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Author	Jimoh, Olatunji Abubakar, Olakanye Blessing Oluwaseun, Ajewole Ayoola Mercy, Akinbuyide Saanu Olajumoke, Adetifa Johnson Sunday, Jimoh Abdul Quadri Ayodeji, Mayowa Adewale Oluwatosin and Adesina Feyisayo Pemisire
Affiliation	[1] Department of Agricultural Technology, The Federal Polytechnic Ado-Ekiti, Ekiti State, Nigeria.
ORCID (for more information, please visit https://orcid.org)	Jimoh, Olatunji Abubakar (https://orcid.org/0000-0001-8204-5816) Olakanye Blessing Oluwaseun https://orcid.org/0000-0002-3377-7137 Ajewole Ayoola Mercy https://orcid.org/0000-0003-0684-0476 Akinbuyide Saanu Olajumoke https://orcid.org/0000-0003-3710-2919 Adetifa Johnson Sunday https://orcid.org/0000-0002-9840-2002 Jimoh Abdul Quadri Ayodeji https://orcid.org/0000-0002-4374-2640 Mayowa Adewale Oluwatosin https://orcid.org/0000-0002-4144-5669 Adesina Feyisayo Pemisire https://orcid.org/0000-0001-7643-5180
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CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	**Abubakar O Jimoh, Ph.D.**
Email address – this is where your proofs will be sent	abubakarjimoh2011@gmail.com
Secondary Email address	jimoh_oa@fedpolyado.edu.ng
Address	Animal Production Technology Option Department of Agricultural Technology, The Federal Polytechnic Ado Ekiti, Ekiti State. Nigeria.
Cell phone number	+234806 605 8134
Office phone number	+234805 978 3712
Fax number	

Potentials of *Phyllanthus amarus, Viscum album* and *Moringa oleifera* supplements to mitigate heat stress in female rabbits in humid tropics

Jimoh, Olatunji Abubakar, Olakanye Blessing Oluwaseun, Ajewole Ayoola Mercy, Akinbuyide Saanu Olajumoke, Adetifa Johnson Sunday, Jimoh Abdul Quadri Ayodeji, Mayowa Adewale Oluwatosin and Adesina Feyisayo Pemisire

[1] Agricultural Technology Department, The Federal Polytechnic Ado Ekiti, Nigeria.

***Corresponding author:** abubakarjimoh2011@gmail.com. +234806658134.

Running title: Physiology of Does influenced by tropical herbs in humid Tropics

Potentials of *Phyllanthus amarus, Viscum album* and *Moringa oleifera* supplements to mitigate heat stress in female rabbits in humid tropics

Abstract

Global warming is a key challenge subjecting animals to heat stress conditions resulting in multiple physiological alterations in tropical climate. Dietary approach seems to be the more friendly approach to curb the adverse effects of heat stress in rabbits. Some herbs have been categorized to have high potential for promotion of immune responses for amelioration of heat stress. Thus, this research aims to evaluate the potential of Mistletoe (*Viscum album),* Moringa *(Moringa oleifera)* and Phyllanthus *(Phyllanthus amarus)* leaf meal as herbal supplements for the alleviation of heat stress in female rabbits by measuring improvement in sex and stress hormonal responses in serum biochemistry.

80 Rabbit does were exposed to 4 dietary groups supplemented with each of Mistletoe, Moringa, Phyllanthus and a control in an 84-day trial at the summit of thermal stress in South west Nigeria. Growth indices were monitored throughout the study, blood samples were compiled at the end of the trial to assess serum biochemistry, stress and sex hormonal responses of the Does using standard protocols.

The results revealed that final weight and weight gain of Does fed on Phyllanthus were significantly ($p<0.05$) higher (11.46% and 14.25%, respectively) than Does on on control. The herbal supplements enhance glucose, protein, albumin and globulin, reduced cholesterol, and creatinine of Does under heat stress conditions. Among the herbal treatment groups, mistletoe, moringa and phyllanthus had 12.42%, 18.39% and 16.90%, respectively, lower corticosterone than control groups which had 39.76ng/ml. Triiodothyronine of Does fed control were significantly ($p<0.05$) lower than Does on *Moringa oleifera* and *Phyllanthus amarus* supplements. Estradiol and Follicle stimulating hormone (FSH) of rabbit Does fed on moringa supplement were significantly ($p<0.05$) higher other treatments.

In conclusion, the herbal supplements tend to mitigate the detrimental outcome of thermal stress on Does by suppressing stress hormones. *Moringa oleifera* and *Phyllanthus amarus* enhanced sex hormones while *Phyllanthus amarus* confered growth promoting effects on the Does.

Keyword: Heat-stress; Rabbits; herbal supplements; nutritional intervention; Phytogens

INTRODUCTION

Current trends in global warming will cause hotter climate which will induce heat stress in rabbits [1]. Heat stressed rabbits has been reported to possess suboptimal productivity, compromised immunity, inefficient thermoregulatory system and subfertility [2], reduced thyroid hormones, secretion of corticosterone [3]. Environmental stress adversely influences the immunity of the animals and invokes inflammation [4], and inadequate productive and reproductive performances due to altered animal behaviour. Heat stress perturbs the normal condition of female sex steroids at hypothalamus and ovarian plane [5].

Herbs are good enhancer of follicular advancement and immune status [6], and they have beneficial effects on enteric and digestive enzyme status which increases appetite and production rate [7]. Thermal stress-induced oxidative damage could be mitigated by dietary supplementation of herbal additives to mitigate the detrimental incursions of environmental stress on animals [8]. There is a notion that natural feed additives ameliorate the negative effects on heat stress of animals by improving the immune system, oxidative status and pro-inflammatory cytokines on stressed rabbits due to the antioxidant, antimicrobial, and immune-booster effects of these herbal plants [4]. Wide range of natural feed resources have been evaluated for potentiality to counter negative impacts of heat stress with emphasis on practicality, safety and economical application [9]. Dietary Supplementation with suitable phytochemical-containing feed could potentially mitigate the deleterious impact of heat stress [10]. El-Desoky et al. [10] reported that phytogenic composite with anti-inflammatory, antioxidant and antibacterial properties may expedite the capability of animals to retain their body homeostasis by agitating domestic cellular fortification mechanisms to handle oxidative assault and inflammation lured by thermal stress. Phytochemicals with antioxidant activity such as polyphenols, are vital secondary metabolite found in plants, are anticipated to resolve heat stress in tropical animals [9,11]. *Phyllantus amarus* contains phyto-nutrients that serves as healthy supplements and growth enhancer in animal feed, Mistletoe act effectively as antioxidants and peroxyl radical scavengers, possess pharmacological properties having immunomodulatory, anti-inflammatory, cardiovascular, and antimicrobial effects, while Moringa is a potent antibiotic and has been employed in the treatment of reproductive, cardiac and circulatory problems [11]. Previous reports had documented the beneficial role of *Moringa oleifera*, *Phyllanthus amarus*, and *Viscum album* individually as tropical medicine in promoting health, welfare and productivity of tropical livestock and poultry [11]. They have been reported to influence reproductive health, as the three herbs elicits different response which implied that strategic herbal supplementation would be required to meet different reproductive desires in rabbit breeding programme [11]. Different natural feed resources have been evaluated for potentiality to counter negative impacts of heat stress with emphasis on practicality, safety and economical application [11]. Dietary supplementation of *Moringa oleifera*, *Phyllanthus amarus*, and *Viscum album* leaves at 10% inclusion had the potency to promote good health and well-being [11]. This study was targeted at evaluating the effect of herbal supplements on performance, serum biochemicals and hormonal profile of rabbit Does expose to heat stress in a hot tropical climate.

MATERIALS AND METHODS

Experimental site

This research was conducted in south-western agro-ecological belt of Nigeria, from January to March, 2020. Severity of heat stress positioned on a sequence of humidity and temperature (Temperature-Humidity Index -THI) was established to peak in southern Nigeria between February and March [2,9,12,13]. Relative humidity and temperature of the rabbit house was documented at 08:00 h, 12:00h and 18.00 h daily in the course of the study with a Thermo-Hygrometer. The daily records were used to compute the temperature humidity index as outlined in Jimoh et al. [14] .

Herbal supplements processing and Evaluation

Fresh *Viscum album* (MILM), *Phyllanthus amarus* (PHLM) and *Moringa oleifera* (MOLM) leaves were harvested from an established orchard within the Ado-Ekiti metropolis of Ekiti State. The plants were identified and indexed with herbarium voucher numbers; *Viscum album* UILH/002/084/1210/2021; *Moringa oliefera* UILH/001/1008/2021; *Phyllanthus amarus* UILH/003/1109/2021.

Leaves were detached from twigs and shade-dried until it was crumbly to touch while keeping their greenish coloration. They were milled and stored in air tight containers until incorporation into the diet. Phytochemical screening and proximate composition of the leaf meals were carried out using standard analytical procedures.

Experimental animals and management

Eighty rabbits Does (527.99 ±10.35g) of 4 weeks old were used for the investigation. The rabbits were allotted to the four groups (10 replicates of 2 Does per replicate) and they were housed individually in a Completely Randomized Design for an 84-day feed trial.

Experimental diet

Four diets were compounded to appropriate the nutrient demand for growing rabbit and were pelleted. Diet 1(control) without the leaf meal apportioned as the control diet and Diets 2, 3 and 4 with 10% Mistletoe (MILM), 10% Moringa (MOLM) and 10% Phyllanthus (PHLM), respectively as presented in Table 1 as a follow up to our earlier study of Jimoh et al., [11]. The does were fed at 4% of their body weight and offered clean water ad libitum for 12 weeks. The weight changes and feed consumed were recorded all through the study to evaluate their performance.

Sample collection and analysis

At the end of the feed trial, samples of blood were collected from all does via jugular venipuncture into plain sample bottle serum biochemical. Samples were centrifuged and serum obtained using standard procedures and stored at -20^0C before analysis. Serum biochemical assay; glucose, total protein, albumin, globulin, aspartate amino transferase (AST), alanine amino transferase (ALT), urea, creatinine, cholesterol, high density lipoprotein (HDL), triglyceride, low density lipoprotein (LDL) were carried out using fortress diagnostics commercial assay kits (Fortress Diagnostics Ltd, Unit 2C Antrim Techn. Park, Antrim, BT41 1QS, Great Britain) and its procedures.

Hormonal assay

Serum samples obtained were assayed for triiodothyronine, insulin, follicle stimulating hormone (FSH), corticosterone, luteinizing Hormone (LH), and estradiol using ELISA, with commercial ELISA kits and its protocol for each assay; Estradiol (E2) ELISA Kit, (Catalog No. ES180S), Calbiotech Inc. 10461 Austin Dr, Spring Valley, CA 91978, USA. Follicle Stimulating Hormone (FSH) ELISA kit, (Catalog No.: FS232, Luteinizing Hormone (LH) ELISA kit, (Catalog No.: LH231F), Triiodothyronine (T3) ELISA (Catalog No. T3225T) and Insulin ELISA kit, (Catalog No. IS130D) the quadruplets by Calbiotech Inc., 1935 Cordell Ct., El Cajon, CA 92020. Corticosterone ELISA Kit, (Cat.No

E0496Ra), Bioassay Technology Laboratory, www.bt-laboratory.com, 1008 Junjiang Inter. Bldg. 228 Ningguo Rd. Yangpu Dist. Shanghai. China.

Statistical analysis

The statistical model applied is as follows:

$Y_{xyz} = \mu + B_i + e_{xyz}$

Where Y_{xyz} denotes the value of performance, serum biochemicals and hormonal profile estimated in the x^{th} animal; μ is the comprehensive mean for each character; B_i is the fixed effect of x^{th} herbal supplement; and e_{xyz} is the random residual effect.

Data obtained was tested using generalized linear model procedure of one-way ANOVA according to statistical software IBM SPSS 20.

RESULTS

Phytochemical and Proximate analysis of the herbs

Proximate and phytochemical analysis of the leaf meals is shown in Table 2. Moringa possesses higher crude protein, saponins, glycosides, steroids among the three leaf meals. Mistletoe possesses higher crude fibre, ash, nitrogen free extract, alkaloids, flavonoids and tannins among the three leaf meals. Of the three leaf meals, Phyllanthus possesses the least crude fibre, ash, saponin and tannin.

Temperature humidity index of the rabbit microclimate

The THI of the rabbit pen is shown in Figure 1. The THI obtained at 8am indicates that the Does were exposed to average THI values of 24.05 (absence of heat) with a range between 18.15 (absence of heat stress) – 32.63 (very severe heat stress). At noon, average THI values obtained 30.89 indicates very severe heat stress with a range of THI of 28.14 (severe heat stress) – 33.04 (very severe heat stress). The average THI values at 1800 hours are 33.26 (very severe heat stress), and a range of values 24.45 (absence of heat stress) – 30.15 (very severe heat stress).

Performance characteristics of heat-stressed Does fed on herbal supplement

Performance characteristics of Does fed on herbal supplement during heat stress condition is shown in Table 3. The weight gain and final weight of Does fed on PHLM were significantly ($p<0.05$) higher than other treatments. The weight gain and final weight of Does on MILM and MOLM were not significantly ($p>0.05$) different from the control. The feed intake of Does on control were significantly ($p<0.05$) higher than Does on supplements, with the significantly ($p<0.05$) least value obtained in Does on MOLM. The Feed conversion ratio (FCR) of Does on control and MILM were significantly ($p<0.05$) higher than other treatments, while Does on PHLM had significantly ($p<0.05$) least values.

Serum biochemistry of heat-stressed Does fed on herbal supplement

Serum biochemistry of Does fed on herbal supplement during heat stress condition is shown in Table 4. Does fed PHLM and MILM had significantly ($p<0.05$) higher glucose than Does on MOLM and control. Total protein was significantly ($p<0.05$) higher in Does fed on MILM and MOLM based diets compared to PHLM and control. Albumin of Does fed on MILM was significantly ($p<0.05$) higher than Does on other treatments, with the statistically ($p<0.05$) least value recorded in Does on PHLM. Globulin of Does on MILM and MOLM were significantly ($p<0.05$) higher than Does on PHLM, while Does on control had the ($p<0.05$) least value. Cholesterol of Does on PHLM was significantly ($p<0.05$) lower than Does on MOLM, MILM and control which had statistically ($p>0.05$) similar values. Triglycerides of Does on PHLM was not significantly ($p>0.05$) different from Does on control and were significantly ($p<0.05$) higher than Does on MOLM and MOLM, and both share statistically ($p>0.05$) similar values. HDL of Does on MOLM and PHLM were significantly higher than Does on MILM, while Does on control had statistically ($p>0.05$) least values. LDL of Does on control were significantly ($p<0.05$) higher than Does on supplement with the significantly least value obtained in PHLM. Creatinine of Does on control were statistically ($p<0.05$) higher than Does on herbal supplement. AST and ALT of Does on MOLM were significantly ($p<0.05$) higher than Does fed on other treatments. Urea of Does on MILM based diets were significantly ($p<0.05$) higher than Does on MOLM and PHLM based diets, while Does on control had the significantly ($p<0.05$) least values.

Stress and metabolic hormones of heat-stressed Does fed on herbal supplement

Stress and metabolic hormones of Does fed on herbal supplement during heat stress condition is shown in Figure 2. Triiodothyronine of Does fed control (0.68ng/ml) were significantly ($p<0.05$) lower than Does on MOLM (0.77ng/ml), PHLM (0,80ng/ml) and MILM (0.79ng/ml) which had statistically ($p>0.05$) similar values. Insulin of Does on PHLM (5.22mIU/ml) were not significantly ($p>0.05$) different from other treatments. However, Does on MILM (4.39mIU/ml) and MOLM (4.79mIU/ml) were significantly ($p<0.05$) lower than the control (6.25mIU/ml). Corticosterone of Does on supplemented groups (34.82ng/ml, 32.45ng/ml and 33.04ng/ml for MILM,MOLM and PHLM, respectively) were significantly ($p<0.05$) lower than Does on control (39.76ng/ml).

Sex hormones of heat-stressed Does fed on herbal supplement

Sex hormones of rabbit Does fed on herbal supplement during heat stress condition is shown in Figure 3. Does fed PHLM supplement (2.73 mIU/ml10^2) had significantly ($p<0.05$) higher LH than Does on MILM (1.17 mIU/ml10^2), MOLM (1.20 mIU/ml102) and control (1.63 mIU/ml10^2), which share statistically ($p>0.05$) similar values. Estradiol and FSH of rabbit Does fed on moringa supplement (1.90mIU/ml and 14.02pg/ml)were significantly ($p<0.05$) higher other treatments.

DISCUSSION

The trend of THI obtained in the rabbitry microclimate reveals that the Does were exposed to very severe environmental stress occasioned by the hot dry climate.

The performance indices of Does fed on phyllanthus supplement was better than other treatments during the heat stress period caused by hot dry climate. This is occasioned by its better final weight, weight gain and least feed conversion ratio. This is supported by Jimoh et al. [15] that reported that phyllanthus supplement act as a growth promoter without adversely affecting the health status of rabbits. Similarly, Khalil et al. [16] reported on the beneficial effects of herbs on growth of growing heat stressed rabbits. This could be due to phytochemicals such as flavonoids present, which has been found to affect the nutrient digestibility and activity of several digestive enzymes [17], while mitigating physiological disorders that can compromise animal productivity. However, Does on other treatments had similar growth during heat stress in this study.

The herbal supplements tended to improve serum biochemical of Does under heat stress conditions, chiefly by enhancing glucose, protein, albumin and globulin, reducing cholesterol, and creatinine. This agreement with claims of Xie et al. [18], which recorded that the heat-stress downregulated total protein was restored by ginger supplementation via the elevated synthesis and mobility of reproductive hormones [19]. Tayer et al. [20] stated that the favorable reactions of flavonoids in therapeutic herbs relates to the hypoglycemic and hepatic glucokinase activity of the liver.

Other reports affirming that moringa improves serum albumin concentration [10] was attributed up to 80% of the vascular colloidal osmotic pressure as an essential component in maintaining equilibrium with tissue fluids. The antioxidant activity of albumin which administer membrane shelter has been reported to promote cell viability by modulating cholesterol efflux from the cell membrane [21]. The herbal supplements could induce pancreatic cholesterol esterase to hydrolyze dietary cholesterol esters which releases free cholesterol in the lumen of the small intestine, the suppression of cholesterol esterase would limit the absorption of dietary cholesterol and thereby reduce cholesterol concentration [22].

Mistletoe fed Does had better serum glucose, total protein, albumin, globulin, lowered cholesterol profile and creatinine compared to Does without supplements. Moringa supplemented Does had higher total protein, globulin, lowered cholesterol profile and creatinine compared to Does without supplements. Reports attribute the high antioxidant content of herbs to elevates total protein by reducing corticosterone suppuration which could curb protein catabolism under thermal stress situation [23].

Phyllanthus supplement fed to Does induced better glucose, cholesterol profile (lower cholesterol, LDL and higher HDL), lower creatinine compared to Does without supplements. *Phyllanthus amarus* have been reported to reduce cholesterol and low-density lipoprotein, inhibit fat accumulation in cells and reduced oxidative stress and inflammation [24]. The herbal supplements improved cholesterol profile by lowering LDL and improving HDL fractions of the total cholesterol. Hypercholesterolemia is linked with elevated lipid peroxidation and the instrument of contraction of cholesterol also lowers lipid peroxidation [25], thus suggesting the capacity of the supplements to enhance oxidative stability in the Does during heat stress. Thus, the better cholesterol profile could promote oxidative stability in herbal supplemented groups, an indication of better productivity and most likely account for superior performance obtained in phyllanthus supplemented Does.

Moringa supplements enhanced AST and ALT of Does exposed to heat stress condition. This is incongruence to claims that *M. oliefera* leaves reduced the status of alkaline phosphatase aspartate amino transferase and alanine amino transferase, in rats [26,27]. The supplements enhanced serum urea of Does during heat stress conditions in comparison to Does on control diet, this could be attributed to the high serum protein profile in all supplemented groups, which could attract higher protein catabolism and elimination of excess protein via urea formation.

Glutathione is a key cofactor of twain antioxidant enzymes and deiodinases, the enzymes culpable for the transformation of thyroxine (T4) to triiodothyronine (T3) [28].

The trends of result obtained showed that triiodothyronine was enhanced in serum of Does fed on herbal supplement during heat stress condition. T4 and T3 increase metabolism by reducing the rate of glucose oxidation and elevating the load of metabolic heat generated [29]. Insulin and corticosterone were reduced in serum of Does fed on supplements compared to those on control, and could attest to Wang et al. [30] that reported heat disclosure increases blood cortisol, which is convoluted primarily in carbohydrate, lipid, and protein metabolism. The corticotropin-releasing factor which stimulates the synthesis of cortisol as part of hormonal stress response inhibits feed intake [31]. This could explain the increased corticosterone and low triiodothyronine in Does on control diet during the heat stress condition, was mitigated by the inclusion of herbal supplements. Change in blood T3 levels fail to cause symbolic variation in growth except for Does on phyllanthus supplements, possibly linked to better FCR [17]. Environmental stress has been documented to have adverse impact glucose level, which reveals that circulating insulin and glucagon formulation was constrained due to their control on glucose metabolism, or owing to lower concentration of thyroxin, which is highly associated with energy metabolism during thermal stress [18]. Polyphenolic compounds present in herbs curb carbohydrate breakdown (α-amylase and α-glucosidase) and absorption of glucose (glucose transporters), arouse insulin secretion from the pancreatic cells, inflect glucose liberation from the liver, stimulate insulin receptors and glucose uptake in the insulin-sensitive tissues, and regulate intracellular signaling pathways and gene expression [32].

Similar to results obtained in Does not fed on herbal supplements, are claims of lowered enzymatic antioxidant response and heightening of blood corticosterone and heterophil:lymphocyte ratio in acute thermal stressed chickens [33]. The claims that heat stress induce increase in serum cholesterol, due to increased corticosterone, via activation of the hypothalamic-pituitary-adrenal [19], could explain the higher cholesterol profile and corticosterone obtained in Does on control diet. Thyroidal hormones trigger cholesterol synthesis and hepatic mechanisms that eliminate cholesterol from circulation [34], a mechanism for the decrease in cholesterol associated with higher triiodothyronine in Does fed on herbal supplements in this study.

Triiodothyronine plays a key part in controlling metabolism and thermogenesis [29], and is strongly correlated to reduction of feed consumption in thermal stress situation [35]. Thus, exposure of rabbits to thermal stress situations reduce T3 level as obtained in Does on control, in order to decrease heat production to sustain homeothermy [35]. The reduction in serum glucose during environmental stress has been attributed to lower thyroxine as obtained in Does on control in this study, could be due to its association with energy metabolism during heat stress [22] and was reversed in Does fed moringa, mistletoe and phyllanthus supplements. The excess of blood glucose is uptaken by the liver, adipose, and muscle tissues under the control of insulin where it will be converted to glycogen with the accumulation of triglyceride in the adipose tissues [36]. Thus, lower insulin in herbal supplemented Does could account for their higher serum glucose compared to Does on control in this study. Higher triiodothyronine could account for better FCR in herbal supplemented groups and growth enhancement in Does fed on phyllanthus supplement. The inclusion of the herbal supplements in Does' diet in this study, ameliorated the effects of heat stress by lowering corticosterone.

Follicle-stimulating hormone are instrumental in gonadal development, sexual maturity at puberty and gamete production by stimulate growth and maturation of ovarian follicles. Luteinizing hormone surge stimulates ovulation of mature follicles in the ovary, and any substance proficient to alter its release may enrage interruption of ovulation by influencing the number of graffian follicles, as Amen and Al-Daraji [37] claimed that heat stress lowered LH and FSH hormones.

Lutenizing hormone of Does were enhanced by phyllanthus supplement, FSH and estradiol of Does were enhanced by moringa supplements during heat stress conditions in a hot dry climate of this study. The elevated FSH observed would enhance conception in Does, as reduction in the levels of follicle-stimulating hormone due to an inhibitory effect on the release of the gonadotropin, hamper folliculogenesis and actively delay maturation of the follicle in the pre-ovulatory phase by garlic extract [38]. This corroborates claims that high free radical scavenging activity of natural antioxidants, have been widely sourced as candidate antidote to cure oxidative stress and anomalies in hormone functions [39]. Similarly, enhancement of reproductive hormone synthesis by dietary glutamine inclusion in heat stress hens has been reported [5]. Similarly, some flavonoids have been found to act as xenoestrogens, in association with gonadal hormones binds with oestrogen receptors and sex-hormone binding globulin, which is involved in the mobility of steroid hormones [17].

Although, heat stress exposure in animals points to a reduction in frequency of gonadotropin-releasing hormone pulse generator in the hypothalamus, lessen secretions of follicle stimulating hormone and luteinizing hormone from the pituitary gland [1]. But flavonoids have reported to affect the activity of few enzymes involved in androgen, progestin and oestrogen metabolism [40]. Similar to result obtained in this study, Ogbomade et al. [41] reported an increase in follicle-stimulating hormone of Wistar rats administered oral doses of *Phyllanthus amarus.*

CONCLUSION

The range of THI obtained in the study revealed that the Does were exposed to heat stress. The three herbal supplements fed to Does ameliorated effects of heat stress by reducing stress hormones, improve serum biochemical of Does chiefly by enhancing glucose, protein, albumin and globulin, reducing cholesterol and creatinine. *Moringa oleifera* and *Phyllanthus amarus* enhanced sex hormones while *Phyllanthus amarus* confered growth promoting effects on the Does.

Abbreviations

Temperature - Humidity Index-THI
relative humidity – RH
Mistletoe – MILM
Moringa – MOLM
Phyllanthus – PHLM
Alanine amino transferase – ALT
Aspartate amino transferase – AST
High density lipoprotein - HDL
Low density lipoprotein – LDL
Luteinizing Hormone – LH
Follicle stimulating hormone – FSH
Enzyme linked immunosorbent assay – ELISA
Thyroxine - T4
Triiodothyronine - T3
Feed conversion ratio – FCR
Analysis of variance - ANOVA

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Availability of data and material

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Authors' contribution

Conceptualization: Jimoh O.A., Adesina F.P.
Data curation: Jimoh O.A., Olakanye B.O.
Formal analysis: Ajewole A.M., Adetifa J.S
Methodology: Jimoh O.A., Akinbuyide S.O., Mayowa A.O.,Jimoh A.A.
Software: Adetifa J.S., Akinbuyide S.O.
Validation: Jimoh A.A., Olakanye B.O.
Investigation: Jimoh O.A., Mayowa A.O., Adesina F.P.
Writing - original draft: Jimoh O.A., Ajewole A.M.
Writing - review & editing: Jimoh O.A.

Ethical Approval and Consent to participate:

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 care and use of animal for research 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.

Competing Interest

No competing interest exist in the research outcome presented in this article

Consent for publication

Not applicable

Author's information:

Jimoh, Olatunji Abubakar PhD (*Registered Animal Scientist; Nigerian Institute of Animal Science)*
Lecturer and consultant,
Specialist in Animal Reproductive Physiology and bioclimatology,
Department of Agricultural Technology, Federal Polytechnic Ado Ekiti, Ekiti State. Nigeria.
Email: abubakarjimoh2011@gmail.com
https://orcid.org/0000-0001-8204-5816

Olakanye Blessing Oluwaseun
Technologist
Animal Production Technology Option
Department of Agricultural Technology,
Federal Polytechnic Ado Ekiti, Ekiti State. Nigeria
Email: olakanye12@gmail.com
https://orcid.org/0000-0002-3377-7137

Ajewole Ayoola Mercy
Technologist
Animal Production Technology Option
Department of Agricultural Technology,
Federal Polytechnic Ado Ekiti, Ekiti State. Nigeria
Email: ajewoleayoolamercy@gmail.com
https://orcid.org/0000-0003-0684-0476

Akinbuyide Saanu Olajumoke
Technologist
Animal Production Technology Option
Department of Agricultural Technology,
Federal Polytechnic Ado Ekiti, Ekiti State. Nigeria
Email: sakinbuyide@gmail.com
https://orcid.org/0000-0003-3710-2919

Adetifa Johnson Sunday
Technologist
Animal Production Technology Option
Department of Agricultural Technology,
Federal Polytechnic Ado Ekiti, Ekiti State. Nigeria
Email: adetifajohnson34@gmail.com
https://orcid.org/0000-0002-9840-2002

Jimoh Abdul Quadri Ayodeji
Technologist
Animal Production Technology Option
Department of Agricultural Technology,
Federal Polytechnic Ado Ekiti, Ekiti State. Nigeria
Email:ayodejijimoh53@gmail.com
https://orcid.org/0000-0002-4374-2640

Mayowa Adewale Oluwatosin
Technologist
Animal Production Technology Option
Department of Agricultural Technology,
Federal Polytechnic Ado Ekiti, Ekiti State. Nigeria
Email: mayowaoluwaseun0@gmail.com
https://orcid.org/0000-0002-4144-5669

Adesina Feyisayo Pemisire
Technologist
Animal Production Technology Option
Department of Agricultural Technology,
Federal Polytechnic Ado Ekiti, Ekiti State. Nigeria
Email: adesinafeyisayo2019@gmail.com
https://orcid.org/0000-0001-7643-5180

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

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
Brewer Dry Grain	5.0	0	0	0
Grower premix	0.25	0.25	0.25	0.25
Maize	25	25	25	25
Methionine	0.40	0.40	0.40	0.40
Soybean meal	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

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/100g)	14.68	8.5	10.34
Saponins(mg/100g)	31.01	39.81	23.96
Glycosides(mg/100g)	75.08	95.92	81.66
Steroids(mg/100g)	18.82	25.00	20.54
Flavonoids(mg/100g)	62.93	25.03	44.86
Tannins(mg/100g)	114.81	96.53	95.98

Table 3: Performance characteristics of rabbit Does fed herbal supplement during heat stress condition

	Control	MILM	MOLM	PHLM	SEM
Initial weight (g)	494.10	514.70	506.80	499.40	16.49
Final weight (g)	2333.70^{b}	2348.42^{b}	2395.96^{b}	2601.08^{a}	27.78
Average Weight Gain (g/doe)	21.90^{b}	21.83^{b}	22.49^{b}	25.02^{a}	0.63
Average Feed Intake (g/doe/day)	60.25^{a}	60.02^{b}	59.25^{d}	59.50^{c}	0.12
Feed Conversion Ratio	2.78^{a}	2.75^{a}	2.66^{b}	2.38^{c}	0.08

abc: means with different superscripts are significantly ($P<0.05$) different; MILM - Mistletoe leaf meal MOLM - Moringa leaf meal PHLM - Phyllanthus leaf meal

Table 4: Serum Biochemistry of rabbit Does fed herbal supplement during heat stress condition

	Control	MILM	MOLM	PHLM	SEM
Glucose (mmol/l)	2.15^{b}	3.05^{a}	2.33^{b}	3.34^{a}	0.33
Total Protein (g/l)	63.79^{b}	112.20^{a}	112.89^{a}	45.20^{b}	11.88
Albumin (g/l)	44.23^{b}	53.04^{a}	45.93^{b}	31.08^{c}	3.83
Globulin (g/l)	19.56^{c}	59.16^{a}	66.96^{a}	43.24^{b}	10.03
Cholesterol (mmol/l)	2.85^{a}	2.52^{a}	2.46^{a}	1.67^{b}	0.16
Triglyceride (mmol/l)	2.78^{a}	2.07^{b}	2.02^{b}	2.64^{a}	0.32
High density lipoprotein (mmol/l)	0.37^{c}	0.55^{b}	0.75^{a}	0.78^{a}	0.07
Low density lipoprotein (mmol/l)	1.36^{a}	0.86^{b}	0.85^{b}	0.32^{c}	0.18
Creatinine (mg/dL)	2.50^{a}	1.07^{b}	1.10^{b}	0.95^{b}	0.47
Aspartate amino transferase (U/I)	12.82^{b}	18.21^{b}	24.80^{a}	6.65^{c}	2.88
Alanine amino transferase (U/I)	5.21^{b}	4.25^{b}	12.30^{a}	6.38^{b}	1.23
Urea (mmol/l)	2.68^{c}	7.40^{a}	4.17^{b}	4.63^{b}	0.70

abc: means with different superscripts are significantly ($P<0.05$) different; MILM - Mistletoe leaf meal MOLM - Moringa leaf meal PHLM - Phyllanthus leaf meal

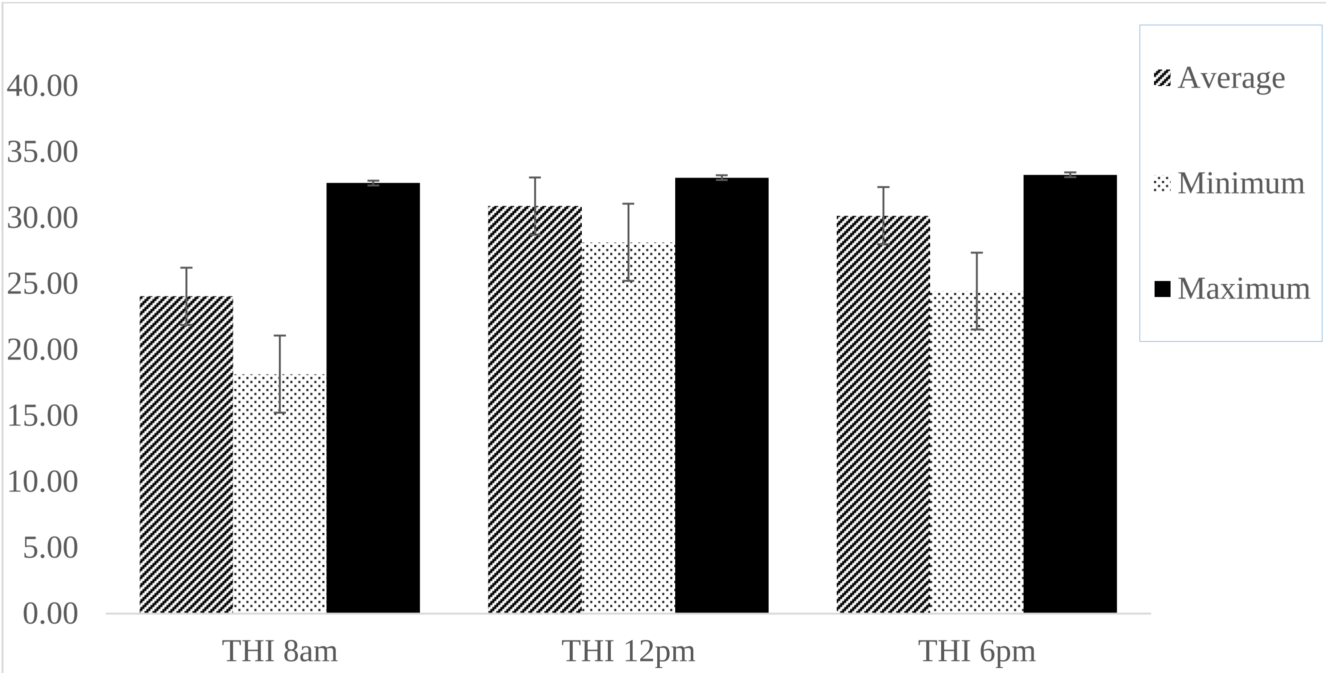

Figure 1: Temperature humidity index of Rabbit pen

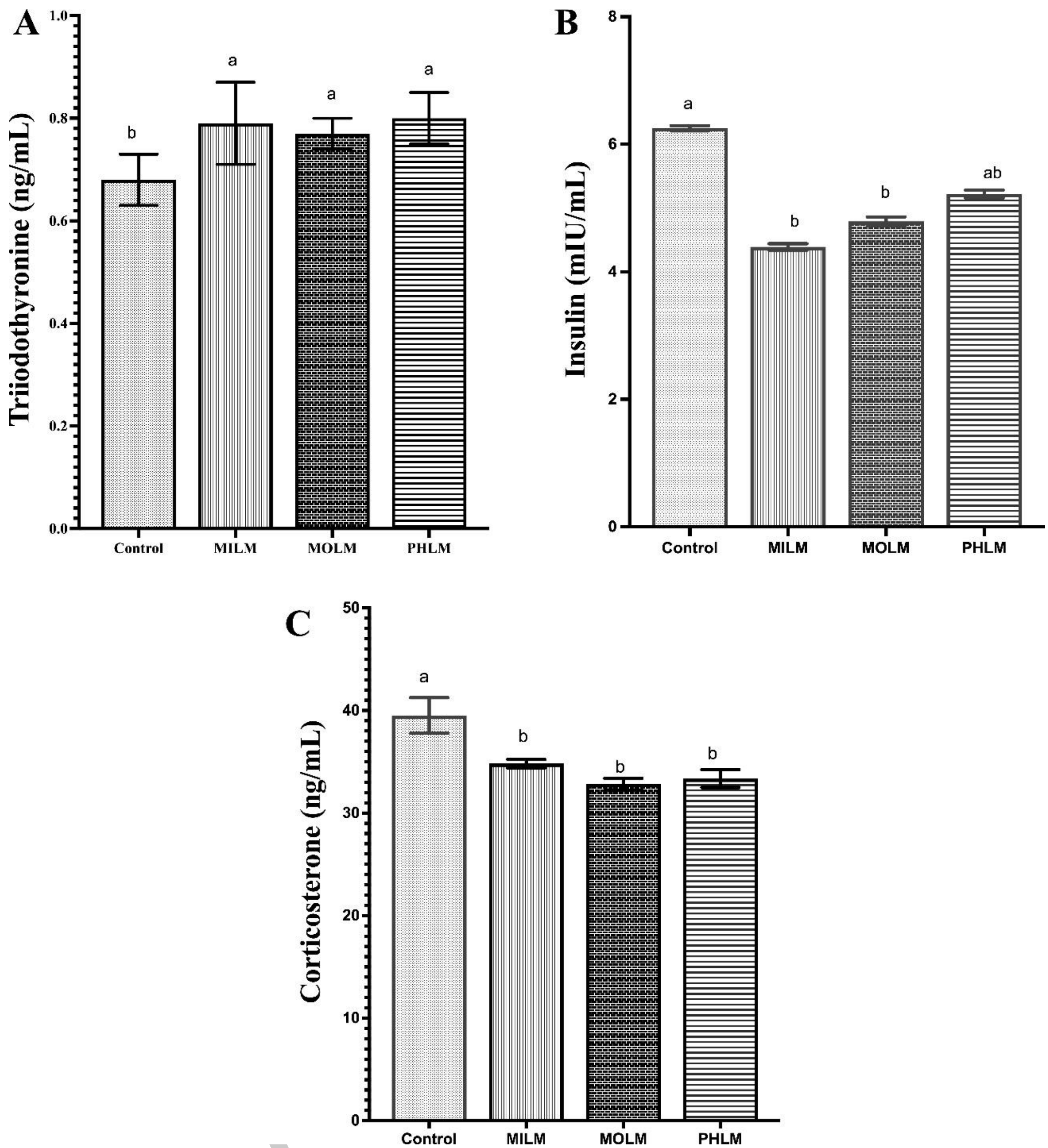

Figure 2: Stress and Metabolic hormones of rabbit Does fed herbal supplement during heat stress condition. The effects of treatment are shown on (A) Triiodothyronine, (B) Insulin, (C) Corticosterone. abc: means with different superscripts are significantly (P<0.05) different; Does received herbal supplemented diets ; MILM - Mistletoe leaf meal MOLM - Moringa leaf meal PHLM - Phyllanthus leaf meal

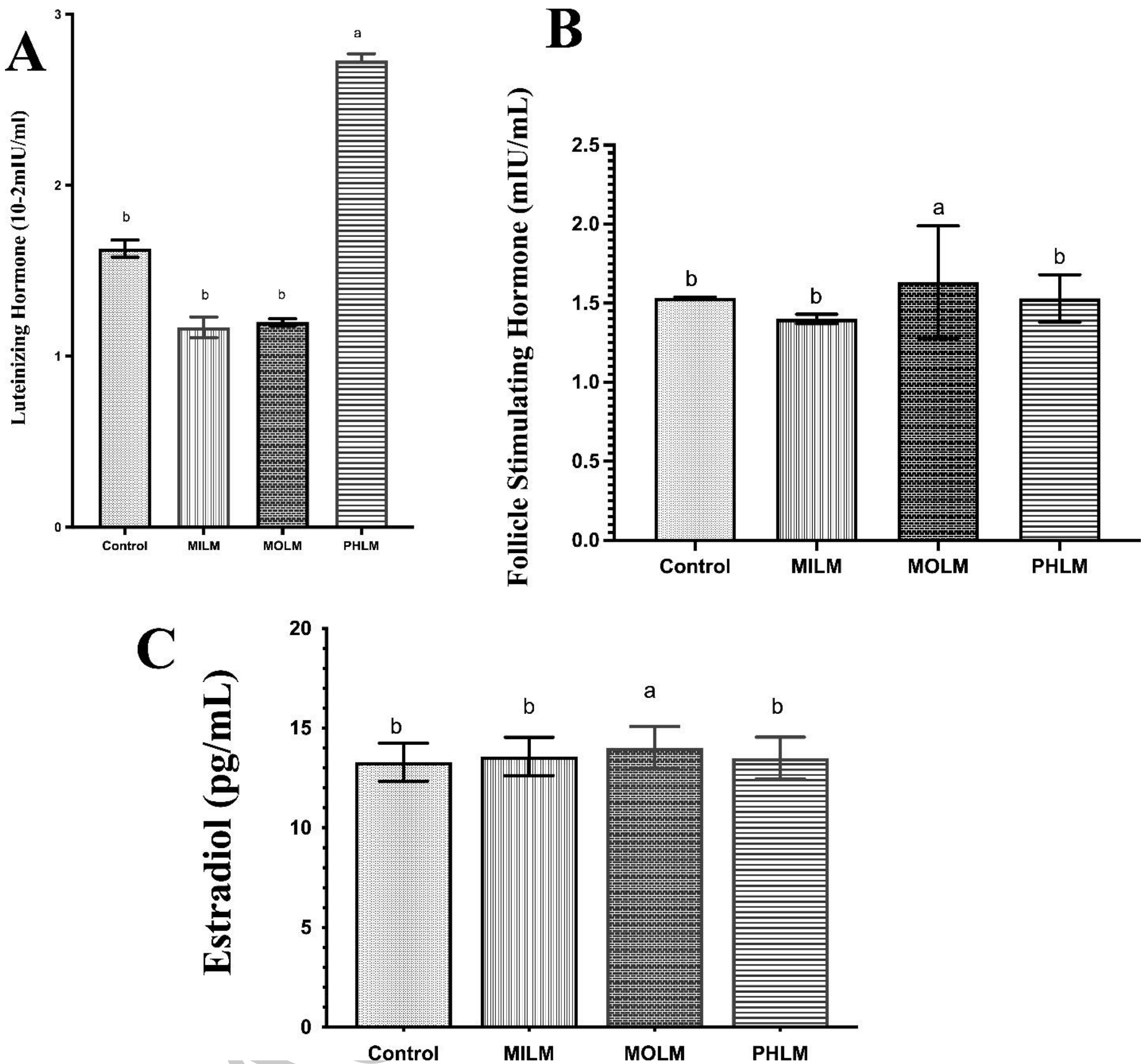

Figure 3: Sex hormones of rabbit Does fed herbal supplement during heat stress condition. The effects of treatment are shown on (A) Luteinizing Hormone, (B) Follicle Stimulating Hormone, (C) Testosterone. abc: means with different superscripts are significantly (P<0.05) different; Does received herbal supplemented diets with MILM - Mistletoe leaf meal; MOLM - Moringa leaf meal; PHLM - Phyllanthus leaf meal