Tropical Animal Health and Production (2023) 55:227
https://doi.org/10.1007/s11250-023-03640-1

REGULAR ARTICLES

Herbal supplements suppress pro-inflammatory cytokines, boost humoral immunity, and modulate adipokines to enhance the productivity traits of rabbit bucks in hot climatic conditions

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Received: 19 August 2022 / Accepted: 16 May 2023
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Abstract

Thermal stress is the main stressor accounting for reduced productivity, compromised immunity, and collapse of thermoregulatory measures in rabbits in the tropics. The current climate change depicts worsening assault of heat stress in the time ahead; hence, the need to develop combative measures for animal productivity. This research investigates the influence of herbal supplements of three tropical herbs *Viscum album* (mistletoe), *Moringa oleifera* (Moringa), and *Phyllanthus amarus* (Phyllanthus) on immune response, oxidative status, adipokines, and growth of eighty weaned rabbits during heat stress in tropical climate. The bucks were fed with four standard diets; a control and others supplemented with each of Moringa, Phyllanthus, and mistletoe for an eight-week feed trial. Performance indicators were monitored and blood were sampled and assayed for hematology, pro-inflammatory cytokines, adipokines, and oxidative status. The result shows that the performance of bucks fed with Phyllanthus and mistletoe supplements was superior to other groups. The neutrophil/lymphocyte ratio was significantly ($p < 0.05$) lower in the bucks fed with Moringa supplement, with significantly ($p < 0.05$) highest values obtained in the control group. Total antioxidant activity of the bucks fed with supplements was significantly ($p < 0.05$) higher than those on control, with the significantly ($p < 0.05$) highest value recorded in bucks fed with Phyllanthus. Serum lipid peroxidation of the bucks on control was significantly ($p < 0.05$) highest and significantly ($p < 0.05$) least value was obtained in bucks on mistletoe. Heat shock protein 70, adiponectin, and leptin of the bucks on control were significantly ($p < 0.05$) higher than bucks on herbal supplements. Interleukin 6, interleukin β, and tumor necrosis factor α of bucks on control were significantly ($p < 0.05$) higher than bucks fed on herbal supplements. In conclusion, the inclusion of herbal supplements Moringa, Phyllanthus, or mistletoe suppressed pro-inflammatory cytokines, boost humoral immunity, enhance the anti-oxidative status, and promote the growth of rabbit bucks during thermal discomfort.

Keywords Appetite · Immunity · Cytokines · Free radicals · Thermal discomfort

Introduction

The present tide of global warming shows that more exploitation of extremely high temperatures resulting from climate change will lead to a worsening assault of heat stress in the time ahead (Vandana et al., 2020). Thermal stress is the paramount stressor in the tropics, accounting for reduced productivity, compromised immunity, and subside inefficient thermoregulatory apparatus in rabbits bucks (Jimoh et al., 2018a, b). Mammals deploy a host of thermoregulatory mechanisms to achieve homeostasis and combat the deleterious consequence of thermal stress (Abdelnour et al., 2020). An increase in neutrophil/lymphocyte ratio has been widely reported to be a highly reliable index of the consequence of thermal assault in

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Published online: 25 May 2023

animals (Prieto and Campo 2010). Jimoh and Ewuola (2018) reported that heat stress in rabbit bucks is associated with an elevated free radical production, which in turn decreases antioxidant levels. Heat stress-induced inflammatory response in rabbits triggers cytokines as intimate beckoning molecules to conciliate the primary defense system (Hietbrink et al., 2006), leading to higher pro-inflammatory cytokines (Sheiha et al., 2020). The stimulation of pro-inflammatory cytokines associated with heat stress can profoundly give rise to oxidative assault, as improved immunology churn out enormous reactive oxygen species (ROS) (Yun et al., 2012).

Heat shock protein (HSP) 70 is most associated with thermal tolerance among HSPs and is the key to stress recovery, fixing impaired cells, supervising the rebuilding of adulterated proteins, and hindering oxidation and apoptosis (Shehata et al., 2020). It is hypothesized that the tools through which oxidative stress is induced by heat stress is intractably corresponded with the institution of cell-mediated immunity through the anti-oxidative defense structure; thus, suggesting that anti-oxidant enrichment will influence humoral responses and cellular stress markers, to mitigate the adverse incursions of thermal assault (Jimoh et al., (2017). Leptin is an intimate sensing components that affords a vital connection among the environment, metabolism, and immune function and conducts a crucial role in the metabolic control of satiety, appetite, food intake, activity, and energy expenditure (Andrade-Oliveira et al., 2015).

In developing countries like Nigeria, the dietary approach seems to be the more user-friendly approach to curb the adverse effect of heat assault in rabbits. Some plants are potent to inflict biochemical indices, promote defense apparatus, and influence molecular path to ameliorate stress (Hu et al., 2019). Moringa possesses attributes capable of enhancing performance and reducing oxidative stress in rabbits (Ewuola et al., 2012a). *Phyllanthus amarus* has been reported as herbal supplement growth promoter and antibiotic replacement in rabbits without adverse effects on animal health (Jimoh et al., 2020). Mistletoe properties reported to enrich antioxidant stability, as well as enhance biochemical indices (Jimoh et al., 2018a, b) without adverse effects on the health of rabbit bucks (Jimoh et al 2021a).

There is a heightening shift in addressing the curative of HSPs through nutritional and pharmacological agents as regards to mollify heat stress in animals (Yin et al., 2020). The anti-inflammatory activity of *Moringa oleifera*, *Viscum album*, and *Phyllanthus amarus* and isolated compounds have already been reported (Mueller et al., 2010). These are coupled with claims that polyphenols can boost the expression of stress response proteins (HSP and antioxidant enzymes) that can impede ROS (Hu et al., 2019) during heat stress. These indicate that herbal supplements of *Moringa oleifera*, *Viscum album*, and *Phyllanthus amarus* could be explored as mitigating strategies for heat stress in rabbits. This study is aimed at assessing the effects of the three herbal supplements on performance, immune response, adipokine, and pro-inflammatory response of rabbit bucks during heat stress conditions.

Materials and method

Experimental site

This research was conducted from January to March 2020, in the south-western agro-ecological zone of Nigeria, when heat stress is at its peak (Jimoh and Ewuola 2016). Relative humidity and temperature of the rabbit house environment was recorded at 08:00 h, 12:00 h, and 18:00 h daily via a thermo hygrometer. Daily records of the relative humidity and temperature determine the temperature-humidity index as outlined in Jimoh et al. (2021b).

Herbal processing

Fresh *Viscum album*, *Moringa oleifera*, and *Phyllanthus amarus* leaves were collected within an orchard in Ado-Ekiti metropolis of Ekiti State, Nigeria. The plants were identified and indexed with herbarium voucher nos. UILH/001/1008/2021, *Moringa oleifera*; UILH/003/1109/2021, *Phyllanthus amarus*; and UILH/002/084/1210/2021, *Viscum album*.

Leaves were detached from twigs and shade dried till it became friable and retained their greenish color. The leaves were milled and reserved in airtight canisters until ready for use.

Experimental animals and management

Eighty weaned rabbits (weighing 615.06 ± 5.27 g; mean $\pm$ SEM) were arbitrarily assigned to four treatments (20 bucks per treatment), two bucks per replicate, and bucks were housed in pairs in a completely randomized design over an eight weeks feed trial.

Experimental diet

Four experimental diets were compounded to target the nutrient demand for growing rabbits. Diet 1 (R1) without the leaf meal regarded as the control diet and diets 2 (R2), 3 (R3), and 4 (R4) composed of 10% each of Moringa, Phyllanthus, and mistletoe, respectively, as shown in Table 1. The diets were pelleted and were fed to all bucks at 4% of their body weight and offered clean water ad libitum for 8 weeks, during which growth indices;

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

Ingredient	R1	R2	R3	R4
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
Maize	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 Haulms	40.0	40.0	40.0	40.0
Moringa	0	10	10	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	81.2	87.45
Crude protein %	16.47	16.58	17.94	17.667
Digestible energy kcal/kg	2721.6	2515.35	2479.6	2509.4
Ether extract %	3.344	3.022	3.754	2.419
Crude fiber %	16.5	17.325	15.378	16.32
Lysine %	1.0085	0.9385	0.9385	0.9385
Methionine %	0.7482	0.7162	0.7162	0.7162
Calcium %	1.5999	1.7479	1.7159	1.5979
Phosphorus %	0.4491	0.5181	0.5211	0.4211

R1 indicates diets fed to animal on Treatment 1; R2 indicates diets fed to animal on Treatment 2; R3 indicates diets fed to animal on Treatment 3; R4 indicates diets fed to animal on Treatment 4

weight changes, feed intake, feed conversion ratio, and liveweights were monitored.

Sample collection and analysis

At the completion of the feed trial, blood were sampled from a buck per replicate via jugular venipuncture into plain bottles and were centrifuged (4000 rpm) to obtain serum using standard procedures and stored at -20 °C before analysis. Serum biochemical was assayed for alanine aminotransferase (ALT), glucose, cholesterol, aspartate aminotransferase (AST), total protein, urea, albumin, creatinine, triglyceride globulin, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) using fortress diagnostics assay kits and its procedures.

Determination of serum lipid peroxidation, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and total antioxidant capacity activities assayed as outlined in Jimoh (2019).

Pro-inflammatory cytokine assay

Serum samples were assayed for interferon gamma (IFN-γ), interleukin 6 (IL-6), interleukin 1 beta (IL-1β), and tumor necrosis factor-alpha (TNF-α), using ELISA and its protocol for each assay.

- IFN-γ ELISA kit, Catalog no.: E-EL-R0009 (Elabscience Biotechnology Inc., USA)
- TNF-α ELISA kit, Catalog no.: E-EL-R0019 (Elabscience Biotechnology Inc., USA)
- IL-1β ELISA kit, Catalog no.: E-EL-R0012 (Elabscience Biotechnology Inc., USA)
- IL-6 ELISA kit, Catalog no: E-EL-R0015 (Elabscience Biotechnology Inc., USA)

Heat shock proteins and adipokine assay

Serum samples were assayed for heat shock protein 70, leptin, and adiponectin using ELISA and its protocol for each assay.

- Adiponectin ELISA kit, Cat. no. E0758Ra (Bioassay Technology Laboratory, www.bt-laboratory.com, 1008 Junjiang Inter. Bldg. 228 Ningguo Rd. Yangpu Dist. Shanghai. China)
- HSP 70 ELISA kit, Cat. no. E0522Ra (Bioassay Technology Laboratory, www.bt-laboratory.com, 1008 Junjiang Inter. Bldg. 228 Ningguo Rd. Yangpu Dist. Shanghai. China)
- Leptin ELISA kit, Cat. no. E0561Ra (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: $Y_{ijl} = \mu + B_i + e_{ijl}$, where Y_{ijl} represents the value of growth performance, hemato-biochemical, oxidative stress markers, pro-inflammatory cytokines, and adipokines measured in the l^{th} animal; μ is the overall mean for each character; B_i is the fixed effect of i^{th} herbal supplement, and e_{ijl} is the random residual effect. For this study, $p < 0.05$ was declared significant, and experimental data values are presented as mean ± pooled SEM. The experiment animals treated alike were replicated ten times within the experiment (i.e., 10 replicates per treatment of two bucks per replicate). Data obtained from the study were analyzed using the general linear model procedure of one-way analysis of variance and significant means separate using the New Duncan multiple range test of IBM SPSS software.

Table 2 Temperature-humidity index (THI) of rabbit pen

	THI 8:00	THI 12:00	THI 18:00
Average	24.05	30.89	30.15
Minimum	18.15	28.14	24.45
Maximum	32.63	33.04	33.26

Results

THI of the rabbit microclimate

The temperature-humidity index of the rabbit pen is shown in Table 2. The THI obtained at 8 am indicates that the bucks were exposed to average THI values of 24.05 (absence of heat) with a range between 18.15 (absence of heat stress) and 32.63 (very severe heat stress). At noon, average THI values obtained indicate very severe heat stress with a range of THI of 28.14 (severe heat stress) and 33.04 (very severe heat stress). The average THI value at 18:00 h is 33.26 (very severe heat stress) and a range of values from 24.45 (absence of heat stress) to 30.15 (very severe heat stress).

Performance characteristics of bucks fed on herbal supplements

The performance characteristics of bucks fed on herbal supplements are shown in Table 3. The feed intake was not significantly ($p > 0.05$) affected by the treatments. The final weights of bucks in R2, R3, and R4 were significantly ($p < 0.05$) higher than bucks on R1. The average weight gain of bucks in R3 and R4 was similar ($p > 0.05$) to bucks in R2 but significantly ($p < 0.05$) higher than bucks on R1. The feed conversion ratios of bucks fed R3 and R4 were significantly ($p < 0.05$) better than bucks fed on R1 and R2.

Hematological indices of bucks fed on herbal supplements

The hematological indices of bucks fed on herbal supplements are shown in Table 4. The packed cell volume (PCV) and hemoglobin of bucks on R1 and R3 were significantly ($p < 0.05$) higher than bucks on R2 and R4. Erythrocyte of buck on R2 was significantly ($p < 0.05$) lower than bucks on R1, R3, and R4. Mean cell hemoglobin

Table 3 Performance characteristics of bucks fed herbal supplement in a humid tropical environment

Parameters	R1	R2	R3	R4	SEM	*p* value
Initial weight (g)	611.97	627.30	611.40	609.55	24.75	0.73
Final weight (g)	1624.30^{b}	1658.97^{a}	1694.37^{a}	1682.48^{a}	27.31	0.02
Average weight gain (g/buck/day)	17.55^{b}	18.42^{ab}	19.33^{a}	19.16^{a}	1.98	0.03
Average feed intake (g/buck/day)	59.19	61.96	61.19	60.95	3.05	0.50
Feed conversion ratio	3.37^{a}	3.36^{a}	3.17^{b}	3.18^{b}	0.41	0.04

RI—control (non-additive); R2—10% *Moringa oleifera* supplementation; R3—10% *Phyllanthus amarus* supplementation; R4—10% *Viscum album* supplementation

$^{a, b}$Mean in the same row with different superscripts are significantly ($p < 0.05$) different

Table 4 Hematology of bucks fed herbal supplement in a humid tropical environment

Parameters	R1	R2	R3	R4	SEM
Packed cell volume (%)	37.67^{a}	33.00^{b}	36.67^{a}	34.33^{b}	1.14
Erythrocyte ($\times 10^{6}$/L)	5.83^{a}	3.17^{b}	5.33^{a}	5.53^{a}	0.49
Hemoglobin (Hbg/dl)	12.56^{a}	11.00^{b}	12.22^{a}	11.44^{b}	0.38
Mean cell hemoglobin concentration (g/dl)	32.97	32.27	31.93	32.93	0.19
Mean cell volume (fL)	69.68^{b}	116.34^{a}	73.98^{b}	62.69^{b}	9.21
Mean cell hemoglobin (pg/cell)	23.23^{b}	38.78^{a}	24.66^{b}	20.90^{b}	3.07
Leukocytes ($\times 10^{9}$/L)	8.03^{b}	6.70^{b}	11.00^{a}	7.93^{b}	1.00
Neutrophils ($\times 10^{9}$/L)	4.23^{a}	1.57^{c}	3.76^{b}	3.24^{b}	0.47
Lymphocytes ($\times 10^{9}$/L)	5.16^{b}	4.98^{b}	7.03^{a}	4.55^{b}	0.77
Monocytes ($\times 10^{9}$/L)	0.05^{b}	0.15^{a}	0.21^{a}	0.14^{a}	0.05
Neutrophil/lymphocyte ratio	0.82^{a}	0.35^{c}	0.58^{b}	0.62^{b}	0.11

RI—control (non-additive); R2—10% *Moringa oleifera* supplementation; R3—10% *Phyllanthus amarus* supplementation; R4—10% *Viscum album* supplementation

$^{a, b, c}$Mean in the same row with different superscripts are significantly ($p < 0.05$) different

and mean cell volume of bucks on R2 were significantly ($p < 0.05$) higher than bucks on other treatment. The leukocytes and their differential counts were significantly ($p < 0.05$) highest in R3. However, monocytes of the herbal supplemented groups were significantly ($p < 0.05$) higher than the control. The neutrophil/lymphocyte ratio was significantly ($p < 0.05$) least in bucks on R2, with the significantly ($p < 0.05$) highest values obtained in R1.

Serum biochemistry of bucks fed on herbal supplements

The serum biochemistry of bucks fed on herbal supplement is shown in Table 5. The total protein of bucks fed on R3 and R1 was significantly ($p < 0.05$) higher than bucks fed on R2 and R4. Serum albumin of bucks fed on R2 was significantly ($p < 0.05$) highest, and the least values were obtained in R1. Contrariwise serum globulin of bucks fed on R1 was significantly ($p < 0.05$) highest, and the least values were obtained in bucks fed on R2 and R4. The total cholesterol of bucks fed on R1 was statistically similar to bucks fed on supplements (R2, R3, and R4). Bucks fed on R2 and R4 had significantly ($p < 0.05$) cholesterol than bucks fed on R3. Triglyceride of bucks fed on R2 was significantly ($p < 0.05$) highest across the treatment, and the least value was obtained in bucks fed on R3. HDL of bucks fed on R2 was higher than bucks fed on other treatment (R1, R3, and R4), while the LDL of bucks fed on herbal supplements (R2, R3, and R4) was lower than bucks fed on control (R1). Serum creatinine of bucks fed on R1, R2, and R4 was not significantly ($p > 0.05$) different but was significantly ($p < 0.05$) lower than bucks fed on R3. AST of bucks fed on R2 and R4 was significantly ($p < 0.05$) lower than the bucks fed on the control and on R3. ALT of bucks on supplements fed groups was significantly ($p < 0.05$) higher than bucks on control. Urea of bucks fed on R1 was significantly ($p < 0.05$) highest, and the least value was obtained in bucks fed on R4.

Oxidative status of bucks fed on herbal supplements

Serum oxidative status of bucks fed on herbal supplement is shown in Table 6. Total antioxidant activity of bucks fed on supplements was significantly ($p < 0.05$) higher than the control, with the significantly ($p < 0.05$) highest value recorded on bucks fed on R3. Serum

Table 5 Serum biochemistry of bucks fed herbal supplement in a humid tropical environment

Parameters	R1	R2	R3	R4	SEM
Glucose (mmol/l)	2.76	2.54	3.19	2.93	0.45
Total protein (g/l)	109.67^{a}	89.02^{b}	102.56^{a}	83.06^{b}	10.10
Albumin (g/l)	25.52^{c}	56.44^{a}	42.37^{b}	42.84^{b}	5.02
Globulin (g/l)	84.16^{a}	49.37^{c}	60.19^{b}	40.22^{c}	11.48
Cholesterol (mmol/l)	1.64^{ab}	1.87^{a}	1.29^{b}	2.00^{a}	0.19
Triglyceride (mmol/l)	1.73^{b}	2.10^{a}	1.16^{c}	1.48^{bc}	0.47
High density lipoprotein (mmol/l)	0.53^{b}	0.67^{a}	0.48^{b}	0.44^{b}	0.06
Low density lipoprotein (mmol/l)	1.50^{a}	0.74^{b}	0.28^{c}	0.97^{b}	0.17
Creatinine (mg/dL)	1.00^{b}	0.83^{b}	1.83^{a}	1.00^{b}	0.24
Aspartate amino transferase (U/I)	16.39^{a}	8.74^{c}	18.09^{a}	12.31^{b}	1.70
Alanine amino transferase (U/I)	3.66^{b}	6.94^{a}	8.49^{a}	8.86^{a}	1.08
Urea (mmol/l)	15.73^{a}	9.26^{b}	8.33^{bc}	7.59^{c}	2.42

RI—control (non-additive); R2—10% *Moringa oleifera*; R3—10% *Phyllanthus amarus*; R4—10% *Viscum album*

[a, b, c]Mean in the same row with different superscripts are significantly ($p < 0.05$) different

Table 6 Oxidative stress markers of bucks fed herbal supplement in a humid tropical environment

Parameters	R1	R2	R3	R4	SEM
Total anti-oxidant activity (mmol/L)	1.03^{c}	3.15^{b}	3.69^{a}	2.73^{b}	0.32
Lipid peroxidation ($\times 10^{-5}$MDA/mg protein)	1.84^{a}	0.96^{b}	0.78^{b}	0.35^{c}	0.17
Superoxide dismutase (U/min/mg protein)	1.67^{b}	2.00^{b}	2.44^{b}	5.33^{a}	0.88
Glutathione peroxidase (mg GSH/min/mg protein)	63.68^{b}	73.22^{b}	139.21^{a}	61.92^{b}	16.77
Catalase (nm H_2O_2/min/mg protein)	0.27^{b}	3.86^{a}	3.61^{a}	0.32^{b}	0.64

RI—control (non-additive); R2—10% *Moringa oleifera*; R3—10% *Phyllanthus amarus*; R4—10% *Viscum album*

[a, b, c]Mean in the same row with different superscripts are significantly ($p < 0.05$) different

lipid peroxidation of bucks fed on R1 was significantly ($p < 0.05$) highest across the treatments, and the significantly ($p < 0.05$) least value was obtained in bucks fed on R4. Serum SOD of bucks fed on R4 was significantly ($p < 0.05$) higher than bucks fed on other treatments. GPx of bucks fed on R2 was statistically ($p < 0.05$) higher than bucks fed on other treatments. Catalase of bucks fed on R2 and R3 was significantly ($p < 0.05$) higher than bucks fed on R1 and R4.

Adipokines of bucks fed herbal supplements

The adipokines of bucks fed herbal supplements are shown in Table 7. HSP 70, adiponectin, and leptin of bucks on R1 were significantly ($p < 0.05$) higher than bucks on R2, R3, and R4, which had statistically similar ($p > 0.05$) values. Adiponectin of bucks on R4 was significantly ($p < 0.05$) lower than bucks on R2 and R3, which were statistically ($p > 0.05$) similar.

Pro-inflammatory cytokines of bucks fed on herbal supplements

Pro-inflammatory cytokines of bucks fed on herbal supplements are shown in Table 8. IL-6, IL-1β, and TNF-α of bucks fed on R1 were significantly ($p < 0.05$) higher than bucks fed on R2, R3, and R4. IFN-γ was not significantly ($p > 0.05$) affected by the herbal supplements.

Table 7 Adipokines of bucks fed herbal supplement in a humid tropical environment

Parameters	R1	R2	R3	R4	SEM
Heat shock protein 70 (ng/ml)	0.99[a]	0.25[b]	0.19[b]	0.17[b]	0.19
Leptin (ng/ml)	9.09[a]	1.70[b]	1.53[b]	1.33[b]	1.92
Adiponectin (mg/L)	16.29[a]	14.23[b]	13.69[b]	10.31[c]	0.89

RI—control (non-additive); R2—10% *Moringa oleifera*; R3—10% *Phyllanthus amarus*; R4—10% *Viscum album*

[a, b, c]Mean in the same row with different superscripts are significantly ($p < 0.05$) different

Discussion

The temperature-humidity index of the rabbitry microclimate during this study reveals that the bucks are exposed to a very high magnitude of heat stress for the longer part of the day. This supports previous claims of Jimoh et al. (2021b, c, and d) that the peak of thermal discomfort in the study region is between January and March annually. Hence, efforts to enhance the welfare and productivity of the rabbits at this critical period are imperative.

Performance indices

The trend of the outcome of this study reveals that the herbal supplement enhanced weight gain and final weight of bucks. The feed intake tended to increase apparently, and the feed conversion ratio improved in birds fed on Phyllanthus and mistletoe supplements during heat stress conditions in a humid tropical environment. This suggests that the bioactive compounds in the herbal supplements confer its phyto-activities such as antimicrobial on the microbiota in the gastrointestinal tract, which thereupon is engaged in improving the digestibility of feed and subsequent performance (Saleh et al., 2018). This is demonstrated by Khalil et al. (2019) who reported the beneficial effects of dietary inclusion of Moringa on the growth performance of growing heat-stressed rabbits. This is in agreement with Selim et al. (2021) that Moringa had a positive encounter on performance of rabbit by playing a vital part in perpetuating the nutrient utilization, owing to its bioactive compounds. Similarly, Phyllanthus has been indicated to promote growth in rabbits (Jimoh et al., 2020), and Saleh et al. (2018) suggested that medicinal plants improve appetite and digestibility and improve the secretion of the digestive enzymes; have coccidiostat, antimicrobial, and antifungal properties; and improved digestibility; and these account for improved growth performance and digestibility in animals. The combination of phytochemicals or bioactive compounds present in these herbs might have acted synergistically to produce this outcome.

Table 8 Pro-inflammatory cytokines of bucks fed herbal supplement in a humid tropical environment

Parameters	R1	R2	R3	R4	SEM
Interleukin 6 (ng/L)	200.40[a]	93.73[b]	71.19[b]	50.45[b]	31.58
Interleukin 1 beta (pg/ml)	2.31[a]	0.59[b]	0.52[b]	0.61[b]	0.37
Tumor necrosis factor alpha (pg/ml)	62.03[a]	37.48[b]	39.21[b]	37.33[b]	5.54
Interferon gamma (pg/ml)	20.56	19.87	19.72	19.56	0.41

RI—control (non-additive); R2—5% *Moringa oleifera*; R3—*5% Phyllanthus amarus*; R4—5% *Viscum album*

[a, b]Mean in the same row with different superscripts are significantly ($p < 0.05$) different

Hematological response

This study reveals that the neutrophil and neutrophil–lymphocyte ratio of bucks fed the control diet (non-supplemented diet) were higher during heat stress. This buttress the claims that elevation in neutrophil/lymphocyte ratio is a crucial index of stress response in animals (Prieto and Campo 2010). Our result shows that the herbal supplements did not enhance the erythrocyte indices of buck, while leukocyte and its differential counts were enhanced by Phyllanthus supplementation during heat stress. The neutrophil/lymphocyte ratio (N/L) of bucks was reduced in bucks on herbal supplements during the heat stress condition. Reduction in N/L values in bucks consuming herbal supplements is an indication that it boosts humoral immunity and a pointer to stress alleviation in the animals (Abd El-Hack et al., 2020).

Similar to this is the report of Prieto and Campo (2010) that increased heterophil/lymphocyte (H/L) ratio recorded in heat-stressed chicks were lowered by dietary capsaicin or allicin supplementation.

Serum biochemical response

High total protein was observed in bucks without feed additive during heat stress conditions; this was also accompanied by high globulin. These signifies that the high serum protein in the bucks is due to humoral response in the bucks due to heat load. Similar to the result obtained in the R1 groups of this study, heat stress reduces protein deposition, increases protein catabolism (marked by increased serum urea), and reduces protein synthesis and nitrogen retention (Slimen et al., 2015).

Higher albumin levels were observed in herbal supplemented bucks suggesting that higher protein digestion and absorption are encouraged in bucks fed herbal supplement during heat stress. This agrees with claims that albumin and total protein raised in relation to dietary folic acid and vitamin C supplementation to Japanese quails on heat stress (Hajati et al., 2015).

The Moringa and mistletoe supplements tended to reduce LDL fractions of cholesterol and enhance HDL, which accounted for the serum cholesterol levels in the bucks. This is contrary to bucks on R1 (without herbal supplement) during heat stress conditions, whose high cholesterol is associated with high LDL fractions and low HDL fractions of cholesterol. This could be explained by lots of bioactive compounds present in the supplements, that had influenced lipid equilibrium, by reducing and delaying cholesterol absorption (the inhibition of pancreatic cholesterol esterase activity), and tying bile acids by producing insoluble complexes and promoting excretion, resulting to decreased cholesterol (Vergara-Jimenez et al., 2017). Also, Ariana et al. (2011) claimed that herbal extracts act by diminishing hepatic 3- hydroxy-3-methylglutaryl coenzyme A reductase needed for cholesterol synthesis in the liver to decrease total lipid and cholesterol. Bucks fed on Phyllanthus supplement-based diets had the least serum cholesterol, triglyceride, and LDL cholesterol. Similarly, Selim et al. (2021) claimed that Moringa in rabbits' diet reduced serum blood lipids, cholesterol, LDL, and triglycerides.

The high creatinine in bucks fed on Phyllanthus could be associated with its apparently higher glucose in comparison with other treatments. The trend of the result of ALT, AST, creatinine, and urea is within the normal range for healthy animals (Boyd 1984) and does not suggest the phytogenics possess adverse effects on the rabbits' organs as previously reported by Ewuola et al. (2012b).

Oxidative status

The trend of result shows that total antioxidant activity was enhanced by herbal supplement and led to its reduced lipid peroxidation in comparison with buck without supplementation during heat stress conditions. Among the herbal supplement, bucks fed Phyllanthus had better antioxidant enrichment during heat stress conditions. The herbal supplements possess high-level antioxidants (Mbikay, 2012) and are valuable source of β-carotene and vitamins B-complex, C, D, and K (Abd El-Hack et al. 2020). This could account for the antioxidant enrichment in the herbal supplemented groups. Similar reports reveal the antioxidant status improvement of ginger in heat-stressed animals via regulation of antioxidant status (Ibtisham, et al., 2019). At thermal comfort, rabbit oxidative stability is better and influences immune status and productivity. However, oxidative assault due to high temperature apparently decreases antioxidant enzymes activities and impairs vital cellular functions (Jimoh and Ewuola 2019). Moringa leaves are claimed to reduce lipids and lipid peroxidation in rats (Halaby et al., 2013) and improve blood antioxidant capacity in rabbits (Selim et al., 2021). Also, Liu et al. (2014) claimed that resveratrol had positive effects on serum oxidative status during heat stress.

Adipokine response

In stressed conditions, HSPs had been found to collaborate with denatured proteins and impede the creation of cytotoxic protein agglomerate in the cell; thus sustaining equilibrium the protein homeostasis of the cell (Mayer and Bukau, 2006). The result obtained in this study for bucks without supplements is in line with reports of Mehaisen et al. (2017) on the elevation of HSP70 expression in quails on heat stress, as a repercussion of higher MDA to defend cell peroxidation casualty. The extracellular HSP70 released in response to thermal stress activates innate and adaptive immunity via its synergy with TLR2/4 and

CD14 receptors (Mansilla et al. 2014). De Nadal et al. (2011) reported that exposure to heat stress promotes the diction of genes related to survival (rapid expression of heat shock factors and heat shock protein), while not expressing fewer essential genes (Cedraz et al., 2017). The pronounced expression of intracellular HSPs revealed to promote cellular resistance to inflammatory cytokines released under heat-stress conditions (Shehata et al., 2020).

The adipokines (leptin, adiponectin, and HSP 70) of bucks were reduced by the herbal supplements during heat stress conditions. Also, *M. oleifera* gold nanoparticles were reported to lower the secretion of HSP70 mRNA and protein levels (Tiloke et al. 2016).

Similarly, Hajati et al. (2015) claimed that ascorbic acid supplementation decreases the synthesis of HSP70 gene expression in heat stressed birds. Liu et al. (2014) reported that resveratrol reduced the expression of HSP90 and HSP27 mRNA in the thymus following thermal assault (Akbarian et al., 2014). Contrariwise, glutamine supplementation promoted HSP70 protein or mRNA expression in thermal stress (Hao et al., 2012).

HSP70 delays the expression of pro-inflammatory cytokines and promotes the secretion of anti-inflammatory cytokines (Siddiqui et al., 2020). Under acute thermal stress, digestive enzyme activity increases via overexpression of HSP 70, which could improve digestion and absorption function in the intestine (Archana et al., 2017). However, the reduction in HSP70 in herbal supplements fed groups could indicate the reversal of cellular activities following mitigation of the biochemical effects of heat stress, as compared to the control groups which had higher HSP 70.

The increase in leptin has been reported with heat stress, to stimulate prevention of food intake or satiety signals (Schwartz et al. 2000), which accounts for the reduction in feed intake during the thermal assault.

However, similar to the result of this study, administration of *Moringa oleifera* causes the mitigation of satiety signals and incentivizes hunger (Abdel-Latif et al., 2018) to improve feed intake. The reduction in leptin and adiponectin in herbal supplemented bucks during heat stress conditions could be responsible for the stimulation of appetite and explain the improvement in feed consumption and growth obtained in the bucks. As reported, hypoadiponectinemia impedes satiety and enhances appetite in fattened birds (Decara et al., 2018).

Similarly, a decrease in serum leptin concentration has been reported following phytogenic compound administration (Yang et al., 2010). Contrariwise, are reports of increased serum leptin and adiponectin with saponin administration (Elekofehinti et al., 2017).

Inflammatory cytokines

This study shows that bucks raised during heat stress conditions had higher pro-inflammatory cytokines and was reduced by dietary herbal supplements included in the diets of the bucks. This is in line with reports of increased TNF-α and IFN-γ in heat-stressed animals (Abdel-Latif et al., 2018). A potential ameliorating mechanism could be deduced from the reduction in TNFα of bucks fed on herbal supplements during heat stress. It has been hypothesized that TNFα overexpression promotes a reduction in feed intake and body weight. Also, the lowered TNFα expression has been reported to depict the weight loss and promoted metabolic status in obese animals (Decara et al., 2018). In line with the result of this study, hydroethanolic *M. oleifera* flower extract has decreased the secretion of IL-6, IL-1β, and TNF-α (Tan et al., 2015).

A similar trend observed in adipokine and pro-inflammatory cytokines in this study is supported by Siddiqui et al. (2020) that HSP70 interacts with IL-6 and TNF-α, to decrease inflammation by mending the immunity of the small intestine.

Decara et al. (2018) reported that an elevation in TNFα occurs as a feedback apparatus to impede the secretion of more adiponectin and reduce feed intake and weight by promoting satiety. This explains the mechanism for better performance in herbal supplemented bucks during heat stress conditions in this study. Spices and herbs possess anti-inflammatory effects via decline in IL-6, TNF-α secretion, and promotion of IL-10 release (Mueller et al., 2010). Several herbal compounds have been reported to be largely effective in the lowering of IL-6 and TNF-α production. In the same trend, *M. oleifera* has been reported to decrease the production of TNF-α, IL-6, and IL-8 in different disease conditions, confirming *M. oleifera* as an efficient therapy for inflammatory reactions (Kou et al., 2018). Tea saponin has been documented to reduce the protein profile of TNFα and IL-6 (Elekofehinti et al., 2017).

Similar to the trend of the result obtained in herbal supplemented bucks during heat stress in this study is a report by Jang et al. (2014) that heat-stressed birds fed on vitamin E supplement improved immunity under stressed conditions. And that the addition of Moringa oleifera promoted broiler growth, physiology, and improved resistance to thermal stress conditions (Abd El-Hack et al. 2020). In tandem, Helal et al. (2017) reported the beneficial effect of Moringa on performance indices, improved immune responses, and reduced lipid peroxidation owing to its antioxidant potency.

De Nadal et al. (2011) reported that heat stress promotes the expression of genes involved in survival, while not expressing essential genes (Cedraz et al., 2017). Thus, the implications of the inclusion of phytogenic supplements in the buck diet during heat stress achieved a reduction of HSP 70, leptin, and adiponectin, which culminated in reducing pro-inflammatory cytokines and enhancing antioxidant and serum biochemical to boost immunity. These reveal that survival has been met and led to suppression of survival-related HSP genes and a switch to express more productivity-related genes, which led to growth promotion of bucks.

Conclusion

During heat stress conditions, the inclusion of herbal dietary inclusion of Moringa, Phyllanthus, or mistletoe leaves ameliorates the deleterious effects of thermal assaults by suppressing pro-inflammatory cytokines, boosting humoral immunity and enhancing the anti-oxidative status and biochemical profile. It mobilizes adipokines to boost feed consumption and improve productive traits in rabbit bucks. Further research should focus on understanding the effects of the herbal supplement on behavioral response of rabbits bucks during heat stress.

Abbreviations *HSP*: Heat shock protein; *ROS*: Reactive oxygen species; *ALT*: Alanine aminotransferase; *AST*: Aspartate aminotransferase; *HDL*: High-density lipoprotein; *LDL*: Low-density lipoprotein; *IFN-γ*: Interferon gamma; *IL*-6: Interleukin 6; *IL*-1*β*: Interleukin 1 beta; *TNF-α*: Tumor necrosis factor-alpha; *SOD*: Superoxide dismutase; *GPx*: Glutathione peroxidase; *N/L*: Neutrophil/lymphocyte ratio; *THI*: Temperature humidity index

Acknowledgements The authors appreciate Adesina feyisayo, Ajewole Ayoola, Akinbuyide Saanu, Adetifa Johnson, Jimoh Ayodeji, Olutade Busayo, Odedele Ayobami, and Mayowa Adewale for the care and animal handling.

Author contribution Jimoh, Olatunji Abubakar: conceptualization, formal analysis, funding, and writing original draft. Daramola Olajumoke Temidayo: co-supervised the study, data curation, and review and editing of manuscript. Okin-Aminu, Hafsat Ololade: validation, visualization, review and editing, project administration, and software. Ojo Olayinka Abosede: funding, data curation, and review and editing of manuscript.

Funding The research outcome presented in this article was funded by the authors.

Data availability The dataset used and/or analyzed during the current study is available from the corresponding author on reasonable request.

Declarations

Ethical approval and consent to participate. The study was approved by the institutional committee on the care and use of animals for experiment, and in accordance with NIH guide for the care and use of laboratory animals.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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