	36.67	32.00	33.67	32.17	1.48
RBC (x10^{6}/µl)	4.57	4.23	4.30	4.15	0.21
WBC (x10^{3}/µl)	6.41^{c}	7.80^{b}	9.17^{a}	9.69^{a}	0.24
Platelets	343.83^{b}	242.00^{b}	292.67^{ab}	316.83^{ab}	28.35
MCV (fl)	65.43^{b}	77.17^{a}	78.85^{a}	81.77^{a}	3.44
MCH (µg)	23.08^{b}	25.55^{ab}	27.23^{a}	27.55^{a}	1.94
MCHC (%)	34.83^{a}	33.00^{b}	34.17^{a}	34.67^{a}	0.36

^{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

Table 3: Effect of vitamin E supplementation on the haematological prarameters of pre-pubertal rabbit bucks fed cottonseed cake based diets

Parameter	-Vitamin E	+Vitamin E	SEM
Hb (g/100 ml)	11.39	11.39	0.36
PCV (%)	32.58	34.67	1.05
RBC (x10^{6}/µl)	4.24	4.38	0.15
WBC (x10^{3}/µl)	8.51	8.03	0.17
Platelets	313.58	284.08	20.04
MCV (fl)	72.05^{b}	79.56^{a}	2.43
MCH (µg)	24.43^{b}	27.28^{a}	0.85
MCH (%)	33.83^{b}	34.50^{a}	0.26

^{a,b}Means along the same row with different superscript 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 4: Interaction effect of CSC and vitamin E supplementation on the haematological parameters of pre-pubertal rabbit bucks

		CSC Level				
Parameter	Vitamin E	0%	5%	10%	15%	SEM
Hb (g/100 ml)	-Vitamin	12.10	1.13	11.37	11.97	0.35
	+Vitamin	13.53	11.23	11.77	11.17	0.45
	SEM	0.46	0.40	0.61	0.53	
PVC (%)	-Vitamin	35.67	30.67	32.67	31.33	0.98
	+Vitamin	37.67	33.33	34.67	33.00	1.13
	SEM	0.96	1.03	1.73	1.72	
RBC (x10^{6}/Nl)	-Vitamin	4.53	4.27	4.02	4.15	0.42
	+Vitamin	4.60	4.20	4.59	4.14	0.15
	SEM	0.59	0.21	0.30	0.08	
WBC (x10^{3}Nl)	-Vitamin	8.73^{a}	7.73^{ab}	9.23^{ax}	6.50^{b}	0.38
	+Vitamin	7.90	7.10	6.40^{y}	7.00	0.25
	SEM	0.43	0.32	0.68	0.28	
Platelet	-Vitamin	350.67	235.67	291.33	376.67^{x}	24.40
	+Vitamin	337.00	248.33	294.00	257.00^{y}	19.27
	SEM	16.90	34.40	22.29	36.60	
MCV (fl)	-Vitamin	49.20^{cy}	73.00^{b}	82.00^{a}	84.00^{a}	4.33
	+Vitamin	81.67^{x}	81.33	75.70	79.53	2.78
	SEM	7.42	5.26	1.98	3.23	
MCH (Ng)	-Vitamin	16.83^{cy}	23.77^{b}	28.67^{a}	28.43^{a}	1.49
	+Vitamin	29.33^{x}	27.33	25.80	26.67	1.03
	SEM	2.86	1.87	1.01	0.94	
MCHC (%)	-Vitamin	33.67^{ay}	32.00^{b}	34.67^{a}	35.00^{a}	0.41
	+Vitamin	36.00^{ax}	34.00^{b}	33.67^{b}	34.33^{ab}	0.36
	SEM	0.60	0.45	0.54	0.33	

^{a,b}Means along the same row with different superscript differ significantly ($p<0.05$).

^{x,y}Means within the same column with different superscripts differ significantly ($p<0.055$). Hb = Haemoglobin concentration: PCV = Packed Cell Volume; RBC = Red Blood Count; WBC = White Blood Cell Counts; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Haemoglobin; MCHC = Mean Corpuscular Haemoglobin Concentration

CSC had significantly ($p<0.05$) higher values than the control group but they were not different from each other. Rabbit group on 5% CSC exhibited a comparable value to the control and those of higher CSC levels (10% and 15%). For MCHC, rabbit groups fed 10% and 15% CSC showed similar values to that of the control, but were

significantly ($p<0.05$) higher than that of 5%CSC group. The PCV and RBC were not significantly ($p>0.05$) affected by CSC. The result that the haematological constants (MCV, MCH and MCHC) revealed significant differences among 0, 5, 10 and 15% CSC groups suggests variations in the sizes of the RBC. Probably gossypol caused alterations in the sizes of the RBCs. The values for MCV and MCH were higher than normal physiological range reported for the young adult bucks (Mitruka and Rawnsley, 1977). However, MCHC values for 0, 5, 10 and 15% CSC were within the normal range. The effect of vitamin-E supplementation on the haematological parameters of rabbit bucks that were fed CSC based diets is shown in Table 3. Vitamin-E supplementation did not have any significant ($p>0.05$) effect on Hb, PCV, RBC, WBC and platelet count; although bucks that were supplemented had higher values for Hb, PCV and RBC and lower value for WBC than those bucks that were not supplemented with vitamin-E. However the three haematological constants (MCV, MCH and MCHC) were significantly ($p<0.05$) affected by vitamin E supplementation with the supplemented group exhibiting higher values. Supplementation of CSC based diets with vitamin E exhibited a lowering effect on the WBC values. This suggests that vitamin E possibly countered the effect of gossypol such that the WBC levels became normalized. Table 4 shows the interaction effects of CSC level and vitamin-E supplementation on the haematological parameters or pre-pubertal rabbit bucks. Haemoglobin concentration, PCV and RBC were not significantly ($p>0.05$) affected by the interaction of the CSC and vitamin-E supplementation, although at all CSC levels, vitamin-E tended to increase the Hb, PCV and RBC values. White blood cell count was significantly ($p<0.05$) affected by the interaction. At 10% CSC levels, vitamin-E lowered WBC significantly ($p<0.05$), while the reduction at 0% and 5% was not significant ($p>0.05$). It could be concluded that CSC had adverse effect on some haemotological parameters. Vitamin-E showed tendency to ameliorate the adverse effect of CSC. However, probably there is need for higher dose of vitamin-E to elicit a greater ameliorative effect on the haematological parameters. The interaction effect of CSC and vitamin E supplementation revealed that Hb, PCV and RBC were not significantly ($p>0.05$) affected. However, MCV, MCH and MCHC were significantly affected. Bucks that were fed 0, 5, 10 and 15% CSC without vitamin E supplementation had significantly ($p<0.05$) different MCV and MCH, while their counterparts that were supplemented with vitamin E were not significantly different from one another. This result indicates that vitamin E exhibited a normalizing effect on the variables.

Conclusion: It could be concluded from this study that, cottonseed cake had adverse effect on some haematological parameters of pre-pubertal rabbit bucks. Vitamin E supplementation at 30 mg/kg diet showed a tendency to correct the adverse effect. However, it appears that a higher level of vitamin E might be required to elicit a greater ameliorative effect. Otherwise it is not advisable to feed cottonseed cake to pre-pubertal rabbit bucks.

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