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Effect of phytogenic supplements on the reproductive physiology and metabolic hormones of rabbits exposed to heat stress conditions

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ABSTRACT

This study investigated the role of phytogenic supplements on the reproductive physiology and metabolic hormones of rabbits exposed to heat stress conditions. Fresh Moringa oleifera, Phyllanthus amarus and Viscum album leaves were obtained and processed into a leaf meal using standard procedure and served as a phytogenic supplements. Eighty rabbit bucks of 6 weeks old (514.84 ± 14.10 g) were randomly assigned to the four dietary groups consisting of Diet 1 without the leaf meal (control diet) and Diets 2 (D2); 3 (D3) and 4 (D4) contained 10% Moringa, 10% Phyllanthus and 10% Mistletoe, respectively, in an 84-day feed trial at the peak of thermal discomfort. Semen kinetics and seminal oxidative status, reproductive and metabolic hormones were assessed using standard procedure. Results reveal that sperm concentration and motility traits of bucks on D2, D3 and D4 were significantly ($p < 0.05$) higher than bucks on D1. Spermatozoa speed traits of bucks on D4 were significantly ($p < 0.05$) higher than bucks on other treatments. Seminal lipid peroxidation of bucks in D2-D4 was significantly ($p < 0.05$) lower than bucks on D1. Corticosterone of bucks on D1 was significantly higher than bucks on other treatments (D2-D4). Lutenizing hormone of bucks on D2 and testosterone of bucks on D3 was higher ($p < 0.05$) than in other groups, while follicle stimulating hormone of bucks on D2 and D3 were higher ($p < 0.05$) than bucks on D1 and D4. In conclusion, the three phytogenic supplements improved sex hormones, enhanced spermatozoa kinetics, viability and seminal oxidative stability of bucks during heat stress condition.

1. Introduction

Rabbits can be considered as one of the several species quite suitable for meat production, for mitigating protein deficiencies in developing countries. Rabbit meat contains protein with high biological value, low sodium and cholesterol, highly unsaturated lipids 60% of the total fatty acids), low in calories and fat. The current global warming trends indicate that climate change will occur in more incidences of severely high temperatures. It is therefore cogent to assume that the assault of heat stress will worsen in the future (Vandana et al., 2020). Thermal assault is one of the highly lethal stressors and a primary environmental disruptor to the reproductive physiology of male rabbits, and this is associated with accumulation of free radicals and infertility (El-Desoky et al., 2017).

Heat stress leads to inadequate productive and procreative performances due to altered animal behavior, metabolic and sexual hormonal imbalances, and promotes the production of free radicals (Abdelnour et al., 2021). Heat stress-induced changes on thyroid activity would affect reproductive performance, as the thyroid plays a vital role in sexual maturity and on the procreative exercise (Lara and Rostagno, 2013). Environmental heat stress associated with oxidative stress ends in inequality in reactive oxygen species (ROS) along with defensive antioxidants, by increasing lipid peroxidation raising both thiobarbituric acid reactive substances (TBARS) along with malondialdehyde (MDA) status (Jimoh et al., 2017, 2018). It also controls the integral reproductive lifespan in both sexes of rabbits (Adeoye et al., 2018).

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Herbal supplements loaded with antioxidant feature plays a pivotal role in enhancing the reproductive status of rabbits (Jimoh et al., 2021a). Such herbal supplements as *Viscum album, Moringa oleifera* and *Phyllanthus amarus* have been identified as having strong therapeutic attributes and enhances an animal's overall performance (Jimoh et al., 2021a), hence could be utilized to enhance productivity of rabbits under heat stress.

They contain flavonoids, alkaloids and several other compounds with synergistic beneficial effect that promotes animal performance. Phytochemicals in these herbs are vital secondary metabolite, in complements of animal diets to form phytochemical-containing feed, could definitely ameliorate the shock of heat stress in rabbit production. Reports reveal that male infertility characterized a decline in semen quality, which also results in lower spermatozoa concentration, abnormal spermatozoa and endocrine inequality could be mitigated using herbal therapies (Jimoh et al., 2021a). This suggests that thermal stress-induced oxidative damage could be mitigated by the dietary addition of medicinal feed supplements to alleviate the detrimental encounter of thermal assault in animals (Abd El-Hack et al., 2020).

The administration of herbal supplements has been reported to enhance semen quality, male sex hormone, conceptive index, and sperm viability (Jimoh et al., 2021a; Noh et al., 2020). Organic antioxidants with high free radical scavenging activity have been widely sourced as capable alternatives for mitigating oxidative stress and irregular androgen functions (Noh et al., 2020). Previous reports have indicated that phytogenic supplements can ameliorate oxidative stress (Jimoh et al., 2018) and reverse semen deficiencies by enhancing seminal oxidative status in heat-stressed rabbits (Jimoh et al., 2021b). There are reports that Moringa oleifera enhanced sexual behavior and positively influence spermatogenesis in male rats (Zade et al., 2013). *Viscum album, Moringa oleifera* and *Phyllanthus amarus* has been reported to enhance antioxidant defense and mitigate peroxide accumulation in rabbits (Jimoh et al., 2021a). These three herbs had recently been recommended to promote the potential of rabbits through improved spermatozoa production, boost sperm kinetics and enhance steroid hormones synthesis (Jimoh et al., 2021a). The mechanism of action was adduced to the three herbal supplements enriching semen antioxidant profile and reduced its lipid peroxidation, a key mechanism to maintain oxidative stability in stress conditions (Jimoh et al., 2021a). Based on the recommendations of our previous study (Jimoh et al., 2021a), 10% inclusion of *Moringa oleifera, Phyllanthus amarus and Viscum album* cofer phytogenic effect in rabbits. Hence, they are potent candidates for mitigating heat stress associated with reproductive deficiency of rabbits. This study is intended to elucidate the role of *Moringa oleifera, Phyllanthus amarus and Viscum album* as phytogenic supplements on the reproductive physiology of bucks during thermal stress conditions.

2. Materials and method

2.1. Experimental site

This research was carried out in the Rabbit rearing section of the Research and Teaching Farm, The Federal Polytechnic Ado-Ekiti, Ekiti State, in south-western agro-ecological belt of Nigeria. This research was conducted from January to March 2020. Severity of heat stress relying on the contribution of humidity and temperature (Temperature - Humidity Index; THI) was established to peak in southern Nigeria between February and March (Jimoh and Ewuola, 2016, 2018; Jimoh et al., 2021c). Relative humidity and temperature of the rabbit microclimate was documented at 08:00 h, 12:00 h and 18.00 h daily during the study with a Thermo-Hygrometer. Daily records of the ambient temperature and relative humidity (RH) were noted to enumerate the temperature humidity index as outlined in Jimoh et al. (2018). Briefly,

$$THI = t - [(0.31 - 0.31 \times RH)(t - 14.4)]$$

where RH = relative humidity/100. t =ambient temperature.

THI are classified as: <27.8 °C = absence of heat stress, 27.8–28.9 °C = moderate heat stress, 28.9–30 °C = severe heat stress and above 30 °C = very severe heat stress.

2.2. Phytogenics processing and evaluation

Fresh *Viscum album* (Mistletoe), *Moringa oleifera,* and *Phyllanthus amarus* leaves were harvested from a well-established orchard in the Ado-Ekiti metropolis of Ekiti State. The plants were identified and indexed with herbarium voucher numbers; *Moringa oliefera* UILH/001/1008/2021; *Phyllanthus amarus* UILH/003/1109/2021; *Viscum album* UILH/002/084/1210/2021. Leaves were detached from twigs and shade dried until it was friable to touch while preserving their greenish coloration. The leaves were pounded and saved in air tight containers until incorporation into the diet. Phytochemical analysis and proximate composition of the leaf meals were carried out using Association of analytical chemistry standard procedures 1990.

2.3. Experimental animals and management

Eighty mixed bred (Chinchilla X New Zealand white X California) rabbit bucks of 6 weeks old weighing 514.84 ± 14.10 g were utilized for the research. The rabbits were arbitrarily alloted to the four groups and they were housed in pairs in a Completely Randomized setup for a twelve-week feeding trial.

2.3.1. Treatment diet

Four treatment diets were standardized to meet the nutrient for growing rabbits. Diet 1(D1) without the leaf meal presented as a control diet and Diets 2 (D2); 3 (D3) and 4 (D4) contained 10% Moringa, 10% Phyllanthus, and 10% Mistletoe, respectively as shown in Table 1. The diets were pebbled, fed at 4% of the animal body weight (with an average feed intake buck/day of 59.20 g, 61.45 g, 61.34 g and 60.86 g, respectively), and offered clean water *ad libitum* for 12 weeks.

2.3.2. Semen assessment

Adult bucks were skilled to mount a rabbit's artificial vagina, prior to the end of the experiment, and ejaculates were harvested between

Table 1
Gross composition of experimental diets (g/100 g).

Ingredient	Control	Moringa	Mistletoe	Phyllanthus
Rice bran	6	1	1	1
Salt	0.25	0.25	0.25	0.25
Wheat offal	5	5	5	5
BDG	5.0	0	0	0
Grower premix	0.25	0.25	0.25	0.25
Maise	25	25	25	25
Methionine	0.40	0.40	0.40	0.40
SBM	17.0	17.0	17.0	17.0
Lysine	0.10	0.10	0.10	0.10
Bone meal	1.0	1.0	1.0	1.0
Groundnut Hauluns	40.0	40.0	40.0	40.0
Moringa	0	10	0	0
Mistletoe	0	0	10	0
Phyllanthus	0	0	0	10
Vegetable oil	0.20	0.20	0.20	0.20
Total	**100**	**100**	**100**	**100**
Calculated nutrient composition				
Dry matter %	88.05	85.41	86.20	87.45
Crude Protein%	16.47	16.58	17.94	17.667
Digestable Energy kcal/kg	2721.60	2515.35	2479.6	2509.4
Ether Extract %	3.34	3.02	3.75	2.42
Crude Fibre %	16.5	17.33	15.38	16.32
Lysine %	1.01	0.94	0.94	0.94
Methionine %	0.75	0.71	0.71	0.71
Calcium %	1.60	1.75	1.72	1.60
Phosphorus %	0.45	0.51	0.52	0.42

0700hrs and 0800hrs from each buck over three weeks. On each collection cycle, the first collection for semen quality and the other were centrifuged at 4000 g for 15 min to isolate seminal fluid and kept at – 20 °C for analysis.

Libido was determined according to Jimoh et al. (2021b). The pH of semen collected ranges from 7.86 to 8.12 and were milky in colour. Semen volume, spermatozoa kinetics and viability indices were assayed as described in Jimoh et al. (2021a).

2.3.3. Seminal biochemical indices

Seminal fluid was assessed for total protein using fortress diagnostics commercial assay kits and its procedure, while seminal superoxide dismutase, glutathione peroxidase (GPx), total antioxidant capacity, catalase activities and lipid peroxidation were assayed as outlined in Jimoh et al. (2019).

Briefly, Determination of serum total antioxidant capacity activities involved the reactive mixture contain 0.5 mL of a (10 mmol/L) Na-Benzoate, 0.2 mL of H2O2 (10 mmol/L), 0.49 mL of phosphate buffer (100 mmol/L, pH = 7.4) (prepared by mixing 19.5 mL of KH2PO4 (100 mmol/L) with 80.5 mL of Na2HPO4 (100 mmol/L), then adjusted the pH to 7.4 and 0.2 mL of Fe-EDTA complex (2 mmol/L) (prepared freshly by mixing equal volumes of EDTA (2 mmol/L) and ammonium ferrous sulfate (2 mmol/L), then left at 25 °C for 60 min. Ten microliters of the blood serum were added to the latter reactive mixture and were incubated at 37 °C for 60 min. Finally, 1 mL glacial acetic acid (20 mmol/L) and 1 mL thiobarbituric acid (0.8% w/v in 100 mL of 50 mmol/L NaOH) was added and the absorbance at 532 nm was measured spectrophotometrically after incubation at 100 °C for 10 min. Total antioxidant capacity was calculated according to the following formula:

$$\text{TAcapacity (mmol/L)} = \text{CUA (K – A) / (K – UA)}$$

Where; CUA (mmol/L); Concentration of uric acid.

K; Absorbance of control (K1 - K0)
A; Absorbance of the sample (A1 - A0).
UA; Absorbance of uric acid solution (UA1 - UA0) (Koracevic et al., 2001)

The assay for lipid peroxidation; the reaction mixture in a total volume of 3.0 ml contained 1.0 ml serum, 1.0 ml of Trichloroacetic acid (0.67%). All the test tubes were placed in a boiling water bath for a period of 45 min. The tubes were shifted to the ice bath and, then centrifuged at 2500 rpm for 10 min. The amount of malondialdehyde (MDA) formed in each of the samples was assessed by measuring the optical density of the supernatant at 532 nm.

Superoxide dismutase (SOD); to 2.1 ml of 50 m M butter, add 0.02 ml of enzyme source and 0.86 ml of distilled water. The reaction is initiated with 0.02 ml of 10 mM pyrogallol and change in absorbance monitored at 420 mm. One unit of SOD is defined as that amount of enzyme required to inhibit to auto-oxidation of pyrogallol by 50% in the standard assay system of 3 ml. The specific activity is expressed as unit/min/mg protein.

Glutathione peroxidase activity (GPx); reaction mixture include 0.5 ml 0.4 M buffer pH 7.0), 0.2 ml enzyme source, 0.2 ml 2 mM glutathione (GSH), 0.1 ml 0.2 mM H2O2 added and incubated at room temperature for 10 min along with a control tube containing all reagents, except enzyme source. The reaction arrested by adding 0.5 ml of 10% Trichloroacetic acid, centrifuged at 4000 rpm for 5 min and the GSH content in 0.5 ml of supernatant was estimated. The activity expressed as µg of GSH consumed/min/mg protein.

Catalase activity in the samples were assay by adding 1.9 ml 0.05 M buffer pH 7.0 and 1.0 ml 0.059 M H2O2. The reaction is initiated by addition of 0.1 ml enzyme source. The decrease in absorbance is monitored at 1 min interval for 5 min at 240 nm and activity is expressed as nmoles of H2O2 decomposed/min/mg protein.

2.3.4. Sample collection and analysis

At the expiration of the feeding trial period, blood was harvested from a buck per replicate via jugular venipuncture into plain sample bottle for serum hormonal assay. Blood samples were centrifuged and serum obtained using standard procedures and stored at −20 °C before analysis.

2.3.5. Stress and metabolic hormonal assay

Serum samples obtained were assayed for triiodothyronine (T3), insulin and corticosterone using enzyme linked immunosorbent assay (ELISA), with commercial ELISA kits (T3: Calbiotech Inc, catalog No. T3225T; Insulin: Calbiotech Inc, catalog No. IS130D; Corticosterone: Bioassay Technology Laboratory, catalog No. E0496Ra) and its protocol for each assay, with inter-intra assay covariance of 6.2%.

2.3.6. Sex hormonal assay

Serum samples obtained were examined for follicle stimulating hormone (FSH), luteinizing Hormone (LH), testosterone adopting enzyme linked immunosorbent assay, with commercial ELISA kits (LH: Calbiotech Inc, catalog No. LH231F; FSH: Calbiotech Inc, catalog No. FS232F; Testosterone: Calbiotech Inc, catalog No. TE187S) and its protocol for each assay, with inter-intra assay covariance of 5.7%.

2.3.7. Statistical analysis

Data collected on semen characteristics, oxidative status, sex and stress hormones in the study were exposed to GLM procedure for one-way Analysis of variance in a Completely Randomized Design according to statistical analysis software IBM SPSS 20. The treatments (phytogenic supplements) were incorporated as main effect in the statistical model.

The statistical model is as follow:

$$Yijl = \mu + Bi + eijl$$

Where Yijl represents the value of semen quality, biochemicals, oxidative status and hormonal responses measured in the ith animal; µ is the overall mean for each character; Bi is the fixed effect of ith, phytogenic supplements (i = control, *Viscum album, Phyllanthus amarus* and *Moringa oleifera*); and eijl is the random residual effect.

Treatment means are expressed along with the standard error of means. Significant means differences were assessed at $P < 0.05$ with Duncan multiple range test.

3. Results

Proximate and phytochemical analysis of the leaf meals is shown in Table 2. Moringa possesses higher crude protein, saponins, glycosides, and steroids among the three leaf meals. Mistletoe possesses higher ash,

Table 2
Proximate and Phytochemical analysis of the leafmeals

	Mistletoe leaf meal	Moringa leaf meal	Phyllanthus leaf meal
Dry matter %	89.82	90.76	90.50
Crude Protein%	18.81	31.06	27.13
Ether Extract %	2.10	2.7	2.5
Crude Fibre %	12.10	11.30	9.90
Ash (%)	14.90	12.40	12.06
Nitrogen free extract (%)	52.09	42.54	48.41
Alkaloids(mg/100 g)	14.68	8.5	10.34
Saponins(mg/100 g)	31.01	39.81	23.96
Glycosides(mg/100 g)	75.08	95.92	81.66
Steroids(mg/100 g)	18.82	25.00	20.54
Flavonoids(mg/100 g)	62.93	25.03	44.86
Tannins(mg/100 g)	114.81	96.53	95.98

nitrogen free extract, crude fibre, alkaloids, flavonoids and tannins among the three leaf meals. Of the three leaf meals, Phyllanthus possesses the least crude fibre, ash, saponin and tannin.

3.1. THI estimates for rabbit microclimate

The THI of the rabbit pen is shown in Fig. 1. The THI obtained at 8 a.m. indicates that the bucks were exposed to an average THI value of 24.05 with a range between 18.15 and 32.63. At noon, the average THI value obtained of 30.89 indicates very severe heat stress with a range of THI of 28.14–33.04. The average THI value at 1800 h is 30.15, and a range of values from 24.45 to 33.26.

3.2. Semen output of heat stressed bucks fed on herbal supplements

Libido, semen volume and kinetics of heat stressed bucks fed on supplements are revealed in Table 3. Reaction time of bucks on D1 were significantly ($p < 0.05$) higher than bucks on D2 and D4. Progressive motility and sperm concentration of bucks on D2 D3 and D4 were significantly ($p < 0.05$) higher than bucks on D1. Percentage sperm motility was significantly ($p < 0.05$) higher in herbal supplement fed bucks than the control. Non progressive sperm motility of bucks on D4 was significantly ($p < 0.05$) higher than bucks on D1-D3. Spermatozoa speed traits (average path velocity, curvilinear velocity and straight-line velocity) of bucks on D4 were significantly ($p < 0.05$) higher than bucks on other treatments. Amplitude of lateral head and beat across frequency of spermatozoa of bucks on D4 were significantly ($p < 0.05$) higher than other treatments. Spermatozoa linearity was significantly ($p < 0.05$) highest in bucks on D3 and least in bucks on D4. Spermatozoa straightness of bucks on D3 were significantly ($p < 0.05$) higher than that obtained from other treatments. Sperm viability traits (acrosome integrity and livebility) were significantly ($p < 0.05$) higher in semen of bucks fed herbal supplements than the control.

3.3. Seminal oxidative markers of heat-stressed bucks fed herbal supplements

Seminal oxidative markers of heat-stressed bucks fed on herbal supplements is revealed in Table 4. Total protein of seminal fluid of bucks on D4 was significantly ($p < 0.05$) higher than bucks on D1-D3. The seminal total antioxidant activity of bucks on D4 was significantly ($p < 0.05$) highest and the significantly ($p < 0.05$) least value was obtained from bucks on D1. Seminal lipid peroxidation was statistically ($p > 0.05$) similar in D2-D4 and was significantly ($p < 0.05$) lower than bucks on D1. Seminal SOD and Catalase activity of bucks on D2 –D4 were statistically ($p < 0.05$) superior to bucks on D1. Bucks on D2 had significantly ($p < 0.05$) higher seminal GPx than bucks on D1, D3 and D4.

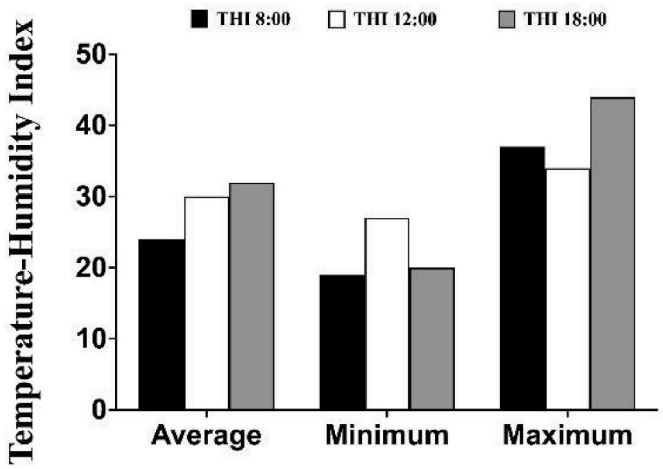


Fig. 1. Temperature - humidity index of Rabbit pen.

Table 3
Libido, semen volume and Kinetics of heat stressed bucks fed phytogenic supplements.

	D1	D2	D3	D4	SEM
Libido (Reaction time in sec)	5.00^{a}	2.00^{b}	3.33^{ab}	2.50^{b}	0.48
Volume (mL)	0.25	0.22	0.23	0.28	0.03
Sperm concentration (x10^{7})	5.33^{b}	9.45^{a}	9.49^{a}	4.07^{b}	1.20
Percentage motility (%)	79.18^{b}	83.86^{a}	84.00^{a}	86.16^{a}	1.08
Progressive motility (%)	69.68^{b}	76.65^{a}	78.09^{a}	76.00^{a}	3.32
Non- Progressive motility (%)	9.50^{b}	7.27^{b}	5.91^{b}	12.10^{a}	2.99
Average path velocity (um/s)	8.83^{b}	10.59^{b}	9.26^{b}	15.88^{a}	1.21
Curvilinear velocity (VCL) (um/s)	15.16^{b}	18.52^{ab}	15.60^{b}	21.88^{a}	1.75
Straight line velocity (um/s)	4.06^{b}	4.78^{b}	4.45^{b}	5.41^{a}	0.39
Amplitude of lateral head (um)	0.44^{b}	0.53^{b}	0.46^{b}	1.28^{a}	0.19
Beat cross frequency (Hz)	3.40^{b}	4.00^{b}	3.22^{b}	7.93^{a}	1.10
Linearity (%)	34.36^{b}	32.59^{b}	40.52^{a}	25.09^{c}	3.37
Straightness (%)	49.60^{b}	49.59^{b}	53.96^{a}	43.63^{b}	2.34
Wobble (%)	65.88	61.99	69.99	67.37	2.96
Acrosome integrity (%)	75.32^{b}	89.62^{a}	86.51^{a}	86.95^{a}	3.50
Liveability (%)	76.84^{b}	91.75^{a}	90.64^{a}	90.15^{a}	2.18

abc: means with different superscripts are significantly ($P < 0.05$) different; D1-control, D2- 10% Moringa, D3- 10% Phyllanthus, D4- 10% Mistletoe.

Table 4
Seminal Oxidative status of heat stressed bucks fed phytogenic supplements.

	D1	D2	D3	D4	SEM
Total Protein (g/L)	27.71^{b}	26.16^{b}	24.09^{b}	43.17^{a}	4.44
Total Antioxidant activity (mmol/L)	4.34^{c}	7.35^{b}	8.89^{b}	10.52^{a}	1.05
Lipid peroxidation (x10^{-5}MDA/mg protein))	1.60^{a}	0.16^{b}	0.35^{b}	0.17^{b}	0.25
Superoxide Dismutase (U/min/mg protein)	0.67^{b}	1.33^{a}	1.96^{a}	2.05^{a}	0.35
Glutathione Peroxidase (mg GSH/min/mg protein)	12.14^{b}	16.05^{a}	12.95^{b}	13.27^{b}	1.40
Catalase (nm H_2O_2/min/mg protein)	0.25^{b}	1.46^{a}	1.76^{a}	2.22^{a}	0.39

abc: means with different superscripts are significantly ($P < 0.05$) different; D1-control, D2- 10% Moringa, D3- 10% Phyllanthus, D4- 10% Mistletoe.

3.4. Stress and metabolic hormones of heat-stressed bucks fed on herbal supplements

Stress and metabolic hormones of heat-stressed bucks fed the herbal supplements is revealed in Fig. 2. Triiodothyronine of bucks on D3 was significantly ($p < 0.05$) highest across the treatments, with the significant ($p < 0.05$) least value obtained in bucks on D1. Insulin of bucks on D3 was significantly ($p < 0.05$) highest across the treatments and the least values were obtained in bucks on D4. Corticosterone of bucks on D1 was significantly higher than bucks on other treatments (D2-D4) which share statistically ($p > 0.05$) similar values.

3.5. Sex hormones of heat-stressed bucks fed on herbal supplements

Sex hormones of heat-stressed bucks fed the herbal supplements is revealed in Fig. 3. LH activity of bucks fed D2 were significantly ($p < 0.05$) higher than bucks on D3 and D4, and the significantly ($p < 0.05$) least value was obtained in bucks on D1. FSH activity of bucks on D2 and D3 was not significantly ($p > 0.05$) different but was significantly ($p < 0.05$) higher than bucks on D1 and D4. Bucks on D3 had significantly ($p < 0.05$) highest testosterone activity than bucks on D2 and D4 which had statistically ($p > 0.05$) similar values, and the significantly ($p < 0.05$) least values were obtained in bucks on D1.

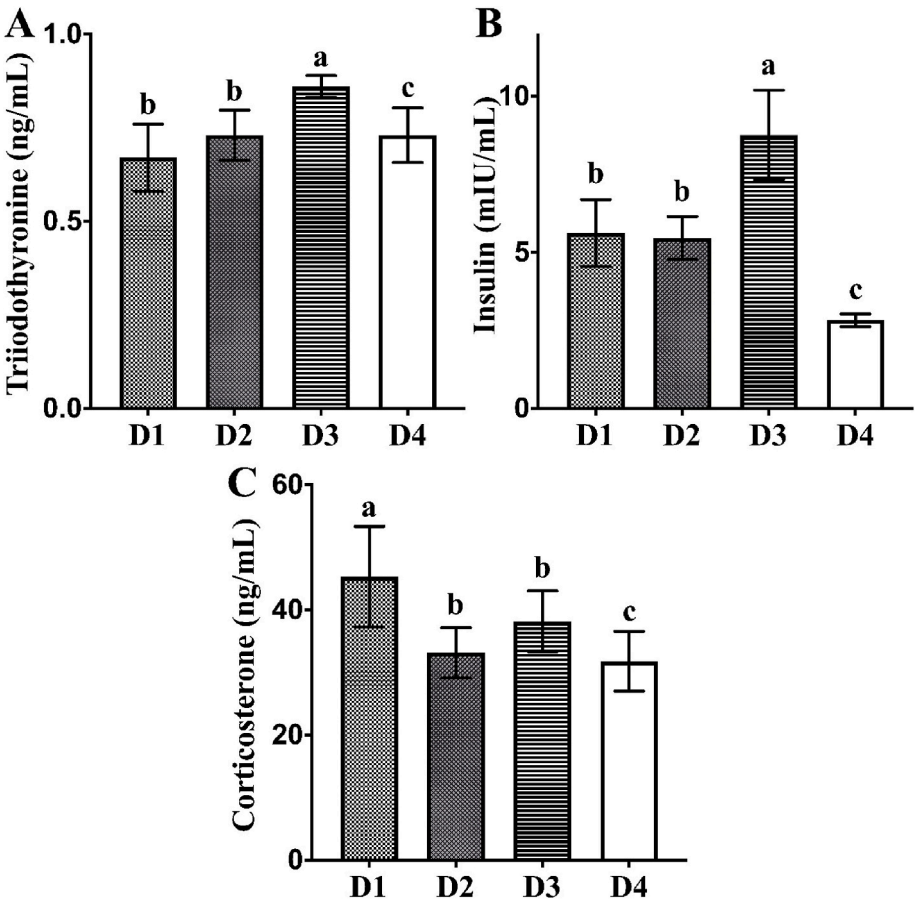


Fig. 2. Stress and metabolic Hormones of heat-stressed bucks fed phytogenic supplements. The effects of treatment are shown on (A) Triiodothyronine, (B) Insulin, (C) Corticosterone. abc: means with different superscripts are significantly ($P < 0.05$) different; heat stressed bucks received herbal supplemented diets D1-control, D2- 10% Moringa, D3- 10% Phyllanthus, D4- 10% Mistletoe.

4. Discussion

The temperature-humidity index of the rabbitry microclimate reveals the magnitude of thermal discomfort the animals were exposed to throughout the study. It confirms earlier reports (Jimoh et al., 2018, 2021b; Jimoh and Ewuola, 2016, 2018) that rabbits are exposed to peak thermal discomfort in southwestern Nigeria between January and March. This is a feature of the tropical region, as against the temperate region where climatic conditions of the summer predispose animals to heat stress usually from July to September.

The trend of results obtained showed that herbal supplementation was beneficial to the libido and spermatozoa kinematics of bucks under conditions of thermal assault. The libido of buck exposed to heat stress was enhanced by moringa and mistletoe supplements. The lower reaction time in the bucks fed on moringa and mistletoe supplements attest to their high libido. Past studies revealed the potency of *Moringa oleifera* to promote sexual urge and spermatozoa quality (Prabsattroo et al., 2012), improve sexual activity and testosterone level in stressed male rats (Prabsattroo et al., 2015). Because Moringa leaves are important fountain of β-carotene, vitamins B-complex, C, D and K (Abd El-Hack et al., 2020).

Sperm concentration and motility traits were higher in bucks fed on moringa and phyllanthus supplements. Spermatozoa speed traits of bucks fed on mistletoe were superior to bucks on other treatments during heat stress. This is in consonance with claims made by Jimoh et al. (Abd El-Hack et al., 2020) that spermatozoa speed traits of non-stressed bucks fed on mistletoe supplements were superior. Similarly, trajectory traits were improved in heat-stressed bucks fed on mistletoe supplement as to other supplements and control. This is connected to the antioxidant potency of mistletoe supplement that enhances spermatogenesis and boosts semen quality leading to remarkable daily sperm production, testicular sperm reserve, spermatozoa kinetics, testosterone and seminal total protein in rabbit bucks (Abd El-Hack et al., 2020).

The linearity and straightness were better in semen obtained from bucks fed on phyllanthus during heat stress conditions. The result of trajectory traits of heat-stressed bucks fed on herbal supplements agrees with earlier reports by Jimoh et al. (Abd El-Hack et al., 2020) who compared the three herbal supplements in normal rabbits. Spermatozoa viability traits (livability and acrosome integrity) were enhanced by the three herbal supplements in heat-stressed bucks. Similar to this result is a report by El Desoky et al. (El-Desoky et al., 2017) which held that herbal supplementation enhanced the spermatozoa quality in heat-stressed rabbits. Contrary to reports by Jimoh et al. (2021a) in bucks fed on Moringa and Phyllanthus supplements had lower Spermatozoa concentration as to bucks fed on mistletoe inclusive diet. The effect of heat stress on these bucks could account for the response obtained in the bucks supplemented with moringa and phyllanthus. A similar result was recorded in rabbit bucks fed on *Phyllanthus amarus*, produce semen with high spermatozoa motility and testosterone (Jimoh et al., 2021a), *Phyllanthus amarus* extracts gavage to male rats (Azubuike et al., 2018) had elevated testosterone, higher spermatozoa motility and concentration.

The seminal oxidation stature of rabbit bucks under heat stress were reduced by herbal supplements due to the enhancements of its seminal antioxidant activities (total antioxidant activity and enzymatic antioxidants) in comparison with the control groups without herbal supplement. The presence of flavonoids in Mistletoe, moringa and phyllanthus

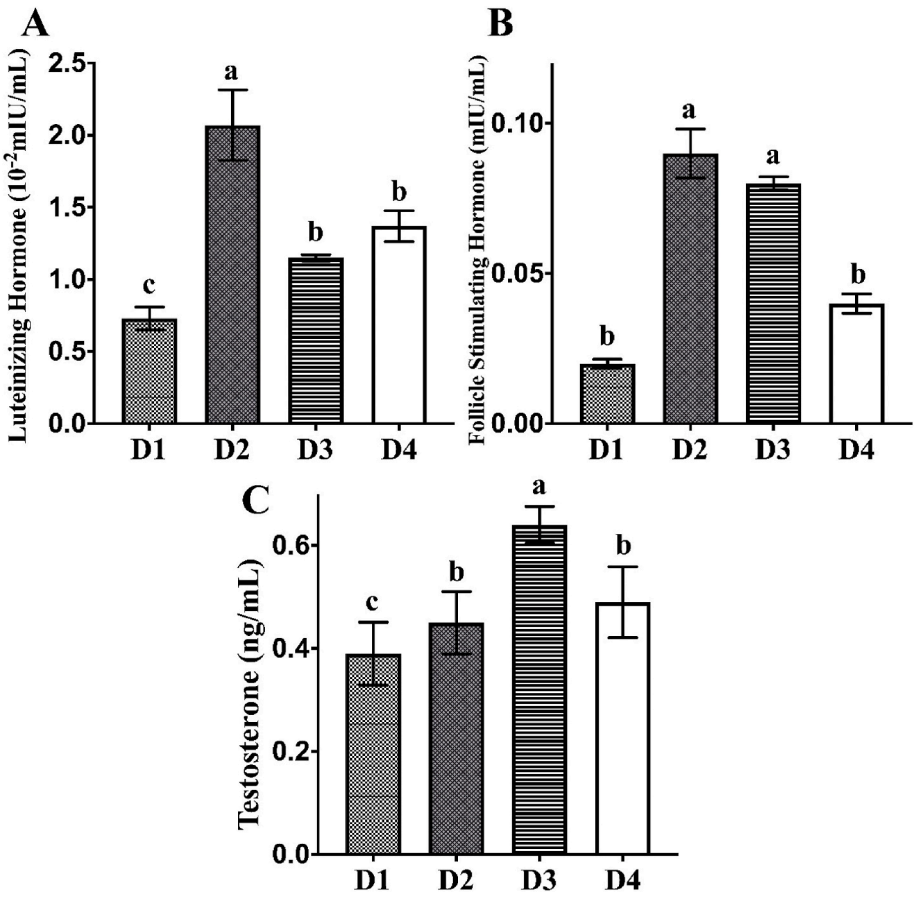


Fig. 3. Sex Hormones of heat-stressed bucks fed phytogenic supplements. The effects of treatment are shown on (A) Luteinizing Hormone, (B) Follicle Stimulating Hormone, (C) Testosterone. abc: means with different superscripts are significantly ($P < 0.05$) different; heat stressed bucks received herbal supplemented diets D1-control, D2- 10% Moringa, D3- 10% Phyllanthus, D4- 10% Mistletoe.

herbal supplements improved rabbit seminal oxidative stability by improving antioxidant enzyme and total antioxidant activity and decreasing lipid peroxidation in semen, and can mitigate oxidative stress-associated testicular damages in animal tissues (Jimoh et al., 2021a). Bucks under heat stress fed on mistletoe supplement had highest antioxidant defence (total antioxidant, superoxide dismutase and catalase activities) amongst the three herbal supplemented groups. This could account for high progressive spermatozoa motility and spermatozoa speed traits obtained in semen from heat stressed bucks fed on mistletoe in this study. Higher glutathione peroxidase in bucks fed on moringa supplements during heat stress would be beneficial to the homeostatic mechanism of the bucks. As glutathione is a crucial cofactor of deiodinases and antioxidant enzymes, the enzymes liable for the transformation of thyroxine (T4) to triiodothyronine (T3) (Mancini et al., 2016). Similarly, Zeweil et al. (2013) reported enhanced seminal plasma antioxidant capability and decline lipid peroxidation in heat stressed rabbit with pomegranate administration. Also in agreement with this study, is the report that soursop juice reversed semen deficiencies by enhancing seminal oxidative status in heat stressed rabbits (Jimoh et al., 2021b).

Seminal plasma affects the viability and membrane integrity of the sperms with regard to its lipid peroxidation level and antioxidant content and influence fertility and litter performance in rabbits (Jimoh and Ayedun, 2020). The result of this study reveals that heat stressed bucks without herbal supplements had high corticosterone and low triiodothyronine and insulin levels. However, the herbal supplements enhanced triiodothyronine in heat stressed bucks with the peak activity obtained in bucks fed phyllanthus. This indicates that reduction in appetite associated with thermal stress (Jimoh et al., 2018) could be combated with herbal supplements during heat stress in rabbits. The mechanism for improving appetite in the rabbit bucks fed on phyllanthus amarus supplements may also explain its high insulin level to combat glucose surge which could increase heat load.

The insulin of bucks fed on phyllanthus supplements were higher than those of other groups while the inclusion of mistletoe supplements in the diets reduced markedly the insulin compared to other treatments. Mistletoe supplements fed to heat stressed bucks lowered insulin levels, and this may be associated with its adaptive mechanism to thermal stress by lowering corticosterone activity. Elevated blood insulin level amid prolong thermal assault is a flexible mechanism used to reduce heat production, which accounts for the declined circulating glucose, the edentated lipolytic activity and the improved adipose tissue lipogenesis (Slimen et al., 2015).

The serum corticosterone of heat stressed bucks without herbal supplementation was higher than those fed on herbal supplements. This could be explained by the report of Wang et al. (2007) that heat exposure elevate peripheral cortisol, which occurs mainly in lipid, protein and carbohydrate metabolism, while T3 and T4 elevates metabolism via reducing the glucose oxidation rate and improving the quantity of metabolic heat generated (Tao et al., 2006). This signifies that the herbal supplements were effective in combating the effect of heat stress by lowering stress hormone levels.

The result obtained in this study shows that heat stressed bucks without herbal supplementation had lower testosterone, LH and FSH, while the herbal supplementation of moringa phyllanthus and mistletoe tended to increased its values in bucks. This is in line with Amen and Doraji (Amen and Al-Daraji, 2011) reports, which affirmed that heat stress reduced LH and FSH hormones. Due to the fact that heat stress

exposure in animals lead to a reduction in the frequency of gonadotropin-releasing hormone pulse generator in the hypothalamus, destroying secretions of FSH and LH from the pituitary gland (Vandana et al., 2020).

Higher LH was observed in bucks on moringa supplements, testosterone peaked in bucks fed on phyllanthus while FSH was highest in bucks fed on moringa and phyllanthus supplements. A similar report by Obianime and Uche (2009) demonstrated that *Phyllanthus amarus* juice accounted for the raise in the testosterone level in Guinea pigs. Previous reports by Jimoh et al. (2021a) affirming that Moringa supplements in diets of bucks increased plasma LH in comparison with phyllanthus and mistletoe, is in line with this study. But, in congruence to this study also is the report by Jimoh et al. (2021a), which compared the three herbal supplements in non-stressed rabbits and recorded higher testosterone in bucks fed on mistletoe supplemented diet. But similar responses were obtained in non-stressed rabbits fed on the three herbal supplements with elevated FSH and LH of Phyllanthus and moringa supplemented bucks as against mistletoe supplements fed bucks (Jimoh et al., 2021a). Similarly, LH declined and increased testosterone in bucks fed on Phyllanthus supplemented reported by Jimoh et al. (2021a) attributed this to negative feedback of testosterone on LH synthesis. Asuquo et al. (2018) reported that decrease in LH and testosterone may be a consequence of the regression of the anterior pituitary cells by *Spondias mombin* in rats, and may explain lower LH and testosterone values of bucks fed on supplement of mistletoe as to higher values obtained in moringa and phyllanthus supplemented bucks.

Testosterone of heat-stressed bucks were enhanced by herbal supplementation with the highest level obtained from bucks fed on phyllantus supplement. This could explain the high libido and spermatozoa kinematics traits recorded in bucks fed on herbal supplements during heat stress conditions in this study. Testosterone bolster anabolic effects and muscular growth and is partly liable for sustaining oxidative stability. Jimoh et al. (2021a) justifies higher oxidative stability and testosterone obtained in this study. Similarly, the improvement of reproductive hormone synthesis in heat stress hens has been attributed to glutamine dietary inclusion (Rizk et al., 2021). Similarly, El-Desoky et al. (2017) reported that moringa juice increased sperm concentration and improved spermatozoa quality by improving testosterone synthesis due to the antioxidant proficiency of moringa may conserve diverse phases of spermatocytes from apoptosis, accompanying improvement in sperm production. Low testosterone in heat-stressed bucks without herbal supplements could explain poor spermatozoa kinematics obtained in this study as Rai et al. (2004) reported that heat stress consumes Leydig cells, reduces plasma testosterone, and diminishes sperm production. Lower testosterone levels in bucks fed on moringa supplements as to bucks fed on phyllanthus supplements could be explained by claims made by Tavares et al. (2016) that reduced serum testosterone owing to the existence of diethyl phthalate in the moringa is notable for posessing an anti-androgenic activity at a higher dose of moringa extract. Similarly, Jimoh et al. (2021a) reported that phyllanthus supplement in bucks' diet promotes weight gain, plasma FSH, testosterone, and epididymal sperm reserves. Similarly, Ginger supplementation restored heat-stressed-induced down regulated total protein by the improved synthesis and movement of sex hormones (Ibtisham et al., 2019), could explain the mechanism of enhanced reproductive hormones obtained in heat stressed bucks fed on herbal supplements in this study.

Increased semen volume, spermatozoa motility and concentration, and, decreased abnormal spermatozoa and an elevated LH and FSH plane were the pro-fertility elements of herbal leaf supplements in rabbits (Ajuogu et al., 2019). This is traceable to leaves that possess high levels of antioxidants like phenolic acids, glucosinolates and flavonoids (Mbikay, 2012). Similarly, Zeweil et al. (2013) reported that defective reproductive status of bucks discovered in heat-stressed rabbits can be improved by pomegranate administration. This affirms that the three herbal supplements are potent ameliorative measure that can be employed in rabbits to mitigate reproductive impairment and metabolic deficiencies during heat stress conditions.

5. Conclusion

The three phytogenic supplement enhance triiodothyronine, sex hormones, spermatozoa kinetics, viability and seminal oxidative stability, but decrease corticosterone of bucks during heat stress condition, with the best outcome recorded in bucks fed on mistletoe supplement. Bucks consumption of phyllanthus supplements recorded elevated insulin and triiodothyronine across the groups. Bucks on moringa supplements having high FSH and LH activity, while bucks consuming phyllanthus supplements recorded elevated FSH and Testosterone activity across the groups.

Animal welfare statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the institutional ethics committee for the care and use of animals for research also approved the study (approval no: FPA/EC/20/0405). The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

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Credit role statement

Jimoh, Olatunji Abubakar: Conceptualization; Investigation; Project administration; original draft; review & editing, Daramola Olajumoke Temidayo: Supervision; Validation; Visualization; Project administration; Resources, Okin-Aminu, Hafsat Ololade: Supervision; Project administration; Resources; Review & editing., Ojo, Olayinka Abosede: Investigation; Methodology; Project administration; Resources, Oyeyemi Wahab Adekunle: Data curation; Formal analysis; Review & editing. All authors read and approved the final manuscript.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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