



Effects of feeding graded levels of whole cottonseed on haemato-biochemical parameters of red Sokoto bucks

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Abstract

Whole cottonseed (WCS) is an important source of protein for ruminants, however, it contains a polyphenolic secondary metabolite gossypol which may reduce its palatability and cause pathophysiological effects. The study was aimed at evaluating the effects of feeding graded levels of WCS on the haemato-biochemical parameters of Red Sokoto bucks (RSB). After a 14-day pre-treatment period, 20 bucks (n = 5) were assigned for 90 days to one of four isonitrogenous treatments: control (A); 0 % (B); 15 % (C); 30 % and (D); 45 %. Blood was collected for Packed Cell Volume (PCV) and serum analyses at days 0, 45 and 90 of the experimental period for concentrations of urea, creatinine, total protein, albumin, globulin and activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). The PCV was decreased in groups fed above 15 % of WCS on days 45. There were no significant ($P > 0.05$) differences in globulin concentration, alanine aminotransferase and alkaline phosphatase activities but aspartate transferase activity was higher ($p < 0.05$) in group D (45% WCS) compared to the various treatment groups. The urea concentration was higher ($p < 0.05$) in groups B and D at day 45 compared to the control group. The creatinine concentration was higher ($p < 0.05$) in group D at days 45 and 90 compared to the control group. The serum total protein and albumin concentration were higher ($p < 0.05$) in groups C and D at day 45 compared to the control group. Our finding revealed that prolonged feeding of bucks above 30 % WCS for more than 60 days exerted considerable deleterious and adverse effects on blood constituents leading to anaemia, hyper-cretenaemia, ureamia, increased levels of total protein, albumin, aspartate transferase activity and death.

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Introduction

The problem of feed shortage has been compounded by the ever-increasing prices of the conventional feedstuffs such as grains and oil seed cakes (groundnut cake and soyabean meal) used in the formulation of goat rations. Goats and poultry compete with man for the available feed grains (Ogunfowora *et al.*, 1980). Consequently, any feeding regime that reduces feed cost ultimately results in reduction of total cost of production and increases the profit margin. Goats in particular are able to survive woody and high fibre forages and have the ability to browse low quality feed than other ruminant animals. There is an increase in the use of agricultural and agro-industrial by products which is also a possible option to ameliorate both the high cost of feed ingredient and scarcity (Oloche *et al.*, 2018). In order to keep the livestock industry in operation, producers often use other less known and cheaper non-conventional protein sources such as whole cottonseeds (WCS) and palm kernel cake (Zeng *et al.*, 2014). They are mostly used as a component ration in feeding most ruminants because they are rich in protein, energy, fibre, phosphorus and vitamin E (He *et al.*, 2015). However, WCS when fed in excess can lead to toxicity because of the presence of gossypol, a naturally-occurring toxic compound, located in the pigment glands of seeds, roots and leaves of cotton plants (Zhang *et al.*, 2016). Although the rumen of small ruminants can detoxify the gossypol to a greater extent (Calhoun, 2003), feeding excessive WCS has been shown to impair various physiological processes in animal's body such as hepatic insufficiency and severely impaired liver and kidney functions, increased levels in aspartate transferase activity, erythrocyte fragility, respiratory distress, impaired body weight gain, low fertility, abortion, still-births, immuno-toxicity, anaemia, anorexia, weakness, apathy, heart failure and death. Haemato-biochemical parameters are important indices of pathological and physiological changes in animals (Mitruka & Rawnsley, 1977). WCS impacts

negatively on blood constituents in cattle by causing erythrocyte fragility leading to anaemia, proteinaemia, increased cholesterol levels and creatinine levels leading to muscle damage and weight loss. (Gadelha *et al.*, 2014a; Camara *et al.*, 2015 and Cao *et al.*, 2018).

The indiscriminate use of WCS or their by-products may decrease the physiological performance of small ruminants, including bucks. Despite the increasing use of WCS as a component ration in feeding most ruminants, there is paucity of information in Nigeria on the effects of feeding graded levels of WCS fed to Red Sokoto bucks on haemato-biochemical parameters, establishing the optimal levels of WCS and how they directly or indirectly affect the physiological parameters of RSB.

The present study sheds more light on the effects of various levels of whole cottonseed on the haemato-biochemical parameters in Red Sokoto bucks.

Materials and Methods

Study location

The study was carried out at the Small Ruminant unit, Faculty of Veterinary Medicine, Ahmadu Bello University, Samaru – Zaria.

Diet preparation

Whole cottonseed was purchased from Sunseed® oil and mills, Sabo-gari, Zaria metropolis. The WCS was crushed into a meal using cereal grinding machine of 5-mm diameter sieve. Four (4) experimental diets were formulated and compounded to contain Whole cottonseed at the rates 0, 15, 30, 45 % (Table 1).

Experimental animals and design

Twenty, apparently, healthy Red Sokoto bucks aged 16 -18 months determined through the pattern of the dentition and weighing 6 -10 kg were randomly used for the experiment. The animals were purchased from goat sellers within Zaria metropolis. They were randomly distributed into four (4) treatment groups of five (5) animals each in a completely randomized design and each animal was a replicate.

Table 1: Proximal Analysis of Whole cotton seed

Groups	DM %	CP %	CF %	EE%	ASH %	NFE%	Calculated gossypol (mg per kg of WCS)
A (Control)	95.12	15.06	3.33	4.16	4.11	73.34	0
B (15 %)	92.36	16.63	15.83	4.87	6.67	56.00	105
C (30 %)	94.18	16.81	19.17	5.87	6.78	51.39	210
D (45 %)	98.00	16.90	25.20	5.95	7.67	48.40	315

DM = Dry Matter, CP = Crude protein, CF = Crude fibre, EE = Ether Extracts, ASH = Ash, NFE = Nitrogen free extract
Ingredients were manually mixed and packaged in 50 kg bags.

Representative samples of each treatment diet were collected and analysed for crude protein (CP); dry matter (DM); CF = Crude fibre; = Ether Extracts (EE); ASH = Ash; NFE = Nitrogen free extract

Management

The bucks were screened and treated with albendazole at 10mg/kg for haemoparasites and helminths before the commencement of the experiment. The bucks were managed under intensive system and kept in separate pens. They were given access to a balanced ration, comprising maize offal, wheat bran, bone meal, common salt, groundnut haulms and water *ad libitum* (Table 2). No vaccine was administered within the two-week pre-conditioned period. Ingredients and samples of the diet were analysed for dry matter, crude protein, ether extract and ash content in animal nutrition laboratory, Faculty of Agriculture, Ahmadu Bello University, Zaria using the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC, 2002) (Table 1). Determinations of the crude fibre and nitrogen-free extract fraction was carried out as described by (Goering & Van Soest, 1970). Each analysis was done in triplicate. Free gossypol content in feedstuffs and diets was determined by aniline reaction procedures according to the AOAC (2002).

Blood evaluation

Five milliliters (5 ml) of blood samples were collected for haemato-biochemical analysis from each buck in each group at days 0, 45 and 90 of the experimental period via jugular veno-puncture using hypodermic needle (23 G). This blood was divided into two. One part of this blood was transferred into ethylenediaminetetraacetic acid (EDTA) - containing sample bottles and was immediately used for the determination of packed cell volume by microhaematocrit method.

Serum chemistry

The second part of this blood was transferred into non-EDTA bottles and allowed to clot. The samples were centrifuged at 3000g for 5 minutes for separation of the serum. The serum parameters analyzed were concentrations of urea, creatinine, total protein, albumin, globulin and activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase

(ALP). The serum concentrations of urea, creatinine, total protein, and albumin, and the activities of ALT, AST and ALP were determined using specific commercial kits (Katal, Belo Horizonte, MG) and an automatic analyzer SBA-2000 (Celm, Barueri, SP). Data on total protein and albumin concentrations were used to calculate the globulin concentrations and the albumin/globulin (A/G) ratio.

Data analysis

Data collected were expressed as means ± Standard error of the mean (SEM). Analysis was done using GraphPad Prism software version 5. One-way analysis of variance (ANOVA) was used to test for differences between groups, followed by Tukey’s post-hoc test for multiple comparisons of the means. Significance of differences between treatment means was determined at P < 0.05.

Results

The Packed Cell Volume (PCV) values did not significantly (P > 0.05) differ among the groups at days 0- 45, but after day 45 to day 90, PCV value increased significantly (P < 0.05) in group B (28.60±1.50 to 39.80 ±3.28) compared to those in group C (30.40±0.07 to 26.33±0.88) and group D (32.20±0.65 to 30.20±2.40). The result for the serum biochemical parameters of Red Sokoto bucks fed whole cottonseed is presented on Table 3. The increased in ALT activity among treatment groups at days 0, 45 and 90 were not significant (P > 0.05). There was no significant (P > 0.05) difference in AST activity among the treatment groups at days 0, and 45. However on day 90, bucks fed 45 % WCS had a significantly (P < 0.05) higher AST activity (41.75 ± 3.80) than those in controls (35.00 ± 2.75) and group B (33.50 ± 2.14).

The ALP activity fluctuated among treatment groups at days 0, 45 and 90 but the differences were not significant (P > 0.05). There was no significant (P > 0.05) difference in urea concentration among the treatment groups on days 0 and 90. However, at day 45, bucks fed the control diet had lower (P < 0.05) urea concentration (3.73 ± 0.69), compared to those in group B (5.72 ± 0.47) and group D (6.84 ± 0.56). Serum

Table 2: Composition of the diets fed to the experimental goats

Group	Levels of inclusion		Ingredient						
	WCS (%)	WO	GNC	MO	BM	CS	TOT	CP	M.E
A	0	10	9.4	78.6	1.5	0.5	100	15	1959.27
B	15	46.53	0	36.47	1.5	0.5	100	15	2363.28
C	30	13.75	0	54.25	1.5	0.5	100	15.01	2482.99
D	45	11.07	0	41.93	1.5	0.5	100	15.04	2601.70

WO = Wheat offal, MO = Maize offal, GNC = Groundnut cake, BM =Bone Meal, CS = Common Salt, TOT = Total, CP = Crude protein and M.E= Metabolisable Energy (Kcal/kg)

creatinine concentration was not ($P > 0.05$) different among the treatment groups at day 0. At day 45, creatinine concentration was significantly ($P < 0.05$) higher in group D (71.20 ± 4.32) compared to the control group (60.50 ± 3.80). At day 90, there was significant ($P < 0.05$) difference in creatinine concentration among the treatment groups and group D had the highest concentration (72.00 ± 3.87) while control bucks had the least concentration (59.0 ± 3.53). Serum protein concentration was not significantly ($P > 0.05$) different at days 0 and 90 among the groups, but at day 45, there was a significant ($P < 0.05$) increase in serum protein concentration in groups C (71.80 ± 3.20) and D (71.80 ± 2.86) compared to the bucks in control group (62.20 ± 1.72). Bucks in group B (63.00 ± 3.53) also had a significant ($P < 0.05$) decrease in protein concentration, compared to those in groups C or D. There was no significant ($P > 0.05$) difference in the albumin concentration at days 0 and 90 among the treatment groups. At day 45, albumin concentration was higher in groups C (39.00 ± 4.54) and D (39.40 ± 3.29) compared to control group A (32.80 ± 1.34). There was significant ($P < 0.05$) decrease in the globulin concentration of bucks in group B (26.00 ± 1.30), compared to bucks in group D (30.75 ± 1.68) at day 0. There was no significant ($P > 0.05$) difference in the globulin concentration at days 45 and 90 among the treatment groups.

Table 3: Mean \pm S.D haemato-biochemical parameters at days 0, 45 and 90 of red Sokoto bucks fed whole cottonseed supplementation

	A (0% WC)	B (15% WCS)	C (30%WCS)	(45% WCS)
Day 0				
PCV	38.60 \pm 5.66	28.60 \pm 1.50	30.40 \pm 1.08	32.50 \pm 0.65
ALT	30.80 \pm 3.80	34.75 \pm 12.03	34.0 \pm 11.42	40.0 \pm 14.18
AST	33.25 \pm 6.42	24.60 \pm 9.84	32.00 \pm 8.81	34.50 \pm 6.15
ALP	69.00 \pm 7.51	71.20 \pm 12.63	73.75 \pm 8.54	79.67 \pm 9.17
Urea	4.86 \pm 0.38	5.26 \pm 0.76	6.0 \pm 0.78	5.93 \pm 1.05
Creatinine	51.80 \pm 8.23	60.60 \pm 3.91	60.00 \pm 4.74	64.0 \pm 10.24
Total protein	54.40 \pm 7.67	55.40 \pm 4.56	60.67 \pm 1.50	58.10 \pm 1.83
Albumin	29.80 \pm 5.72	30.80 \pm 4.65	33.25 \pm 4.70	27.25 \pm 1.68
Globulin	27.33 \pm 3.24	26.00 \pm 1.30 ^a	29.25 \pm 1.90	30.75 \pm 1.68 ^a
Day 45				
PCV	29.00 \pm 3.42	30.60 \pm 2.44	29.80 \pm 3.37	34.00 \pm 3.63
ALT	27.67 \pm 3.7.11	28.75 \pm 4.31	26.00 \pm 2.03	25.50 \pm 1.92
AST	27.00 \pm 3.87	7.00 \pm 6.75	25.25 \pm 6.08	25.67 \pm 3.73
ALP	64.00 \pm 8.40	2.60 \pm 5.41	69.75 \pm 6.98	75.25 \pm 11.05
Urea	3.73 \pm 0.69 ^{ab}	.72 \pm 0.47 ^{acd}	4.24 \pm 0.45 ^{ce}	6.84 \pm 0.56 ^{bde}
Creatinine	60.50 \pm 3.80 ^a	66.80 \pm 3.37	67.40 \pm 1.95	71.20 \pm 4.32 ^a
Total protein	62.2 \pm 3.84 ^{ab}	63.0 \pm 3.53 ^{cd}	71.80 \pm 3.20 ^{ac}	71.80 \pm 2.86 ^{bd}
Albumin	32.60 \pm 1.34 ^{ab}	33.80 \pm 2.40	39.00 \pm 4.54 ^a	39.40 \pm 3.29 ^b
Globulin	29.60 \pm 4.45	39.20 \pm 2.48	32.80 \pm 1.79	32.40 \pm 1.78
Day 90				
PCV	34.75 \pm 2.05	39.80 \pm 3.28 ^a	26.33 \pm 0.88 ^a	30.20 \pm 2.40
ALT	43.33 \pm 3.94	45.50 \pm 12.72	50.67 \pm 7.09	46.67 \pm 5.81
AST	35.00 \pm 2.75 ^a	33.50 \pm 2.14 ^b	36.33 \pm 4.90	41.75 \pm 3.80 ^{ab}
ALP	67.33 \pm 6.10	69.00 \pm 6.39	67.33 \pm 6.10	70.00 \pm 10.20
Urea	4.30 \pm 0.31	4.70 \pm 0.38	4.73 \pm 1.43	4.73 \pm 1.88
Creatinine	59.0 \pm 3.53 ^{ab}	63.40 \pm 3.65 ^c	66.33 \pm 1.97 ^a	72.00 \pm 3.87 ^{bc}
Total protein	69.50 \pm 2.82	64.75 \pm 5.59	66.33 \pm 8.21	65.6 \pm 4.34
Globulin	30.00 \pm 2.41	30.60 \pm 2.03	27.67 \pm 7.78	30.60 \pm 3.04
Albumin	29.50 \pm 2.95	34.25 \pm 5.79	35.33 \pm 3.93	35.00 \pm 2.55

^{abcde}Means in the same row with same superscript letters are significantly different from one another ($P < 0.05$).

PCV – Packed Cell Volume
 ALT - Alanine aminotransferase
 AST -Aspartate aminotransferase
 ALP-Alkaline Phosphatase

Discussion

The present study showed that feeding bucks with 15% WCS did not result in anaemia. This agrees with the finding of Braga *et al.* (2012) who reported that feeding 15% extruded – expelled cottonseed meal to rams did not result in changes in PCV. However, feeding at 30% WCS for a longer duration (90 days) resulted in a decrease in PCV. This could be due to the fact that bucks on 30% WCS in this study were observed to consume more feed thereby making gossypol more readily available which probably caused low PCV, hence anaemia. This is in agreement with the work of Calhoun (2003) that matured cows

and weaned calves given higher WCS of 0.5 % and 0.33 %, respectively of their body weight increased red blood cell fragility and decreased PCV values.

The increase in ALT activity at 30 % inclusion of WCS is in agreement with the findings of Velasquez-Perriera *et al.* (1988) who reported that gossypol acetic acid caused a significant elevation in serum ALT and ALP activities due to hepatic insufficiency and major liver damages. The present investigation showed a significant ($P < 0.05$) increase in AST activity with an increasing level of WCS in the diets of Red Sokoto bucks, compared to bucks fed the control diet (Table 3) this could be due to an increase in impaired liver functions of the bucks. This decrease in AST was an indication of possible hepatic dysfunction attributed to the toxic effect of gossypol. Akingbemi & Aire (1994) reported that AST activity was higher in rats fed on a diet containing 15.7% Easiflo® cottonseed for 140 day. Although AST a plasma membrane-bound enzyme is not a liver-specific enzyme, its activity may be associated with pathological changes in the liver, cardiac, skeletal muscles, kidney and brain tissues (Solaimon *et al.*, 2009). Furthermore, AST activity significantly increased in bucks fed 45 % WCS on day 90. The increase in serum AST activity indicates cellular destruction in acute and chronic injury, predominantly in hepatic and heart muscle cells which may be due to the increase in levels of gossypol in WCS ration (Mena *et al.*, 2004). The result of the present study is in agreement with the findings of Kaneko (1989) who observed that WCS beyond 30 % in the diet increases AST activity and those of liver-related enzymes.

There were no significant changes in ALP activities across the groups, even as days of the treatment progressed. This result is in agreement with the works of Giannini *et al.* (2005), who obtained no significant change in the alkaline phosphatase activity in growing Holstein cows fed 30% WCS for 144 days but though a linear decrease ($P < 0.05$) in the activity was recorded after 430 d of feeding. This result also agrees with that of Colin-Negrete *et al.* (1996), who reported that the activity of alkaline phosphatase fed WCS in dairy cows did not differ among the treatments (Table 3). The present study also showed that feeding bucks with 45 % WCS resulted in increased urea and creatinine concentration, which is consistent with the report of Colovic *et al.* (2019) that the serum of bucks fed WCS above 30 % showed elevated levels of urea and creatinine. Blood urea concentration in ruminants is closely regulated by the rate of protein breakdown and ammonia utilization

for bacterial protein synthesis in the rumen. An increase in the level of blood urea may reflect an accelerated rate of protein catabolism rather than decreased urinary excretion (Kaneko, 1989). Furthermore, Gadelha *et al.* (2014a) showed that blood urea increased with increasing levels of 0 %, 7%, 14% and 21% WCS in the diet of supplemented hieifers. The increase in urea may be due to increase in protein catabolism in mammalian body or from inefficient conversion of ammonia to urea, the bucks in group D had difficulty and shallow breathing, fatigue, inappetence, nausea, anorexia and somnolence. However, in contrast to the present findings, (Gadelha *et al.*, 2014b) reported lower blood urea nitrogen in rats fed 20 % WCS. There was a significant increase in creatinine levels as the percentage of WCS in diets increased. This is in agreement with the findings of Dayani *et al.* (2010), who also reported elevated levels of creatinine in lambs fed gossypol above 30 % WCS of their total feed ration. Increased creatinine levels are generally seen in degenerative muscular diseases, extreme tiredness and fatigue in the bucks in group D at days 70 – 90, nausea, cramping, inactivities, dark, cloudy and bloody urine (Braga *et al.*, 2012). In contrast to the present finding Felekecha *et al.* (2013) showed that serum creatinine kinase is usually high in diseases of the heart, skeletal muscle and brain because there are large quantities of the enzyme in these organs, and even in mild injury, the enzyme is released into the systemic circulation. Pattanaik *et al.* (2003) also demonstrated decreased levels of creatinine with increasing levels of WCS in the supplemented diets of calves, compared to those fed the control diet. This finding may be due to the low effect of the gossypol content of WCS or the relatively high tolerance of the goats to the negative effects of gossypol. On the other hand, Morgan *et al.* (1988) found no variation in the creatinine levels of Holstein lambs fed on 50% WCS in their diet. In the present study, there was a decrease in serum protein levels of the bucks in the control and 15 % WCS group. This finding is in agreement with the reports obtained by Morgan *et al.* (1988), who showed significant decrease in serum level of total protein in Gossypol acetic acid-treated lambs, compared to the control group. Gossypol, a very reactive reagent, easily binds either to proteins (such as the functional proteins) or to the phospholipid bilayers of biological membranes, and then alter their structures. Gossypol readily interacts with proteins through either non-covalent interaction or covalent binding between aldehyde group of gossypol and the amine groups of cellular proteins, by Schiff's base

formation (Strom-Hansen *et al.*, 1989). Gossypol may bind to proteins containing free amino sites, which impair its absorption in the digestive tract (Barrazza *et al.*, 1991). It may also be detoxified in the rumen by binding free form to soluble protein, or by dilution and slowed absorption (Barrazza *et al.*, 1991; Risco & Kutches, 1992), which may result in decreased serum protein levels.

The reduced level of total protein, globulins and albumin below the normal standard values of 6.00 to 8.30 g/dl (60.00 to 83.00 mg/L) as reported by Mitruka & Rawsley (1977) showed that bucks exposed to WCS in group B (15% WCS) suffered significantly from inadequate synthesis and concentration of serum total protein and albumin suggesting hypoproteinaemia, hypoglobulinaemia and hypoalbuminaemia in the animals resulting in fluid retention on both the fore and hind limbs, cirrhosis, malnutrition, rapid heart rate, exhaustion, diarrhoea, thinning and shedding of fur, nausea and inappetence. This implies that there was protein metabolism in the animals, since protein synthesis is related to the amount of available protein in the diet (El-Sharakay *et al.*, 2010; Fonseca *et al.*, 2013; Sun *et al.*, 2013). The gossypol in WCS probably impaired protein metabolism in bucks in the present study. This is in contrast with the findings of Kaneko (1989) that recorded increase in albumin concentration of arsi-bale male goats fed WCS at very low doses. The results also revealed that feeding Red Sokoto bucks above 30 % WCS or its prolonged feeding resulted in more deleterious effects on the biochemical parameters.

It is concluded that increasing WCS at above 30 % inclusion levels resulted in significant alterations in PCV and serum biochemistry parameters such as urea, creatinine, protein, albumin, globulin, ALT and AST of Red Sokoto bucks. Animal fed above 30% Whole cotton seed within a 60-90 days feeding period showed sign and lesion of gossypol poisoning. The animals in group D with 45% WCS exhibited these signs which are pulmonary edema and congestion in the liver, lung and spleen, yellowish liquid in the chest and peritoneal cavities, gastroenteritis, centrilobular liver necrosis, hypertrophic cardiac fiber degeneration respiratory distress, impaired body weight, anorexia, anaemia, weakness, apathy and hepatotoxicity. Food and animal feed industries should minimize the use of cotton derived product levels to prevent haemato-biochemical toxicity. Farmers should adequately process cottonseed to reduce gossypol content

before being fed to Red Sokoto bucks in order to reduce its adverse toxic effects.

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Conflicts of Interest

The authors declare no conflict of interest.

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