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HSP70, adiponectin, leptin, pro-inflammatory cytokines and metabolic hormones of heat-stressed broilers fed herbal supplements

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ABSTRACT

Global warming undoubtedly is a serious challenge facing poultry production especially in tropical climate, which subject animals to heat-stressed conditions leading to multiple physiological alterations. An investigation was conducted to determine the role of herbal supplements on adipokines, pro-inflammatory cytokines and metabolic hormones of broilers exposed to heat stress. 200 a day-old broiler chicks were randomly allotted to standard diets; diet 1 without leaf meal (T1), diet 2 with 5% *Moringa oleifera* (T2), diet 3 with 5% *Phyllanthus amarus* (T3) and diet 4 with 5% mistletoe; *Viscum album* (T4) in a 49-day feed trial during the peak of thermal discomfort in Southern Nigeria, to assess hormones, adipokines and cytokines using standard procedures. Results obtained shows that triiodothyronine of birds fed phyllanthus and mistletoe were significantly ($p < 0.05$) higher than birds on basal diet. Corticosterone of birds fed mistletoe and phyllanthus were significantly ($p < 0.05$) lower than those obtained in birds on basal diet. Heat shock protein of birds fed on moringa supplements were significantly ($p < 0.05$) lower than those in birds on basal diet. The adiponectin of birds fed on basal diet were statistically ($p < 0.05$) higher than other treatments. Interleukin 6 of birds on phyllanthus and mistletoe supplements were not significantly ($p > 0.05$) different from birds on basal diet. Interleukin 1β of birds fed basal diet was significantly ($p < 0.05$) higher than birds fed moringa supplements and the significantly ($p < 0.05$) least values obtained in those of birds fed phyllanthus supplement. Tumor necrosis factor α of birds fed on phyllanthus and mistletoe were significantly ($p < 0.05$) higher than birds fed basal diet and the significantly ($p < 0.05$) least values were obtained in birds fed moringa supplement. In conclusion, three herbal supplements decreased the HSP 70, leptin and adiponectin of broilers exposed to heat stress and moringa supplements lower pro-inflammatory cytokine expression in heat stress birds.

1. Introduction

Heat stress is a major health challenge encountered in poultry farming. Elevated ambient temperature reduces the live performance, product quality, health status, survival, and general welfare of the poultry (Goel, 2021). Heat stress negatively affects the immune responses in the animals and induces inflammatory reactions (Hirakawa et al., 2020; Sheiha et al., 2020; Goel, 2021).

In developing countries, dietary approach seems to be the more user-friendly strategy to curb heat stress. Prolonged heat stress elevates corticosterone level, which suppresses innate and adaptive immunity in birds (Lartey et al., 2020). Chronic heat stress causes immunosuppression, decreases productivity, resulting in severe economic losses in chicken production in the humid tropics (Nelso et al., 2018; Lartey et al., 2020). Among all heat shock protein (HSP) families, HSP 70 is the most associated with thermal tolerance (Shehata et al., 2020). HSP 70 is vital to stress recovery and repairing of damaged cells, regulating the refolding of damaged proteins, inhibiting oxidation and apoptosis, and

Abbreviations: HSP, Heat Shock Protein; ELISA, Enzyme Linked Immunosorbent Assay; T3, Triiodothyronine; IFN-γ, Interferon Gamma; TNF-α, Tumor Necrosis Factor Alpha; IL-1β, Interleukin 1 Beta; IL-6, Interleukin 6; THI, Temperature - Humidity Index; MAPK, Mitogen-Activated Protein Kinase; NF-κB, Nuclear Factor-Kappa B.

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controlling protein assembly, disassembly, and translocation (Nitika et al., 2020). The lack of HSP induction is the cause rather than a consequence of cell death. Its accumulation of HSPs is associated with resistance to cell apoptosis, thus suggesting its importance to the survival of cells under stress (Shehata et al., 2020). Therefore, HSP 70 is utilized in the evaluation of heat resistance of animals to thermal assault.

In view of increased consumers' interest in organic products and to meet the evolving market demands, current production goals focus on improving productivity and regulating the intestinal microflora of birds, and targeted at developing new products less harmful to human health (Tekce et al., 2020). The anti-inflammatory activity of several plant extracts and isolated compounds have already been scientifically demonstrated (Mueller et al., 2010). Thermal stress-induced oxidative damage could be mitigated by dietary supplementation of herbal feed additives to ameliorate the adverse impacts of heat stress in animals (Abd El-Hack et al., 2020). Jimoh et al. (2017) reported that mitigating strategies to ameliorate the adverse effects of heat stress on using cellular stress markers should be explored. HSP70 plays a major role in the adaptive response to thermal stress in broiler chickens by improving antioxidant status, preventing lipid peroxidation, and increasing digestive enzyme activity (Hao et al., 2012). Recently, improving antioxidant defences for poultry in stress conditions is focused on activating a range of vitagenes (via the three levels of antioxidant defence) to maximize internal antioxidant protection and redox balance maintenance (Surai et al., 2019). It has been hypothesized that the mechanism of dietary antioxidants to alleviate heat stress in birds is by the down-regulation of HSP expression associated with the modulation of pro-inflammatory cytokine expression in response to dietary antioxidant (Chang et al., 2010; Jang et al., 2014). Supplementing the poultry diet with a suitable phytochemical-containing feed could potentially mitigate the deleterious impact of heat stress in poultry production (Vandana et al., 2020). Phytochemicals with antioxidant activity such as polyphenols found in plants, are vital secondary metabolite, and these are anticipated to resolve heat stress in poultry (Vandana et al., 2020).

Different natural feed resources have been evaluated for potentiality to counter negative impacts of heat stress with emphasis on practicality, safety and economical application (Jimoh et al., 2021a). El-Desoky et al. (2017) reported that phytogenic compounds with antioxidant, antibacterial and anti-inflammatory properties may facilitate the ability of animals to maintain their body homeostasis by provoking endogenous cellular defence mechanisms to cope with oxidative stress and inflammation induced by heat stress. *Moringa oleifera* has been discovered to be a potent antibiotic and has been employed in the treatment of reproductive, cardiac and circulatory problems, *Viscum album* act effectively as antioxidants and peroxyl radical scavengers, possess pharmacological properties having immunomodulatory, anti-inflammatory, cardiovascular, and antimicrobial effect, while *Phyllantus amarus* rich in phyto-nutrients acting as healthy supplements and growth enhancer in animal feed (Jimoh et al., 2022a). *Moringa oleifera* leaves have a beneficial effect for enhancing the immune responses and improving the intestinal health of broilers (Gouda et al., 2018; Mahmoud et al., 2019; Jimoh et al., 2022b).

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 (Jimoh et al., 2021a,2021b) and to counter negative impacts of heat stress with emphasis on practicality, safety and economical application (Jimoh et al., 2022a, 2022b, 2023). *Moringa oleifera, Phyllanthus amarus* and *Viscum album* have been documented to be of high therapeutic value and promotes general animal performance (Daramola et al., 2018; Jimoh et al., 2018b, 2020). Hence, the consumption of *Moringa oleifera, Phyllanthus amarus,* and *Viscum album* leaves for the maintenance of health and well-being has been encouraged under heat stress condition in the tropics (Jimoh et al., 2023).

The mechanism of action was adduced to the three herbal supplements enriching antioxidant profile and reduced its lipid peroxidation; a pathway to maintain oxidative stability in stress conditions (Jimoh et al., 2023). Dietary supplementation of *Moringa oleifera, Phyllanthus amarus,* and *Viscum album* leaves at 5% confer phytogenic effect, had the potency to promote good health and well-being (Jimoh et al., 2022b). This study aims to assessing the role of herbal supplements on the HSP 70, pro-inflammatory cytokines and hormones of broilers exposed to heat stress.

2. Materials and methods

2.1. Experimental site

The study was approved by the institutional research ethics committee on the care and use of animals for experiment with the approval number FPA/EC/20/0073, and in accordance with NIH guide for the care and use of laboratory animals.

This study was carried out in a poultry facility (N 7°35′15.85788″ and E $5^0$17′55.38588″) Teaching and Research Farm at The Federal Polytechnic Ado-Ekiti, Ekiti State in the Southwest of Nigeria, from February to March 2020 - a period that is the peak of heat stress in southern Nigeria. Severity of heat stress based on the combination of temperature and humidity (Temperature - Humidity Index; THI) was established to peak in southern Nigeria between February and March (Jimoh and Ewuola 2016, 2018; Jimoh et al., 2018a). The poultry facility consist of a single pen (5 × 10 × 3m; width x length x height), naturally lighted (12D:12 L) with a dynamic ventilation system. The poultry pen is evenly distributed with 40 double sided and floored wire mesh cage units of 1 m^2 per unit, 30 cm each feeding and drinking space per unit. Cage rearing was preferred for this study in order to evaluate the response of the animals at better yield and profitability commonly adduced to raising broilers on cages (in terms of crop age, % mortality, FCR, live weight at slaughter and dressing weight) when compared with broilers raised on floor. However, the stocking density (5 birds per 1m2) was low to positively enhance welfare of the birds. Temperature and relative humidity of the poultry microclimate was recorded at 08:00 h, 12:00 h and 18.00 h daily during the study using a Thermo-Hygrometer. Daily records of the ambient temperature and relative humidity were used to compute the temperature humidity index as outlined by Jimoh et al. (2017).

2.2. Phytogenics processing and evaluation

Fresh *Moringa oleifera* (Moringa)*, Phyllanthus amarus* (Phyllanthus) *and Viscum album* (mistletoe) leaves were harvested from an established orchard within 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 became crispy to touch while retaining their greenish coloration. The leaves were milled to powder and stored in air-tight containers until incorporation into the diet along with other ingredients as contained in the feed composition. The proximate composition of the three herbs were assessed and revealed that dry matter, crude protein, ether extract, crude fibre, ash and nitrogen free extract of mistletoe are 89.82%, 18.81%, 2.1%, 12.10%, 14.9% and 52.09%, respectively, and moringa are 90.76%, 31.06%, 2.7%, 11.3%, 12.4%, 42.54%, respectively, while Phyllanthus are 90.50%, 27.13%, 2.5%, 9.90%,12.06% and 48.41%, respectively.

2.3. Experimental animals and management

Two hundred (200) a-day old arbo acre broilers were purchased from a reputable hatchery, weighed and allotted to four treatment diets of 10 replicates and five birds per replicate and were randomly allotted to cage/units in a completely randomized design.

Four experimental diets were formulated to meet the nutrient requirement for broilers at starter and finisher phases as shown in Tables 1 and 2: diet 1 without leaf meal (T1), diet 2 with 5% *Moringa oleifera* (T2), diet 3 with 5% *Phyllanthus amarus* (T3) and diet 4 with 5% *Viscum album* (T4). The inclusion of the supplements account for the slight variation in calculated nutrient composition of the four diets in Tables 1 and 2, which is due to chemical composition of the herbal supplements.

Birds were fed from day old *ad libitum* with jambo feeders and fresh water was offered daily with bell drinkers. The vaccination programme as recommended by the hatchery were followed and no medication was administered throughout the 49-day trial.

2.3.1. Blood sample collection

At the end of the feeding trial, fastened blood samples were collected via jugular venipuncture between 0700hrs and 0800hrs (27.5 °C ambient temperature) to offer minimal discomfort to the animals. Samples were collected from 3 birds per replicate into plain sample. Blood samples in plain tubes were centrifuged at 4000 rpm for 15 min and serum obtained using standard procedures and stored at −20 °C until analysis.

2.3.2. Hormonal assay

Serum samples obtained were assayed for triiodothyronine, insulin, corticosterone using enzyme linked immunosorbent (ELISA) assay, with commercial ELISA kits and its protocol for each assay.

- Triiodothyronine (T3) ELISA Catalog No. T3225T (Calbiotech Inc., 1935 Cordell Ct., El Cajon, CA 92020)
- Insulin ELISA kit, Catalog No. IS130D (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)

2.3.3. Heat shock proteins, adipokines and pro-inflammatory cytokines assay

Serum samples obtained were assayed for heat shock protein 70 (HSP70), leptin, interleukin 6 (IL-6), adiponectin, interleukin 1 Beta (IL-1β), tumor necrosis factor alpha (TNF-α), interferon Gamma (IFN-γ) using enzyme linked immunosorbent assay, with commercial ELISA kits and its protocol for each assay.

- IFN-γ (Interferon Gamma) ELISA Kit, Catalog No: E-EL-R0009 (Elabscience Biotechnology Inc USA)
- TNF-α (Tumor Necrosis Factor Alpha) ELISA Kit, Catalog No: E-EL-R0019 (Elabscience Biotechnology Inc USA)
- IL-1β (Interleukin 1 Beta) ELISA Kit, Catalog No: E-EL-R0012 (Elabscience Biotechnology Inc USA)
- IL-6(Interleukin 6) ELISA Kit, Catalog No: E-EL-R0015 (Elabscience Biotechnology Inc USA)
- Adiponectin ELISA Kit, Cat. No E0758Ra (Bioassay Technology Laboratory, www.bt-laboratory.com, 1008 Junjiang Inter. Bldg. 228 Ningguo Rd. Yangpu Dist. Shanghai. China)
- Heat Shock Protein 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)

Table 1
Gross composition of Broiler Starter Ration.

INGREDIENT	T1	T2	T3	T4
Maize	58.5	58.5	58.5	58.5
Groundnut cake	9.8	8.8	8.8	8.8
Soyabean meal	25	21	21	21
Fishmeal	3.1	3.1	3.1	3.1
Methionine	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Oyster shell	1.5	1.5	1.5	1.5
Limestone	1.1	1.1	1.1	1.1
Premix	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Moringa	–	5	–	–
Phylanthus	–	–	5	–
Mistletoe	–	–	–	5
TOTAL	**100**	**100**	**100**	**100**
CALCULATED NUTRIENT ANALYSIS				
Dry Matter %	85.85	85.88	85.88	85.88
Crude Protein %	22.99	21.41	21.41	21.41
Metabolisable Energy kcal/kg	3031.27	3026.87	3026.87	3026.87
Ether Extract %	3.94	3.81	3.81	3.81
Crude fibre %	3.32	3.60	3.60	3.60
Lysine %	1.38	1.28	1.28	1.28
Methionine %	0.61	0.60	0.60	0.60
Calcium %	1.17	1.31	1.31	1.31
Phosphorus %	0.32	0.30	0.30	0.30

Table 2
Gross composition of Broiler finisher Ration.

INGREDIENT	T1	T2	T3	T4
Maize	66.5	66.5	66.5	66.5
Groundnut cake	8.3	6.3	6.3	6.3
Soyabean meal	19.5	16.5	16.5	16.5
Fishmeal	2	2	2	2
Methionine	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Oyster shell	1.5	1.5	1.5	1.5
Limestone	1.2	1.2	1.2	1.2
Premix	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Moringa	–	5	–	–
Phylanthus	–	–	5	–
Mistletoe	–	–	–	5
TOTAL	**100**	**100**	**100**	**100**
CALCULATED NUTRIENT ANALYSIS				
Dry Matter %	85.546	85.781	85.781	85.781
Crude Protein %	18.575	19.1625	19.1625	19.1625
Metabolisable Energy kcal/kg	3086.43	3088.948	3088.948	3088.948
Ether Extract %	3.9305	3.81995	3.81995	3.81995
Crude fibre %	3.0325	3.33995	3.33995	3.33995
Lysine %	1.17005	2.27055	2.27055	2.27055
Methionine %	0.56059	0.55654	0.55654	0.55654
Calcium %	1.15925	0.748475	0.748475	0.748475
Phosphorus %	0.47845	0.252875	0.252875	0.252875

2.3.4. Data analysis

The statistical model applied is as follows:

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

Where Yijl represents the value of HSP 70, pro-inflammatory cytokines and hormones measured in the lth animal; μ is the overall mean for each character; Bi is the fixed effect of ith herbal supplement administered; and eijl is the random residual effect. Data were tested for normality and homogeneity of variances with levene's test and subjected to one way analysis of variance and significant means were separated using Duncan multiple range test using statistical software IBM SPSS 20 package.

3. Results

The temperature humidity index of the broiler pen is shown in Fig. 1. The range of values obtained indicate that the birds were exposed to an

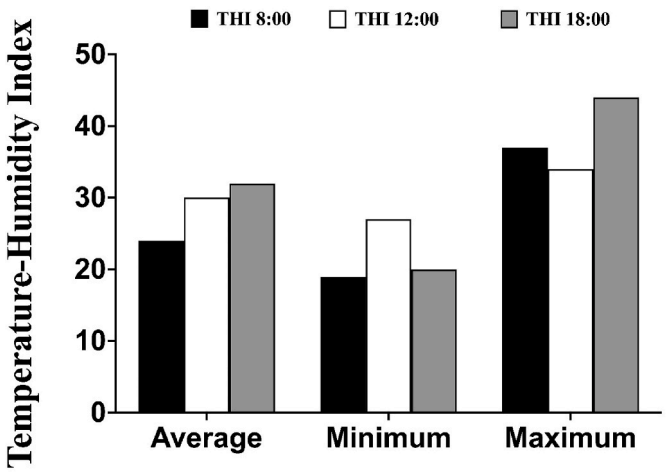


Fig. 1. Temperature-Humidity Index of Broiler pen. (THI values are means of 50 sample size).

average THI of 24.13 (absence of heat stress) in the morning with a range of 19.14 (absence of heat stress) – 36.52 (very severe heat stress). At noon, the birds were exposed to an average THI of 30.35 (very severe of heat stress) with a range of 26.88 (absence of heat stress) – 34.18 (very severe heat stress). At 1800hrs, the birds were exposed to an average THI of 31.95 (very severe heat stress) with a range of 20.00 (absence of heat stress) – 43.90 (very severe heat stress).

Hormonal profile of heat stressed broilers fed on phytogenic feed supplements is shown in Fig. 2. Triiodothyronine of birds fed on phyllanthus and mistletoe were significantly ($p < 0.05$) higher than birds on basal diet. Birds fed on moringa had statistically ($p > 0.05$) similar triiodothyronine as birds on other treatments. Insulin in birds fed on basal diet, moringa and phyllanthus were not significantly ($p > 0.05$) different and were significantly ($p < 0.05$) lower than birds fed on mistletoe supplement. Corticosterone of birds fed on mistletoe and phyllanthus were significantly ($p < 0.05$) higher than those of birds fed on moringa, with the significantly ($p < 0.05$) highest value obtained in birds on basal diet.

Adipokines of heat-stressed broilers fed on phytogenic feed supplements are shown in Fig. 3. Heat shock protein 70 of birds fed on moringa supplements were significantly ($p < 0.05$) higher than those of birds fed on phyllanthus and mistletoe which had statistically ($p > 0.05$) similar values, and the highest values obtained in birds on basal diet. Leptin of birds fed on basal diet were significantly ($p < 0.05$) higher than birds fed on moringa and phyllanthus supplement, with the significantly ($p < 0.05$) least value obtained in birds fed on mistletoe supplement. Adiponectin of birds fed on phyllanthus and mistletoe supplement were significantly ($p < 0.05$) higher than those of birds fed on moringa supplement. The adiponectin of birds fed on basal diet were statistically ($p < 0.05$) higher than other treatments.

Pro-inflammatory cytokines of heat-stressed birds fed on phytogenic feed supplement is shown in Fig. 4. Interleukin 6 of birds fed on phyllanthus and mistletoe supplements were not significantly ($p > 0.05$) different from birds on basal diet, with the significantly ($p < 0.05$) least value obtained in birds fed on moringa supplements. Interleukin 1β of birds fed on basal diet was significantly ($p < 0.05$) higher than birds fed on moringa supplements and the significantly ($p < 0.05$) least values obtained in those of birds fed on phyllanthus supplement. Interleukin 1β of birds fed on mistletoe supplements were not significantly ($p > 0.05$) different from birds fed on basal diet and moringa supplement. Tumor necrosis factor α of birds fed on phyllanthus and mistletoe were significantly ($p < 0.05$) higher than birds fed on basal diet and the significantly ($p < 0.05$) least values were obtained in birds fed on moringa supplement. Interferon γ of birds fed on phyllanthus and mistletoe were not significantly ($p > 0.05$) different and were significantly ($p < 0.05$) higher than birds fed on basal diet and moringa supplement which were statistically ($p > 0.05$) similar.

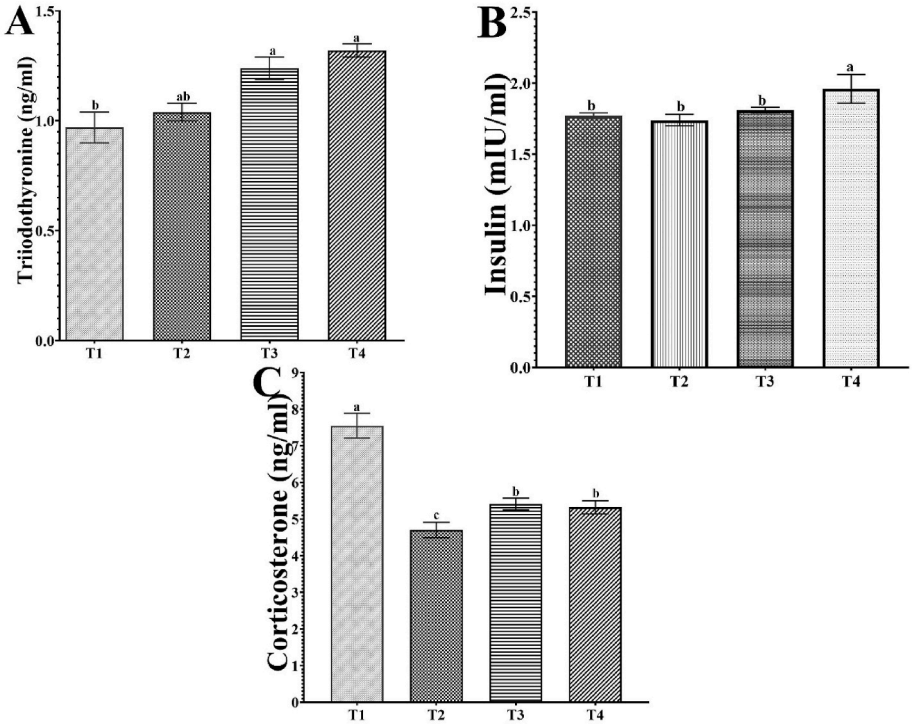


Fig. 2. Hormonal profile of heat stressed broiler fed phytogenic feed supplements. The effects of treatment are shown on (A) Triiodothyronine, (B) Insulin, (C) Corticosterone. Bar and error bars are mean ± SEM, N = 30 per treatment group abc: means with different superscripts are significantly ($P < 0.05$) different; heat stressed broilers fed phytogenic feed supplement T1-control, T2- Moringa, T3- Phyllanthus, T4- Mistletoe.

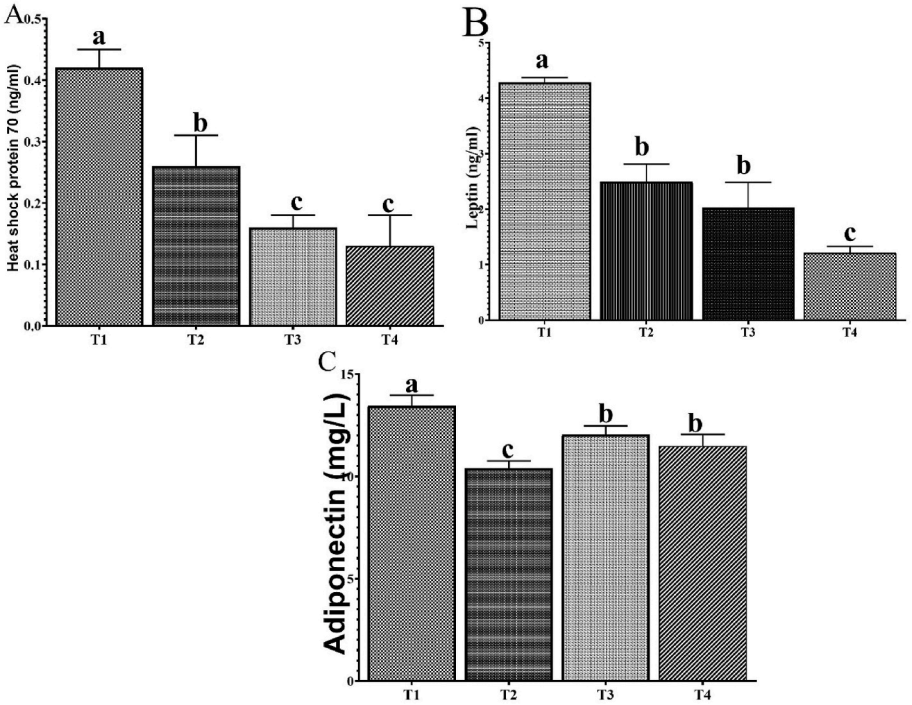


Fig. 3. Adipokines of heat stressed broiler fed phytogenic feed supplements. The effects of treatment are shown on (A) Heat shock protein 70, (B) Leptin, (C) Adiponectin. Bar and error bars are mean ± SEM, N = 30 per treatment group abc: means with different superscripts are significantly ($P < 0.05$) different heat stressed broilers fed phytogenic feed supplement T1-control, T2- Moringa, T3- Phyllanthus, T4- Mistletoe.

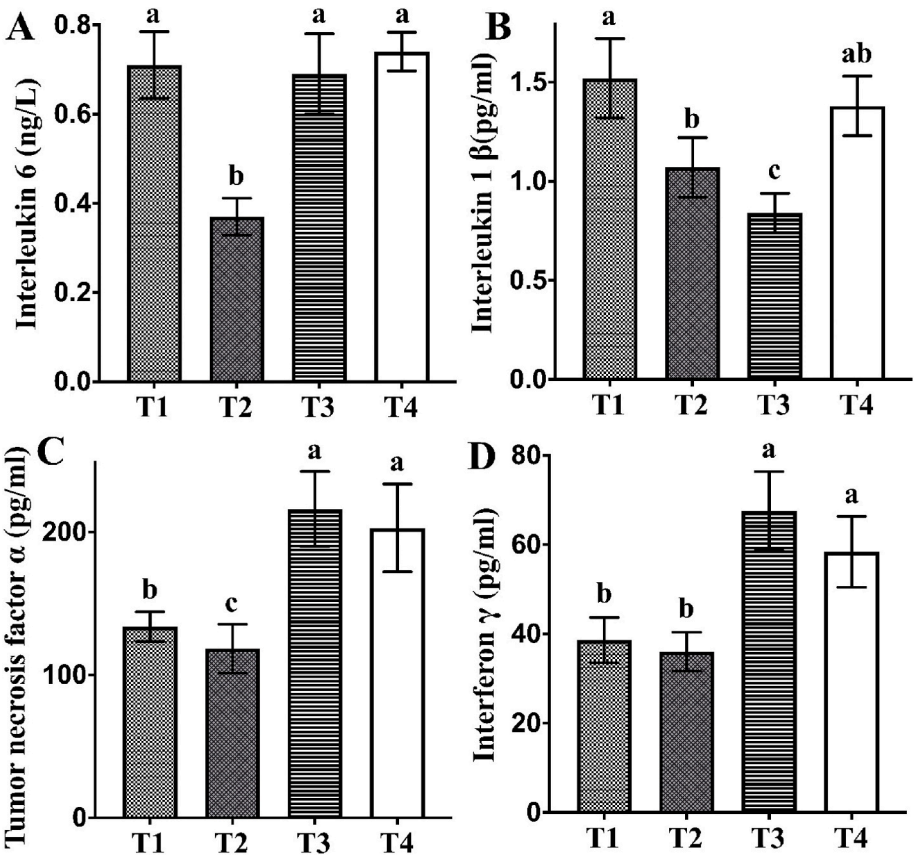


Fig. 4. Pro-inflammatory cytokines of heat stressed broiler fed phytogenic feed supplements. The effects of treatment are shown on (A) Interleukin 6, (B) Interleukin 1β, (C) Tumor necrosis factor α, (D) Interferon γ. Bar and error bars are mean ± SEM, N = 30 per treatment group abc: means with different superscripts are significantly ($P < 0.05$) different heat stressed broilers fed phytogenic feed supplement T1-control, T2- Moringa, T3- Phyllanthus, T4- Mistletoe.

4. Discussion

The temperature humidity index of the broiler microclimate during this study reveals that the birds were exposed to severe heat stress, and this supports earlier claims that the peak of thermal discomfort occur between January and March (Jimoh et al., 2021a). Increased corticosterone levels in animals fed on basal diet is a valuable indicator of stressful conditions such heat stress in these birds, in which is congruent with earlier studies (Sakr et al., 2019). The trend of results obtained shows that the phytogenics supplements reduced corticosterone and enhanced triiodothyronine of birds during heat stress conditions. This could be attributed to arrays of many valuable phytochemical compounds such as lignans, flavonoids, hydrolysable tannins (ellagitannins), polyphenols, triterpenes, sterols and alkaloids (Jimoh et al., 2022b). They possesses a wide spectrum of pharmacological activities including antiviral, antibacterial, antiplasmodial, anti-inflammatory, antimalarial, antimicrobial, anticancer, antidiabetic, hypolipidemic, antioxidant, hepatoprotective nephroprotective and diurectic, to ameliorate problems of stomach, genitourinary system, liver, kidney and spleen (Eweka and Adaze, 2011), and could account for the result in this study. Moringa richly contains various phytoconstituents such as alkaloids, saponins, tannins, steroids, phenolic acids, glucosinolates, flavonoids, and terpenes, which accounts for their antioxidant, anti-inflammatory, anticancer, and antihyperglycemic activities (Ewuola et al., 2012b).

These antioxidant properties and the presence of some vitamins in these herbs, could justify the results obtained it this study. Mistletoe active phytochemicals are lectins and viscotoxins which play prominent role alongside phenolic acids, triterpenes, phenylpropanoids and flavonoids int their antioxidant and anti-inflammatory activities (Hossain et al., 2012; Kienle and Kiene 2003), which protects and ameliorate cellular damage, acts as sedative to elicit anticancer, antimicrobial, antiviral, anti-inflammatory, cardiovascular, apoptosis-inducing and immunomodulatory effects (Shi et al., 2006). This is in line with previous reports that dietary supplementation of broilers with propolis, vitamin E or vitamin C attenuates heat-stress and induced oxidative damage by reducing serum corticosterone levels, as well as improves thyroid hormonal levels (Mahmoud et al., 2014). Similarly, mistletoe, moringa and phyllanthus supplements have been reported to enhance antioxidant system (e.g., glutathione) in animals (Jimoh et al., 2021b; Jimoh et al., 2018a,b), this could account for enhanced triiodothyronine. As reduced glutathione is an important co-factor of both antioxidant enzymes and deiodinases, the enzymes responsible for the conversion of thyroxine (T4) to triiodothyronine (T3) (Mancini et al., 2016). This could be linked to similar report that affirms that the addition of moringa in broiler diet led to an increased level of thyroid hormones (Hassan et al., 2016). Thyroid hormones stimulate cholesterol synthesis as well as the hepatic mechanisms that remove cholesterol from the circulation (Jimoh 2019). Lower blood T3 concentration has been associated with reduced growth of chickens (Abdel-Hamid and Dawod, 2020).

Contrary to T3, corticosterone, which increases as a response to -stressful conditions is an effective biological indicator of severe heat stress response in birds (Sakr et al., 2019). Heat-stress induced elevated serum cholesterol might be due to the increase in corticosterone, via activation of the hypothalamic-pituitary-adrenal (Bishop-Williams, 2015; Ibtisham et al., 2019). In this study, the values of corticosterone obtained from birds fed on moringa supplements were lower than those of birds fed on phyllanthus and mistletoe. Other researchers have reported similar results of heat-stress induced elevated plasma corticosterone, and was reduced with vitamin E supplementation in the diet of Japanese quails (Mahmoud et al., 2014). And Hajati et al. (2015) reported that ascorbic acid supplementation reduces the synthesis of corticoid hormones in birds under heat stress.

Usually, the increased blood insulin level during chronic heat stress is an adaptive mechanism to decrease heat production, which accounts for the decreased circulating glucose concentration, the blunted lipolytic activity and the increased lipogenesis of adipose tissue (Slimen et al., 2015). Insulin levels of birds fed on mistletoe supplements improved during heat stress conditions.

Perturbed HSP is a potent biomarker to evaluate an organism's maintenance of cellular functioning under heat challenges (Mackei et al., 2021; Pamplona and Costantini, 2011). The upregulation of HSP70 in response to stress conditions contributes to inhibiting proinflammatory cytokine expression by preventing the transfer of nuclear factor-κB (NF-κB) to the nucleus (Pritts et al., 2000). Thus, elevated expression of HSPs during heat-stress conditions confer cytoprotection by preventing cytokine production and improving cell tolerance (Shehata et al., 2020). Also, during acute heat stress, digestive enzyme activity increases via overexpression of HSP 70 in broiler, which could improve digestion and absorption function in the intestine (Archana et al., 2017).

Significantly higher HSP70 reported in animals under heat stress without herbal supplement reported here is in line with previous study in Peloco and Caneluda chicken (Adu-Asiamah et al., 2021; Al-Zghoul et al., 2018; Cedraz et al., 2017). This significantly high HSP was reduced by various herbal supplements.

HSP 70 of birds fed on mistletoe and phyllanthus supplements were lower than birds fed on moringa during heat-stress conditions. This could be the effect of antioxidant enrichment of the birds by the phytogenics which mitigated thermal stress in the animals. This is in line with reports that reveal that dietary resveratrol decreased the expression of HSP70, HSP90, and NF-κB in the intestine of heat-stressed chicken after 2 weeks of administration (Liu et al.., 2016, 2014). Similarly, decreased expression of HSP70 was observed in a 35-day-old white Leghorn chicken fed dietary ascorbic acid, vitamin E and lung cancer cells under heat stress (Tiloke et al., 2016; Hajati et al., 2015; Jang et al., 2014; Mahmoud et al., 2003). Conversely, Gu et al. (2012) demonstrated that higher HSP70 response is highly correlated with better antioxidant defense in acutely heat stressed broilers. However, supplementation with rosemary extract was also found to alleviate acute thermal stress effects in broiler chickens via inducing HSP expression and improving the antioxidant capacity in the heart (Tang et al., 2018).

From the above, Moringa, Phyllanthus, Mistletoe supplements have the potential to mitigate heat stress in the birds.

HSP70 can force target proteins to maintain an adequate structure and retain normal function, because its overexpression prevents the oxidative-stress induced decline of mitochondrial permeability transition and the swelling of mitochondria, and in the inhibition of cell apoptosis (Wang et al., 2014). In a different study, dietary rosemary extracts alleviated acute heat shock in broilers by activating HSP70 gene in heart (Tang et al., 2018). This is contrary to our result as HSP was significantly reduced due to plant supplement, this might imply increase oxidative stress in animals on fed with plant supplement.

Leptin secreted by adipocytes suppresses food intake and stimulates energy expenditure (Yuan et al., 2012; Alvala et al., 2013). Intra- and extra-cellular levels of leptin are closely associated with adipocyte size, body fat mass and body weight, and this is influenced by environmental factors or hormones (insulin) (Shim et al., 2017). Leptin levels of birds fed on phytogenic supplements were lower than birds without supplements during heat-stress conditions. The leptin of birds fed on mistletoe supplements were the least amongst birds fed on phytogenic supplement during heat stress conditions. The increased level of leptin during heat stress stimulated satiety signals or prevention of food intake (Schwartz et al., 2000), but moringa oliefera feed led to stimulation of hunger (Abdel-Latif et al., 2018). This could explain the result of increased T3 in birds fed on phytogenics during heat stress in this study. Previous report showed that the administration of *Phyllanthus buxifolius* reduced leptin levels, body weight, intracellular lipid accumulation, abdominal fat weight and cholesterol level in broiler chicken (Wardah, 2012), this supports the trend of the results of this study. Large adipose tissue increases synthesis of leptin due to trigylcerides accumulation (Wardah, 2012). This could account for the effect of the phytogenics reported to

lower cholesterol (Jimoh et al., 2022b), as well as lowers leptin during heat stress in birds.

Adiponectin (adipocyte hormone) modulates a number of metabolic processes such as fatty acid oxidation, glucose regulation, insulin resistance and atherosclerosis (Al-Braich et al., 2014; Elekofehinti et al., 2017). The adiponectin of birds not fed on supplements were higher than birds fed on supplements during heat stress period. Moringa supplement inclusion in birds ration during heat stress decreased the adiponectin levels than birds fed on mistletoe and phyllanthus supplements. The report that adiponectin secretion is reduced in fast growing animals (Hofstedt et al., 2004), could suggest that the birds fed on phytogenics during heat stress condition in this study, had lower adiponectin due to its enhanced appetition (by increase triiodothyronine and decrease leptin) and improves growth rate while mitigating heat stress in the birds.

In response to heat stress, IL-6 expression is upregulated in chickens fed basal diet, Phyllanthus and Mistletoe, which is in tandem with earlier reports (Adu-Asiamah et al., 2021; Al-Zghoul et al., 2018). Consequently, our result implies that both Phyllanthus and Mistletoe did not ameliorate heat stress but Moringa does as reported significantly lowers IL-6. Increased IL-6 in heat-stressed groups could be attributed to its role in tissue protection, healing, and regeneration under stressful situations or after tissue injury (Streetz et al., 2000; McFarland-Mancini et al., 2010; Welc et al., 2013).

The inclusion of phytogenic supplements decreases interleukin 1β of birds during heat stress. Birds fed on moringa and mistletoe supplements were lower than birds not fed on supplements. Birds fed with phyllanthus supplement markedly reduced the interleukin 1β than other supplements during heat stress conditions. This agrees with earlier literatures where *Phyllanthus amarus* and phyllanthin manipulated the mitogen-activated protein kinase (MAPK), nuclear factor-kappa B (NF-κB) and PI3K-Akt signaling pathways to exert their anti-inflammatory effects by disrupting the potential inflammatory signaling mediators (Harikrishnan et al., 2018a,b).

TNFα of birds on moringa supplements are reduced during heat stress compared to other treatments. This is similar to a report that *Moringa oleifera* leaves extract have potent antioxidant properties that reduces TNF-α, a pro-inflammatory cytokine in melamine induced stress (Abu--Zeid et al., 2021). More so, antioxidant enrichment of the phytogenics agrees with the submission of Jang et al. (2014) that reported that antioxidant vitamins vitamin C and E are effective for alleviating summer heat stress due to its beneficial effect on maintaining immunity and antioxidant status under summer conditions. This could be linked to claims that vitamin C during summer conditions showed a significant down-regulation of IL-1β, IL-6, IFN-γ in chicken and IL-6, IL-2 and TNF-α in rodents (Jang et al., 2014). Similarly, hydroethanolic *M. oleifera* flower extract have suppressed production of IL-6, IL-1*β*, and TNF-*α* but enhanced IL-10 by concentration dependently in the Lipopolysacharide induced macrophage (Tan et al., 2015). M. oleifera has also been reported to decrease the production of TNF-α, IL-6, and IL-8 in different disease conditions, confirming it as effective in the management of inflammation (Kou et al., 2018).

TNF-α is the proinflammatory cytokine that defects insulin function in the peripheral tissue (Naowaboot and Piyabhan 2016). In this study, TNFα and IFN-Γ of birds fed on phyllanthus and moringa supplements during heat stress were higher than birds on moringa supplements and birds without supplements. Several plant extracts and compounds have been reported to elevate anti-inflammatory IL-10 production, reducing pro-inflammatory IL-6 or TNF-a production (Mueller et al., 2010). Chili pepper, allspice, basil, bay leaves, black pepper, licorice, nutmeg, oregano, sage or thyme possess anti-inflammatory effects via reduction of IL-6, TNF-α production, enhancement of IL-10 production (Mueller et al., 2010), thus indicating the anti-inflammatory effect of phytogenics on heat stressed birds. Mueller et al. (2010) reported that anti-inflammatory hormone cortisol elevated IL-10 secretion, reduced IL-6 and TNF-a production and is explained by the results of birds fed on phytogenics during heat stress in this study.

As exposure to heat stress increased the protein levels of IL-1β and TNF-α and decreased those of IL-2 and INF-γ in the chicken spleen (Xu et al., 2017). The increased levels of cortisol, adrenaline, leptin (a pro-inflammatory peptide), and TNF-α during heat stress is a result of an interaction between nervous, endocrine, and immune systems towards increased inflammatory status (Abdel-Latif et al., 2018). This explains the trend of result obtained in birds not fed on phytogenic supplements in this study. And the inclusion of moringa, mistletoe and phyllanthus supplements reversed the heat induced effect by eliciting anti-inflammatory response, increase appetite, improve cellular protein response and reduce corticosteroid to mitigate serum biochemical alterations that adversely affects animal physiology during thermal stress. Thermal stress has been implicated to increase the levels of Serum cortisol, adrenaline, TNF-α and leptin (Al-Dawood, 2017) and was reduced by moringa oliefera administration (Abdel-Latif et al., 2018). While, supplementation of moringa in diet modulate physiological stress responses (H:L ratio, corticosterone level, HSP70 expression) and improves production as well as immunity of heat stressed broiler chicken (Rokade et al., 2017). Similar with Mehaisen et al. (2017) who reported that Propolis administration to heat stressed quails maintained normal plasma T3 levels, decreased corticosterone, expressed good immunity status (lower H/L ratio), lowered Hsp70 in the heat-stressed quail.

5. Conclusion

The study reveals that the three herbal supplements decreased corticosterone, HSP 70, leptin and adiponectin of broilers exposed to heat stress and indicated improvement in the welfare of the birds. The triodothyronine of birds under heat stress were enhanced by herbal supplements while mistletoe supplement improved insulin levels in the birds. Birds fed on moringa supplements had lower pro-inflammatory cytokine expression amongst the four groups. Hence, the herbal supplements are recommended for improving welfare and mitigating effects of heat stress in broiler chicken.

CRediT author statement

Jimoh, Olatunji Abubakar: Conceptualization, Formal analysis, Funding and Writing original draft. **Daramola Olajumoke Temidayo:** co-supervised the study, Data curation, review & editing of manuscript. **Okin-Aminu, Hafsat Ololade:** Validation; Visualization, review & editing, Project administration; Software. **Ojo Olayinka Abosede:** Funding, Data curation review & editing of manuscript. All authors read and approved the final manuscript.

Ethical approval and consent to participate

The study was approved by the institutional research ethics committee on the care and use of animals for experiment with the approval number FPA/EC/20/0073, and in accordance with NIH guide for the care and use of laboratory animals.

Consent for publication

Not applicable.

Availability of data and material

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

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Declaration of competing interest

No conflicting interest exist in the research outcome presented in this article.

Data availability statement

The raw/processed data required to reproduce the above findings cannot be shared at this time as the data also forms part of an ongoing study.

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Appendix A. Supplementary data

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