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Physiological Responses of Rabbit Bucks Fed Diets Containing Cotton Seed Cake Supplemented with Carrot (Daucus Carota) or Ginger (Zingiber Officinale)

Amao, O. A[1] Iyanda, A.R.[2] Raheem, L.A.[2]
1.Department of Animal Nutrition and Biotechnology
Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso Oyo State, Nigeria
2. Department of Animal Production and Health, Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso Oyo State, Nigeria

Abstract
Twenty-four (24) weaner rabbit bucks, aged 6 – 7 weeks were used to investigate the physiological responses of rabbit bucks fed diets containing cottonseed cake supplemented with carrot or ginger. The bucks were allotted to four (4) dietary treatments of six (6) rabbits each. The treatment diets (T_1, T_2, T_3 and T_4) contained 16% crude protein such that soyabean meal (SBM) was the main protein source for the control diet (T_1). In T_2, the SBM was completely replaced with Cottonseed cake (CSC). In T_3 (CSC + Carrot), 5g/ kg diet of carrot was added to the composition in T_2; while in T_4 (CSC + Ginger), 5g/ kg diet of ginger was added to the composition in T_2. Animals were fed for nine (9) weeks after which blood was collected for both haematological and serum biochemical analyses. The haematological variables determined were Packed Cell Volume (PCV), Red Blood Cell count (RBC), Haemoglobin concentration (Hb), Platelet count, White Blood Cell count (WBC) and the differential counts. The haematological indices, Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV), and Mean Corpuscular Haemoglobin Concentration (MCHC), were also determined. The serum biochemical variables determined included total protein, albumin, urea, creatinine, aspartate transaminase (AST), alanine transaminase (ALT), cholesterol, triglyceride and glucose. The results indicated that lymphocytes, neutrophils and platelet counts were significantly ($P<0.05$) affected by treatment. Lymphocyte count for T_4 (CSC + Ginger) was significantly ($P<0.05$) higher (78.0%) than for other treatments which were not significantly different from one another. Neutrophils for T_4 was significantly ($P<0.05$) lower (18.0%) than for others (29.0; 30.7 and 30.7% for T_1, T_2 and T_3 respectively). Platelet count was significantly ($P<0.05$) depressed by CSC (T_2). Albumin and urea were depressed ($P<0.05$) by CSC (T_2) and CSC + Ginger (T_4). Creatinine was significantly ($P<0.05$) elevated by T_3 (1.24mg/dl). The AST and ALT increased significantly ($P<0.05$) from 20.77 to 43.30 iu/L and 21.42 to 43.17 iu/L, respectively in T_1 and T_4. Cholesterol level was significantly ($P<0.05$) higher for T_2 than for other treatments. It was concluded that CSC had adverse effects on the serum biochemical parameters of rabbit bucks. Carrot or ginger supplementation demonstrated a potential to correct the adverse effect with ginger exhibiting a greater influence, suggesting the need to always supplement CSC-based diets for rabbits with either of the products to safeguard the health of the animals.
Keywords: Haematology, Serum biochemistry, cottonseed cake, carrot, Ginger, Rabbit buck.

INTRODUCTION

The shortage of animal protein intake below the recommended level in developing countries including Nigeria could be overcome by production of fast growing and early maturing animals such as rabbits. The prolific nature of rabbit coupled with its short gestation period and interval makes it suitable for multiplication and a fast way of increasing animal protein intake in Nigeria (Egbo *et al.*, 2001).

Anthony *et al.* (1999) observed that in commercial production, rabbit is close to modern broiler chicken in terms of growth rate, feed conversion efficiency and meat quality. Although rabbit can be raised on forage-based diet, the poor and unbalanced quality of grasses is a major constraint which limits the commercial production of rabbits. Cheeke and Raharjo (1988) concluded from a review of rabbit production on tropical feed resources that tropical grasses were unsuitable as a sole feed for rabbit due to their low digestibility (less than 10%) and seasonality hence the need for concentrate preparation for the rabbit.

In feed manufacturing, the major sources of protein for non-ruminant feeding such as soya bean meal and groundnut cake are becoming increasingly expensive due to competition between livestock and man for the feedstuffs. Cottonseed cake (CSC) is a potential source of protein for livestock and it is not consumed by man.

According to NCPA, (1995) CSC is an excellent source of protein, energy and fibre for a variety of livestock species. It is rich in crude protein (35 – 46%), contains over 1% phosphorus and 70 – 80% TDN (Coppock *et al.*, 1987; NCPA, 1995). Bamigbose (1995) reported that CSC contained 36.15% crude protein, 19.96% crude fibre, 14.42% crude fat (ether extract) while the biological value was 51.00%. Despite the rich nutritional potential of the CSC, its utilization for animal feeding has been largely restricted to ruminant feeding. This is due to the presence of gossypol, a polyphenolic compound of great physiological implications (Chase *et* *al*., 1994). The negative effect of gossypol in CSC on animal health and reproduction has long been recognized and the adverse effect is much more severe for non-ruminants than ruminants. The ruminants have the ability to bind gossypol to soluble protein in their rumen and so render it harmless in the body (Willard *et al*., 1995).

Gossypol has been reported to impact varying degrees of deleterious effects on animal health. The negative effects of gossypol on animal's body include growth depression, intestinal and other internal organ abnormalities, (Berardi and Goldblatt, 1980; Robinson *et al*., 2001), inhibition of digestive enzymes (Ryan *et al*., 1986), haematological and serum biochemical characteristics (Amao *et al*., 2012) and reproductive impairment (Taha *et al*., 2006; Amao *et al*., 2012). According to Amao *et al*. (2012), increasing level of CSC in diet of rabbit buck depressed packed cell volume (PCV), red blood cell count (RBC), serum total protein and albumin; while it caused significant increase in alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Antioxidants such as vitamin E and selenium have been found to be useful in ameliorating the adverse effects of gossypol in CSC when consumed by non-ruminants (Amao, *et al*., 2010; 2012). This goes on to suggest that if natural sources of antioxidants could be consumed along with or prior to consumption of diets containing CSC, the mechanism that utilized the synthetic antioxidants to ameliorate the adverse effect of CSC, would also take advantage of the natural antioxidant in the feed to either inactivate or completely neutralize the gossypol in the CSC. More so, the recent global campaign is in favour of consumption of natural or organic agricultural products.

Such natural sources of antioxidants include carrot (*Daucus carota*) and ginger (*Zingiber officinale*). Carrot as a source of antioxidants slows down the ageing process, contains beta-carotene and it is broken down to release vitamins A, B, C, K, B_2, B_6, biotin, potassium and thiamine in the body (Gaziano *et al*., 1995). Ginger, a rhizome plant, has high content of anti-oxidant, it inhibits platelets aggregation and so it is an ideal condiment for people predisposed to blood clotting which may lead to either heart attack or stroke.

There is dearth of technical information on the use of carrot and or ginger supplementation in CSC-based diets to mitigate the deleterious effects of CSC occasioned by gossypol for rabbit feeding. The objective of this study therefore, was to evaluate the effect of CSC-based diets with or without carrot or ginger supplementation on the haematological and serum biochemical variables of rabbit buck.

MATERIALS AND METHODS

Experimental Site

The experiments was conducted at Rabbit Production and Research Unit of the Teaching and Research Farm, Ladoke Akintola University of Technology (LAUTECH) Ogbomoso, Oyo State, Nigeria.

Animals and management

Twenty four (24) weaner crossbred (New Zealand White X Chinchilla) rabbit bucks aged 5 – 6 weeks were randomly allotted to four (4) treatment groups of six animals each in a completely randomized design. Treatment diets were prepared to contain 16% crude protein such that soyabean meal was the main protein source for the control diet (T_1). In diet 2 (T_2), the soyabean meal was completely replaced (weight for weight) with cottonseed cake (CSC). In diet 3 (T_3) 5g/ kg diet of carrot was added to the composition in T_2 to give CSC + carrot while in T_4 (CSC + ginger), 5g/ kg of ginger was added to the composition in T_2. The gross composition and calculated nutrients of experimental diets are presented in Table 1.

Animals were housed individually in wooden cages measuring 44cm x 44cm x 34cm and allowed to acclimatize for one week. They were treated against endo- and ecto – parasites. Feed was offered ad-libitum with an allowance of 100g/ rabbit/ day and refusals were measured every morning to determine feed intake. Water was made available throughout the trial period which lasted for nine weeks.

Blood collection and analysis

At the end of nine weeks of feeding, animals were fasted overnight (for 12 hours) and blood was collected from the ear vein of the bucks using syringe and needle (23 gauge). Blood meant for haematological analysis was collected into plastic bottles containing anticoagulant, ethylene diamine tetra acetic acid (EDTA). Blood meant for serum biochemical analysis was collected into plastic bottles that did not contain anticoagulant for serum separation.

Haematological parameters analysed include the packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell (RBC) count, white blood cell (WBC) count, and the WBC differential counts namely, the lymphocytes, neutrophils, monocytes and eosinophils. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were derived variables obtained from PCV, RBC and Hb.

The PCV was analysed by the microhaematocrit method. The RBC, WBC and the differential counts were determined using the improved Neubauer haemocytometer, while the Hb was determined using cyanmethaemoglobin method of Jain (1986).

The serum parameters analysed were aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, albumin, globulin, urea, creatinine, cholesterol, triglycerol and glucose. The liver enzyme activities AST and ALT were determined using spectrophotometric methods as described by Rej and Hoder (1983) and Hoder and Rej (1983). Serum total protein was determined using Biuret method as described by Kohn and Allen (1995), while albumin was determined using Bromocresol green (BCG) method of Peters *et al*. (1982). The serum globulin was determined by subtracting albumin value from total protein value. Serum total cholesterol was determined using enzymatic – colorimetric method (according to Randox diagnostic reagent kit CHOD - PAP®).

Statistical analysis

Data obtained were subjected to one-way analysis of variance (ANOVA) using SAS (1999). Separation of means was done by Duncan's multiple range test option of the same statistical software.

RESULTS AND DISCUSSION

Results

The results of this study are presented in Tables 2 and 3. Table 2 shows the haematological parameters of rabbit bucks fed diets containing cottonseed cake supplemented with carrot and ginger. The PCV, Hb, RBC, WBC, MCV, MCH and MCHC were not significantly (P>0.05) affected by cottonseed cake with or without carrot or ginger supplementation. The platelet counts and the WBC differential counts Lymphocyte and neutrophils were significantly (P<0.05) affected by the treatment. Bucks on T4 had significantly (P<0.05) higher lymphocyte counts than other treatments. The neutrophil counts for T_4 was significantly (P<0.05) lower than T_1, T_2 and T_3. The platelet count for bucks that fed on CSC without supplementation (T_2) was significantly (P<0.05) lower than that of other treatments.

The serum biochemistry of rabbit bucks fed cottonseed cake based diets supplemented with carrot or ginger is presented in Table 3. The albumin, urea, creatinine, AST, ALT, total cholesterol and glucose were significantly (P<0.05) affected by the treatment. CSC without supplementation (T_2) significantly (P<0.05) depressed albumin, which was similar to that of T_4 (CSC + ginger). Supplementing CSC with carrot significantly (P<0.05) increased the albumin level.

Urea level for rabbits that fed on CSC + ginger (T_4) was significantly (P<0.05) lower than that of other treatments which were statistically similar to one another. The creatinine level was highest (P<0.05) for bucks that fed on CSC + carrot, but statistically similar among other treatments.

AST and ALT demonstrated similar pattern across the treatments with T_4 eliciting the highest (P<0.05) levels, while the control bucks had the lowest value. Cholesterol level was significantly (P<0.05) higher for bucks that fed on CSC diet without supplementation than for bucks on other treatment diets. Buck on T_2 and T_4 had statistically similar glucose level which was significantly (P<0.05) higher than that of bucks on other treatments. Total protein and triglycerol were not significantly (P>0.05) affected by treatment.

Discussion

The results obtained from study showed that cottonseed cake with or without carrot or ginger supplementation did not have any significant effect on PCV, Hb and RBC. This is an indication that the test ingredient did not elicit and any inadequacy of nutrient utilization that could have resulted in anaemic condition. Also, the oxygen carrying capacity of the blood was not impaired. Similar observation was reported by Amao *et al*. (2010) in a study where Vitamin E and selenium were used to supplement cottonseed cake. However, this observation contradicts a recent report by Amao *et al*. (2012) that 15% CSC in diets of rabbit buck depressed PCV and Hb. Probably the period of exposure to the test ingredient used in this study was not long enough to generate any observable deleterious effect on the haematological variables. The values obtained for PCV and Hb concentration are comparable to the normal physiological values documented by Mitruka and Rawnsley, (1977).

The observation in this study that WBC was not significantly different among treatments is a reflection that cottonseed cake in diet did not precipitate any immune response in the bucks. This could also be explained by the fact that the period during which the animals were fed with diets containing cottonseed cake was not long enough to elicit a response to the challenge of gossypol in the CSC. This report disagrees with the report of Amao *et al*. (2012) in a study where rabbits were fed for twenty weeks. The toxicity effect of gossypol seems to be cumulative; and so the longer the period that animals are on diets that contain much gossypol, the more likely they are to have toxicity problem. Earlier, Chase *et al*. (1994) had reported that haematological effects of gossypol in CSC diets include anaemia with reduced number of red blood cells and increased red blood cell fragility, decreased oxygen release from oxyhaemoglobin and reduced oxygen carrying capacity of blood. Taha *et al*. (2006) suggested that gossypol may interfere with normal oxygen exchange in animal erythrocytes. Findings from the present study contrast with these reports.

The significant reduction in platelets value for bucks on CSC-based diet suggests that CSC could compromise the ability of blood to clot. This observation supports the report of Amao *et al*. (2012). The trend observed in the present study that serum total protein was not significantly different from among treatment means indicates adequacy of crude protein availability to the animals. It also indicates that gossypol levels in the

CSC used in this study did not interfere with the metabolism of protein in the rabbit bucks. This result is at variance with the report of Amao *et al*. (2010). According to Ryan *et al*. (1986), gossypol in CSC has an inhibiting effect on the enzymes pepsin and trypsin in the alimentary tracts and thus interferes with protein digestion.

The significantly higher values of serum glucose recorded for bucks that fed on CSC-based diets than the control, is a reflection of higher energy content in the feed made available to the experimental animals. This could be related to residual oil in the CSC which invariably increased the energy level of the diets since cottonseed is an oil seed from which oil has been extracted to obtain the CSC. These values were higher than the normal physiological range reported by Mitruka and Rawnsley, (1977) for normal rabbits.

The observation that AST and ALT were significantly higher for the rabbits that were fed CSC-based diets than the control is indicative of liver and kidney damage in the bucks. The AST is a liver enzyme that is associated with liver damage. When liver and or kidney tissues are damaged, the enzymes are liberated into the blood stream thereby increasing their levels in the blood. This could be related to the adverse effect of gossypol. Gossypol has been reported to impact negatively on some vital organs of animal including the liver (Lindsey *et al*., 1980; Gray *et al*., 1990).

The significantly lower value of albumin recorded for T_2 (CSC-based) diet, is an indication of liver dysfunction. This finding confirms the fact that hepatotoxicity must have taken place. Malnutrition and liver dysfunction are two of the factors that could result in decrease in serum albumin. Since the diet has been observed to be adequate in protein and energy, the liver dysfunction could be attributed to gossypol effect.

The observed higher creatinine level for T_2 and T_3 has implication for the kidney. Creatinine is a product of protein metabolism that needs to be excreted by the kidney. Thus a significant increase in serum creatinine is an indication of functional damage to the kidney. This observation corroborates the report of Amao *et al*. (2012).

The significantly higher total cholesterol level obtained for animals on CSC-based diet compared with bucks on other diets tends to confirm the earlier explanation about glucose level in this study. The level of total cholesterol in this study clearly indicates a possibility of residual oil in the CSC. Supplementing the CSC with either carrot or ginger lowered the serum total cholesterol, suggesting an effective anti-cholesterolemic property of these natural products.

It was concluded from this study that CSC had adverse effects on serum biochemical characteristics of rabbit bucks. However, supplementation with either carrot or ginger corrected the adverse effects suggesting the need to always supplement CSC-based diets for rabbits with either of the products to safeguard the health of the animals.

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Table 1: Gross composition and calculated nutrients of experimental diets.

Ingredients (%)	T_1 (SBM)	T_2 (CSC)	T_3 (CSC + Carrot)	T_4 (CSC+Ginger)
Maize	43.57	42.88	42.88	42.88
Soyabean meal	21.43	---	---	---
Cottonseed cake	---	22.12	22.12	22.12
Rice husk	30.00	30.00	29.50	29.50
Fish meal	2.00	2.00	2.00	2.00
Bone meal	0.25	0.25	0.25	0.25
Oyster shell	2.00	2.00	2.00	2.00
Vitamin/ premix*	0.25	0.25	0.25	0.25
Salt	0.30	0.30	0.30	0.30
Lysine	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10
Carrot	---	---	0.50	---
Ginger	---	---	---	0.50
Total	**100**	**100**	**100**	**100**
Calculated nutrients				
Crude protein (%)	16.00	16.00	16.01	15.99
Crude fibre (%)	11.28	12.64	12.51	12.50
Metabolizable energy (ME)(Kcal/kg)	2552.00	2523.00	2517.00	2517.00

* 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: Haematological parameters of rabbit bucks fed diets containing cottonseed cake supplemented with carrot or ginger.

Parameters	T_1 (control)	T_2 (CSC)	T_3 (CSC+Carrot)	T_4 (CSC+Ginger)	SEM
PCV (%)	31.67	30.67	29.67	30.00	0.96
Hb (g/ dl)	9.64	10.22	9.89	10.00	0.19
RBC($x10^6$/ mm^3)	3.79	4.07	3.14	3.33	0.27
WBC ($x10^3$/mm^3)	5.77	5.15	5.23	5.10	0.26
Lymphocyte (%)	67.00^b	64.67^b	65.67^b	78.00^a	2.17
Monocyte (%)	2.33	3.00	2.33	3.00	0.28
Neutrophil (%)	29.00^a	30.67^a	30.67^a	18.00^b	2.13
Eosinophil (%)	1.67	1.67	1.33	1.33	0.19
MCV (fl)	86.32	75.46	100.92	96.58	6.50
MCH (µl)	26.95	25.16	33.63	32.39	2.37
MCHC (%)	30.99	33.34	33.33	33.35	0.59
Platelets ($x10^3$/mm^3)	125.00^a	95.00^b	115.33^{ab}	106.67^{ab}	8.47

a,b: means in the same row with different superscript are significantly different ($P<0.05$).
PCV=Packed Cell Volume; Hb= Haemoglobin concentration; RBC=Red Blood Cell; WBC=White Blood Cell; MCV=Mean Corpuscular Volume; MCH=Mean Corpuscular Haemoglobin; MCHC=Mean Corpuscular Haemoglobin Concentration.

Table 3: Serum biochemistry of rabbit bucks fed diets containing cottonseed cake supplemented with carrot or ginger.

Parameters	T_1 (control)	T_2 (CSC)	T_3 (CSC+Carrot)	T_4 (CSC+Ginger)	SEM
Total protein(g/dl)	7.76	6.45	6.61	6.47	0.31
Albumin (g/dl)	4.04^{ab}	3.64^{b}	4.54^{a}	3.71^{b}	0.14
Urea (mg/dl)	18.17^{a}	17.21^{ab}	17.30^{ab}	14.03^{b}	0.67
Creatinine (mg/dl)	0.92^{b}	1.13^{ab}	1.24^{a}	0.93^{b}	0.05
AST (iu/L)	20.77^{c}	31.83^{b}	31.89^{b}	43.30^{a}	2.15
ALT (iu/ L)	21.42^{c}	32.25^{b}	35.10^{b}	43.17^{a}	2.47
Cholesterol (iu/dl)	68.53^{b}	90.69^{a}	77.42^{b}	77.95^{b}	2.71
Triglycerol (iu/dl)	51.43	47.72	48.06	51.18	1.03
Glucose(mmol/L)	142.64^{c}	197.92^{a}	$150.68b^{c}$	186.14^{ab}	8.55

^{abc} Means on the same row with different superscripts differ significantly ($p<0.05$)

ALT = Alanine transaminase

AST = Aspartate transaminase