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Physiological and Reproductive Characteristics of Rabbit Bucks Fed *Cassia tora* Leaf Meal Diets in the Semi-Arid Zone

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Target Audience*:* Animal scientists and poultry farmers

Abstract

A total of 30, 18 weeks old rabbit bucks with an average weight of 1.5-2kg were allotted into five treatments consisting of six bucks each in a completely randomized design (CRD). The results of this study revealed higher (P<0.05) respiratory rate (175.59 and 167.77 brpm) in bucks fed 10 and 20% Cassia tora leaf meal diets. Rectal temperature (38.79-38.99^{o}C) of the bucks fed Cassia tora leaf meal diets were higher (P<0.05) than other bucks. Average daily feed intake (73.6-84.75 g/d) and White blood cell count of rabbit bucks fed 5, 10 and 20% Cassia tora leaf meal diets were higher (P<0.05). Rabbit bucks fed Cassia tora leaf meal diets had 0% mortality while bucks fed control diet had 16.67% mortality. Packed cell volume of the rabbit bucks fed 0% Cassia tora leaf meal diets was higher (P<0.05); haemoglobin concentration, albumin and low density lipoprotein of the rabbit bucks fed 10% Cassia tora leaf meal diets was higher (P<0.05) than other bucks; Red blood cell count of the bucks fed 0% Cassia tora leaf meal diets was higher (P<0.05). Total proteins and triglycerides of rabbits bucks fed 0 and 5% Cassia tora leaf meal diets were higher (P<0.05). The serum antioxidant activities of catalase and superoxide dismutase of rabbit bucks fed 10, 15 and 20% dietary Cassia tora leaf meal diets were higher (P<0.05). Semen evaluation shows that semen color, semen pH, normal cells, free tail, coiled tail and detached head were statistically (P>0.05) similar in all treatments. Semen volume of rabbit bucks fed 0, 5, 15 and 20% Cassia tora leaf meal diets were higher (P<0.05). Reaction time, sperm concentration and motility of the rabbit bucks fed 20% Cassia tora leaf meal diets were higher (P<0.05) than other bucks. Percent live sperm of bucks fed 0 and 20% Cassia tora leaf meal diets were higher (P<0.05) than rabbit bucks fed 5, 10 and 15% Cassia tora leaf meal diets. The incidence of bent tail was higher (P<0.05) in bucks fed 5% Cassia tora leaf meal diet. In conclusion, the temperature humidity index values of this study indicate absence of heat stress both in the morning and afternoon period. Rectal temperatures of the bucks were within the normal range. Haematological and biochemical indices of all the rabbit bucks were within the normal physiologic range. Rabbits fed Cassia tora leaf meal diets had increased catalase and superoxide dismutase activities. Rabbit bucks fed 20% Cassia tora leaf meal diet had similar reaction time, semen volume, sperm concentration, motility and live sperm with the bucks on control, an indication that it can be fed to breeding bucks without any detrimental effect.

Key words: *Cassia tora* leaf; antioxidant enzymes; semen characteristics

Description of the Problem

The problem of inadequate protein supply in developing countries can be alleviated by increased rabbit production. High demand for animal protein and scarcity of resources has necessitated more research on livestock with short generation interval (1). Recently, the use of herbaceous plants as natural antioxidants has gained increasing interest as a result of the global trend of restricting the use of synthetic substances (2; 3; 4). The richest sources of antioxidants are fruits, vegetables, cereals and legumes, tea, coffee, wine, beer, herbs and spices (5). The leaf of *Cassia tora* plant is rich in phenols and other phytochemical compounds which may be responsible for the observed antioxidant activities of *Cassia tora* leaf. Optimal antioxidant supplementation has shown to be important to maintain high productive and reproductive performances of farm animals (6; 7; 8). Antioxidants regulate various oxidative reactions naturally occurring in tissues, by reacting with reactive oxygen species and neutralizing them. There is however a paucity of information on the physiologic response of rabbit bucks fed *Cassia tora* leaf meal diets. The study aimed at evaluating the effect of *Cassia tora* leaf meal diets on the thermoregulatory, serum oxidative status and reproductive parameters of rabbit in the semi-arid zone.

Materials and Methods

Experimental site

The study was conducted at the Rabbit unit of the Teaching and Research farm of the Department of Animal Science, Faculty of Agriculture, Ahmadu Bello University, Zaria. Zaria is located within the Northern Guinea Savannah zone of Nigeria, at latitude, 11^0 9'45'N and longitude 7^0 38'68' E, at an altitude of 610m above sea level (9).

Experimental animals and management

Thirty mixed breed rabbit bucks, four to five months old with an average weight of 1.5kg were used for this study. The rabbits were individually weighed and randomly assigned into five treatments in a completely randomized design. The bucks were individually caged with each buck serving as a replicate. The *Cassia tora* leaves were harvested fresh, air dried, ground and stored in a polythene bag at room temperature for further use and analysis. Five iso-nitrogenous, iso-caloric diets containing 16%CP and 2500KcalME/Kg were formulated and *Cassia tora* leaf meal was incorporated at 0, 5, 10, 15 and 20% to meet the recommended nutrient requirement for this class of rabbits (10). Feed and water were offered *ad libitum*. During the experimental period, all management practices were strictly observed. The duration of the experiment was three months.

Collection of meteorological data

The ambient temperature and the relative humidity of the pen was recorded in the morning and afternoon period (8:00am and 2:00pm) with the aid of a digital temperature-humidity clock throughout the experimental period. The mean ambient temperature (T) and relative humidity (RH) was recorded. The temperature humidity index (THI) was computed using the equation described by Marai *et al.* (11).

THI = t - {(0.31- 0.31 (RH/100) (t – 14.4)}. Where: t - dry bulb temperature (^{0}C), RH - relative humidity (%). THI less than 27.8 - absence of heat stress, 27.8 – 28.9 - moderate heat stress, 29.0 – 30.0 - severe heat stress and 30.0 and above - very severe heat stress.

Measurement of thermoregulatory parameters

Thermoregulatory parameters of rabbits were measured once a week between the hours of 8:00am to 10:00am. Heart rate was measured by counting the number of contractions of the heart in one minute with a stethoscope firmly placed on the heart region on the left side of the chest. A stop watch was

used to determine the time of the heart beat per minute. Respiratory rate was measured by visual observation and counting of the flank movement of the rabbits for one minute when the rabbits were seated calmly. Rectal temperature was measured using a digital clinical thermometer by inserting the tip into the rectum and tilting it side way at a depth approximately 4cm in order to get the temperature of the rectal wall with an accuracy of $\pm 0.1\ ^0C$.

Serum antioxidant analyses

Catalase activity was determined by Goth method (12). For determination of Superoxide dismutase activity, kit (Cat no SD125) was used and for Glutathione peroxidase, kit was also used. The analyses were conducted at the Department of Human Anatomy, Ahmadu Bello University, Zaria.

Semen collection and analyses

Semen was collected once fortnightly for a period of two months between 8.00am – 10am with artificial vagina and a teaser non pregnant rabbit doe was used to stimulate the bucks. The following parameters were analyzed according to the method described by Rodriguez-De Lara *et al.* (13). Semen volume was measured by collecting the semen in a calibrated test-tube. Semen pH was taken with a pH meter after each collection by placing a drop of the semen on the pH paper, the color change was matched on the pH chart. Sperm motility was determined using a drop of undiluted semen on a pre-warmed slide under a light microscope at x 10 magnification. The sperm concentration was determined by using the red blood cell counting chamber of a haemocytometer. Semen smear was stained with eosin-nigrosin for the determination of % live sperm. Live sperm cells repel the stain and were colorless, while dead cells absorb the stain and appeared reddish.

Statistical analysis

All data generated from this study was subjected to analysis of variance (ANOVA) using the Genera Linear Model (GLM) procedure of SAS (14). Dunnette's test was used to compare means that were significantly different.

Results and Discussion

Meteorological observation during the experimental period is shown in Table 1. The air temperature and THI in the afternoons were higher ($P<0.05$) than the air temperature and THI values in the mornings. On the contrary, relative humidity in the morning was higher ($P<0.05$) than relative humidity in the afternoons.

The mean ambient temperature for morning period was (25.4^0C) and (28.6^0C) during the afternoon period of this study. Mean relative humidity of this study was 73.88 and 65.98% in the morning and afternoon period. The THI value in the afternoon was significantly ($P<0.05$) higher than the THI value in the morning period. Temperature-humidity index (THI) is an indicator of thermal comfort level for animals in an enclosure. The THI values of 24.48 and 27.10 obtained in the morning and afternoon respectively indicated that rabbits were not exposed to heat stress during the study period.

Thermoregulatory parameters of rabbit bucks fed *Cassia tora* leaf meal diet is shown in Table 2. Respiratory rate of the rabbit bucks fed 10 and 20% *Cassia tora* leaf meal diets were higher ($P<0.05$) than other bucks. The respiratory rate observed in this study were within the range indicated for stressed rabbits 60 to 300 bpm (15) which were above the established normal range of 30-60 bpm for domestic rabbits (16). This could be due to failure of thermoregulatory mechanism of the rabbit bucks as highlighted by (17). The increase in respiratory rate of this study was also similar to the results obtained by (18; 19).

Rectal temperature of the bucks fed *Cassia tora* leaf meal diets were also higher (P<0.05) but within the established normal range of 38.6-39.5^{o}C for domestic rabbits (15). This implies that rabbit bucks fed 0% *Cassia tora* leaf meal diets were unable to maintain internal body temperature which may be due to disturbances in metabolism, enzymatic reactions, hormonal secret efficiency and utilization of the feed as highlighted by Marai and Habeeb (20), while bucks fed 5, 10, 15 and 20% *Cassia tora* leaf meal diets were able to maintain normal internal body temperature. Rabbits use general body position, breathing rate and peripheral temperature as means to modify heat loss (11). However, respiratory rate and ear temperature are the most important heat dissipation pathways for rabbits.

Table 3 shows the serum antioxidant indices of rabbit bucks fed different levels of dietary *Cassia tora* leaf meal diets. Catalase and Superoxide dismutase (SOD) activity in rabbit bucks fed 10, 15 and 20% *Cassia tora* leaf meal diets were higher (P<0.05) than other bucks. There was increase in catalase and Superoxide dismutase (SOD) concentration with increase in *Cassia tora* leaf meal in the diets. The finding is in contrast with the study of Joyti *et al*. (21), who reported that there was no significance increase in the concentration of catalase in broiler chickens treated with *Cassia tora* leaf extract, the difference may be due to the fact that the study utilized leaf meal diet of *Cassia tora*. (22) reported that *Cassia tora* leaf extract administration in rats increased SOD concentration significantly. According to (23), SOD is the first line of defense against the action of O_2^- and other ROS. GSH activity of the rabbit bucks fed 5% *Cassia tora* leaf meal diets were higher (P<0.05) than other bucks. Flavonoid is one of the active constituents of *Cassia tora*. *In vitro* studies showed that flavonoid have potent antioxidant and free radical scavenging activities (24), which may have been responsible for the elevated levels of the antioxidant enzymes observed in the study. Flavonoid exhibit the beneficial effect due to the antioxidants and chelating properties of these molecules by preventing the free radicals to damage the biological molecules such as lipid, protein, sugar, DNA, and RNA (21). *Cassia tora* leaves is known to increase the blood levels of antioxidant enzymes (SOD, CAT and GSH) which scavenge the ROS, thereby protecting the sperm cells against depletion caused by oxidative stress (25).

Table 4 shows the semen antioxidant indices of rabbit bucks fed different levels of dietary *Cassia tora* leaf meal diets. Catalase of the rabbit bucks fed 10, 15 and 20% *Cassia tora* leaf meal diets were higher (P<0.05) than other bucks. SOD activity of the bucks fed 5, 10, 15 and 20% *Cassia tora* leaf meal diets were higher (P<0.05) than bucks fed 0% *Cassia tora* leaf. GSH of the rabbit bucks fed 0, 5, 15 and 20% *Cassia tora* leaf meal diets was higher (P<0.05) than bucks fed 10% *Cassia tora* leaf meal diet. In this study, rabbits administered *Cassia tora l*eaf meal diets at 5, 10, 15 and 20% levels amplified the activities of SOD and catalase against free radicals due to the increased concentration of these enzymes in the semen, concurrently decreasing the levels of lipid peroxidation in the testes. The increase in the activities of antioxidant enzymes indicates the response of the primary antioxidant system to act against free radicals, thereby preventing the depletion of sperm cells by ROS through the process of lipid peroxidation (26).

Semen characteristics of rabbit bucks fed different levels of dietary *Cassia tora* leaf meal diets are presented in Table 5. Semen volume of bucks fed 0 5 and 20% were higher (P<0.05) than 10% *Cassia tora* leaf meal diets. The decrease in semen volume observed in 10 and 15% *Cassia tora* leaf meal diets in this study is not clear however it may be due to possibility of contamination of the semen by urine as highlighted by (27) who reported a

13% chance of contamination of rabbits' semen during collection with artificial vagina. Sperm concentration of the rabbit bucks fed 20% *Cassia tora* leaf meal diet was higher (P<0.05) than rabbit bucks fed 0, 5, 10 and 15% *Cassia tora* leaf meal diets. The low sperm concentration observed in buck fed 5, 10 and 15% *Cassia tora* leaf meal diets could be attributed to atrophy or decreased secretory activity in the lumen of the epididymis and seminiferous tubules (28, 29).

The sperm motility of rabbit bucks fed 20% *Cassia tora* leaf meal diet was higher (P<0.05) than the bucks fed 0, 5 10 and 15% *Cassia tora* leaf meal diets. The sperm motility of the rabbit bucks fed 20% *Cassia tora* leaf meal diets was higher (P<0.05) than 0, 5, 10 and 15% *Cassia tora* leaf meal diets. This implies that high inclusion of *Cassia tora* leaf meal could promote sperm motility of rabbit bucks. The increase in sperm motility of this study may be related to the release of metabolic energy and/or the protective effect of antioxidant enzymes against ROS (30, 31). The percent live sperm of the rabbit bucks fed 0 and 20% were higher (P<0.05) than bucks 5, 10 and 15% *Cassia tora* leaf meal diets. Percentage dead sperm and reaction time of bucks fed 20% was lower (P<0.05) than in other bucks. Saponin has some positive effects on libido and spermatogenesis (32). Therefore saponin which is one of the phytochemical constituents of *Cassia tora* may have a positive effect on the libido of the rabbit bucks fed 20% *Cassia tora* leaf meal diets which was indicated as reaction time of the rabbit bucks in this study. Bent tail of bucks fed 5% *Cassia tora* leaf meal diet was higher (P<0.05) than other bucks. However, this result was below allowed percentage (20%) of abnormal sperm cells reported in literature for good reproductive potential and fertility in either normal mating or in artificial insemination (33, 34).

Conclusion and Application

1. The THI values of this study indicate absence of heat stress both in the morning and afternoon period.
2. Rectal temperature, haematological and biochemical indices of all the rabbit bucks were within the normal physiologic range.
3. Rabbits fed *Cassia tora* leaf meal diets had increased catalase and superoxide dismutase activities.
4. Rabbit bucks fed 20% *Cassia tora* leaf had similar reaction time, semen volume, sperm concentration, motility and live sperm with the bucks on control, an indication that it can be fed to breeding bucks without any detrimental effect.
5. For improved antioxidant enzymes activities and reproductive characteristics of rabbit bucks, 20% *Cassia tora* leaf meal diet is recommended.

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Table 1: Meteorological observation during the Experimental Period

Parameters	Morning	Afternoon	SEM	P value
Temperature (^{0}C)	25.4^b	28.6^a	1.12	0.005
RH (%)	73.88^a	65.98^b	3.92	0.033
THI	24.48^b	27.10^a	0.83	0.003

$^{(ab)}$ Means with different superscript within the same row are significantly different at p<0.05, RH (%) - relative humidity, THI - temperature humidity index

Table 2: Thermoregulatory Parameters of Rabbit Bucks Fed *Cassia tora* leaf meal diets

Parameters	0	5	10	15	20	SEM	P Values
HR (bpm)	203.21	207.03	202.71	201.26	206.56	5.41	0.85
RR (brpm)	162.54^b	159.77^b	175.59^a	161.26^b	167.77^a	6.12	0.04
RT (^{0}C)	38.02^b	38.79^a	38.85^a	38.86^a	38.99^a	0.33	0.01

$^{(abc)}$ Means with different superscript within the same row are significantly different at p<0.05, brpm - breath per minute, bpm - beat per minute, HR- heart rate, RR- respiratory rate, RT-Rectal temperature

Table 3: Serum Antioxidant Indices of Rabbit Bucks Fed *Cassia tora leaf meal diets*

Parameters	0	5	10	15	20	SEM	P Values
CAT (ug/ml)	1.84^b	1.85^b	1.99^a	1.93^a	1.95^a	0.02	0.005
SOD (ug/ml)	5.08^b	5.06^b	7.45^a	7.28^a	7.92^a	0.17	0.001
GSH (umol/l)	18.33^b	18.91^a	17.89^b	16.78^c	18.16^b	0.28	0.004

$^{(abc)}$ Means with different superscript within the same row are significantly different at p<0.05, CAT- catalase, SOD- Superoxide dismutase, GSH- Glutathione peroxidase.

Table 4: Semen Antioxidant Indices of Rabbit Bucks Fed *Cassia tora leaf meal diets*

	Inclusion Level of *Cassia tora leaf meal diets* (%)						
Parameters	0	5	10	15	20	SEM	P Values
CAT (ug/ml)	1.26^{bc}	1.01^c	1.74^a	1.66^{ab}	1.98^a	0.15	0.005
SOD (ug/ml)	2.87^b	4.13^a	4.13^a	4.87^a	3.99^a	0.28	0.006
GSH (umol/l)	13.24^a	12.71^{ab}	9.59^b	10.34^{ab}	10.11^{ab}	0.28	0.046

$^{(abc)}$ Means with different superscript within the same row are significantly different at p<0.05, SEM- standard error mean, CAT- catalase, SOD- Superoxide dismutase, GSH- Glutathione peroxidase.

Table 5: Semen Characteristics of Rabbit Bucks Fed *Cassia tora* leaf meal diets

Parameters	0	5	10	15	20	SEM	P Values
Semen volume (ml)	1.36[a]	1.25[a]	0.83[b]	0.85[b]	1.25[a]	0.12	0.043
pH	7.33	7.33	7.50	7.00	6.83	0.17	0.281
Sperm Con.(x10^6/ml)	127.92[a]	73.50[c]	86.42[bc]	102.08[b]	144.08[a]	9.88	0.005
Sperm motility %	68.92[b]	49.33[c]	70.67[b]	74.17[b]	85.00[a]	4.32	0.004
Live sperm (%)	79.20[b]	70.00[c]	75.83[b]	75.83[b]	82.92[a]	1.82	0.005
Dead sperm (%)	20.83[b]	30.03[b]	24.17[b]	24.17[b]	17.13[a]	7.52	0.017
Normal cell	86.58	87.25	86.25	87.17	85.08	1.45	0.950
Reaction time (s)	4.50[bc]	5.25[c]	3.92[b]	3.92[b]	3.02[a]	0.30	0.023
Free tail	5.75	3.67	4.25	3.67	5.83	1.72	0.188
Coiled tail	2.50	2.08	2.83	2.42	1.33	1.11	0.388
Bent tail	1.75[b]	3.17[a]	1.08[b]	1.42[b]	1.17[b]	0.44	0.039
Detached head	3.42	3.33	5.17	3.67	5.50	1.39	0.079

[(abc)] Means with different superscript within the same row are significantly different at $p<0.05$, Sperm Con-concentration.