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Effect of *Yucca Schidigera* Supplementation in Diet on the Health Performances, Blood Biomarkers, and Relative Telomere Length of Broiler Chickens Reared Under Tropical Conditions

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Abstract

The main aim of this study was to elucidate the potential of *Yucca schidigera* supplementation on the health performances, blood biomarkers, and relative telomere length of broilers reared under tropical conditions. A total of 300 one-day-old male Ross 308 broilers were purchased, weighed, and divided randomly into six dietary regimens. The dietary treatments consisted of T1: commercial feed without antibiotics (negative control) and T2: commercial feed added with 100 mg/kg oxytetracycline (positive control). Birds in T3, T4, T5, and T6 were fed with commercial feed supplemented with 25, 50, 75, and 100 mg/kg of powdered *Y. schidigera* saponins. The broilers were monitored daily and the clinical sign and mortality were recorded. On day 42, ten male broilers from each treatment were randomly selected and slaughtered, and blood samples were collected for leucocyte profiling, immunoglobulins, cytokines, and telomere length analyses. All data obtained were subjected to the Chi-Square Test, one-way analysis of variance (ANOVA), and Tukey Post-Hoc Test to determine the significant differences among treatment groups. The data were considered significant at $P < 0.05$. Throughout the 42-day study, a few cases of leg problems, diarrhea, and mortality were observed, but there were no significant associations ($P > 0.05$). However, there were significant differences ($P < 0.05$) in the leucocyte profiling, immunoglobulins, cytokines, and telomere length expression among treatments. Negative control broilers not fed with any additive demonstrated the lowest values as compared to the antibiotic and treatment groups. Overall, T6 broilers fed with a diet supplemented with 100 mg/kg of *Y. schidigera* saponins exhibited the best leucocyte profiling (increased total WBC count, monocytes, and basophil count; decreased H/L ratio), immunoglobulins (upregulated Ig-G, Ig-A, and Ig-M,), cytokines (increased IL-4 and IL-7), and the longest telomere length expression followed by T2 broilers. In summary, this study has established that 100 mg/kg of *Y. schidigera* saponins supplementation in diet can be used to enhance the health performances of broilers raised under hot and humid conditions.

Introduction

Despite the industry growth and monetary benefits, the broiler industry faces numerous challenges to fulfil the needs of demand, which include poor management, high feed production cost, disease outbreak, the uncertainty of government policy, and technological advancement pressure (Alghirani *et al*., 2021a). Moreover, in the tropical region, the nature of high humidity and high temperature-triggered thermal stress in broilers may also hinder optimal health and growth (Kpomasse *et al*., 2021). This defying challenges definitely will harm the meat supply

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consequent in large economic losses, especially on commercial broiler farms (Alghirani *et al*., 2021a). Perceiving these concerns, the broiler industry frequently practiced vaccination, medical treatments, and the inclusion of feed additives such as antibiotics as a prophylactic preventive measure to optimize broiler production and health. Nonetheless, with the strong awareness of food safety reasons, antibiotic has become a global concern and the usage of antibiotic has started to be banned in developed countries (Sarmah *et al*., 2006).

In recent years, phytobiotic draws global attention as a promising alternative to replace antibiotics due to their high content of pharmacologically active compounds that may act as a natural growth promoter in broiler production (Dhama *et al*., 2015). For instance, saponins have become a favorable phytobiotic to be explored due to their physiological, immunological, and pharmacological properties that triggered the clinical interest in this substance. This is explained by the function of saponins, which helps to emulsify oil lipids, promote broiler digestion, and absorb vitamins and minerals, resulting in increased immunological benefits in chicken (Khaskheli *et al*., 2020). Furthermore, saponins were reported to have other pharmacological advantages such as anti-inflammatory, immunostimulant, hypocholesterolemic, antifungal, cytotoxic activities, antimicrobial, anticarcinogenic, and antioxidant (Güçlü - Üstündağ and Mazza, 2007).

Commercial saponins extract from *Yucca schidigera* is now commonly used in the temperate commercial broiler industry as it is capable of enhancing broiler growth and health (Ranjbar *et al*., 2014; Sahoo *et al*., 2015). *Y. schidigera* is an herbaceous dessert shrub that originated in Southwestern United States of America, which is rich in secondary metabolites namely glycol and steroidal saponins (Khaskheli *et al*., 2020). However, there are a few limitations in implying saponins effectively in tropical climates as the effects and mechanisms are not well understood. There is still a lack of information on the effect of saponins in tropical climates as high temperature and humidity are the major factors affecting the growth and health of broilers. Herein, this study was conducted to reveal the potential of *Y. schidigera* saponins as an alternative feed additive on the health performances, leucocyte profiling, immunoglobulins, and cytokines concentrations, as well as the relative telomere length of broilers reared under tropical conditions.

Materials and Methods

Broilers management

All experimental methods were carried out following the Universiti Putra Malaysia (UPM) Institutional Animal Care and Use Committee (IACUC) Research Policy (Approval number: UPM/IACUC/AUP-R005/2020). In a completely randomized design (CRD), 300 one-day-old male Ross 308 broilers were purchased, weighed, and divided randomly into six dietary regimens, with five replicates consisting of ten broilers each replication. The broilers were raised on wired flooring battery cages in an open-sided house for 42 days. During rearing, the mean temperature and relative humidity were 29 °C and 79%, respectively. For the first three days, anti-stress (VP1000) containing various vitamins and amino acids was added to the drinking water. All broilers were intraocularly immunized against Newcastle disease and infectious bronchitis on day 7, and infectious bursal disease on day 14 (Chung *et al*., 2021).

From day 1 to 21, the broilers were provided with a commercial starter diet in crumble form with a corn and soybean meal basal composition, followed by a finisher diet from day 22 to 42. The nutritional requirements were formulated according to Ross 308 nutritional guidelines. The dietary treatments consisted of T1: commercial feed without antibiotics (negative control) and T2: commercial feed added with 100 mg/kg oxytetracycline (positive control). T3, T4, T5, and T6 were fed with commercial feed supplemented with 25 mg/kg, 50 mg/kg, 75 mg/kg, and 100 mg/kg of powdered *Y. Schidigera* saponins. The commercially available *Y. schidigera* extracted powder containing 60.60% of total saponins was purchased from Xi'an Longze Biotechnology Co., Ltd., China. The nutritional composition of starter and finisher diets supplemented with *Y. schidigera* saponins at various doses is shown in Tables 1 and 2. Throughout the 42-day feeding study, feed and fresh water were supplied to the broilers *ad-libitum*. The broilers were monitored daily and the clinical sign and mortality were recorded. Clinical signs observed include depression, ruffled feathers, diarrhea, legs problem, and coughing (Chakma, 2015). From our previous study, T6 broilers treated with 100 mg/kg *Y. schidigera* saponins outperformed the other treatment broilers in terms of growth efficiency, nutrient digestibility, digestive health, carcass features, and meat quality (Alghirani *et al*., 2021b). The present study, therefore, will further determine the health performances, blood biomarkers, and telomere expression of those broilers to support the positive findings of *Y. schidigera* supplementation towards broilers reared under tropical conditions. On day 42, ten broilers were chosen at random from each treatment and slaughtered (two in each replicate). Blood samples were collected at the time of slaughter to be analyzed for leucocyte profiling, immunoglobulins, and cytokines concentrations, as well as telomere length expression.

Table 1. Composition and nutrient content of broiler starter diet supplemented with *Y. schidigera* saponins at different concentrations

Parameters	Treatments					
	T1	T2	T3	T4	T5	T6
Ingredient (%)						
Corn (%)	41.27	41.27	41.27	41.27	41.27	41.27
Soybean meal (%)	40.60	40.60	40.60	40.60	40.60	40.60
Palm oil (%)	6.00	6.00	6.00	6.00	6.00	6.00
Wheat pollard (%)	6.88	6.88	6.88	6.88	6.88	6.88
Dicalcium phosphate (%)	2.28	2.28	2.28	2.28	2.28	2.28
Calcium carbonate (%)	1.75	1.75	1.75	1.75	1.75	1.75
Salt (%)	0.30	0.30	0.30	0.30	0.30	0.30
L-Lysine (%)	0.25	0.25	0.25	0.25	0.25	0.25
D,L-Methionine (%)	0.20	0.20	0.20	0.20	0.20	0.20
Mineral premix (%)	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix (%)	0.10	0.10	0.10	0.10	0.10	0.10
Antioxidant (%)	0.02	0.02	0.02	0.02	0.02	0.02
Choline chloride (%)	0.10	0.10	0.10	0.10	0.10	0.10
Toxin binder (%)	0.10	0.10	0.10	0.10	0.10	0.10
Calculated analysis						
Metabolizable Energy (kcal/kg)	3107	3076	3081	3129	3081	3147
Dry matter (%)	89.77	90.77	90.33	91.33	90.57	90.43
Crude protein (%)	23.10	23.57	23.17	22.90	23.07	23.13
Crude fibre (%)	3.40	3.40	3.47	3.13	3.23	2.97
Ether extract (%)	6.85	7.20	7.35	7.15	7.20	7.00
Ash (%)	5.90	5.67	5.77	5.80	6.00	5.80

Note: T1: No additive; T2: 100 mg/kg oxytetracycline; T3: 25 mg/kg *Y. schidigera*; T4: 50 mg/kg *Y. schidigera*; T5: 75 mg/kg *Y. schidigera*; T6: 100 mg/kg *Y. schidigera* in diet.

Table 2. Composition and nutrient content of broiler finisher diet supplemented with *Y. schidigera* saponins at different concentrations

Parameters	Treatments					
	T1	T2	T3	T4	T5	T6
Ingredient (%)						
Corn (%)	49.50	49.50	49.50	49.50	49.50	49.50
Soybean meal (%)	33.44	33.44	33.44	33.44	33.44	33.44
Palm oil (%)	6.00	6.00	6.00	6.00	6.00	6.00
Wheat pollard (%)	6.35	6.35	6.35	6.35	6.35	6.35
Dicalcium phosphate(%)	1.61	1.61	1.61	1.61	1.61	1.61
Calcium carbonate (%)	1.83	1.83	1.83	1.83	1.83	1.83
Salt (%)	0.30	0.30	0.30	0.30	0.30	0.30
L-Lysine (%)	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine (%)	0.20	0.20	0.20	0.20	0.20	0.20
Mineral premix (%)	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix (%)	0.10	0.10	0.10	0.10	0.10	0.10
Antioxidant (%)	0.02	0.02	0.02	0.02	0.02	0.02
Choline chloride (%)	0.10	0.10	0.10	0.10	0.10	0.10
Toxin binder (%)	0.15	0.15	0.15	0.15	0.15	0.15
Calculated analysis						
Metabolizable Energy (kcal/kg)	3203	3265	3248	3286	3251	3253
Dry matter (%)	90.30	90.20	89.57	90.43	89.97	90.43
Crude protein (%)	19.33	19.77	19.37	19.87	19.00	19.30
Crude fibre (%)	3.95	4.53	3.70	3.40	4.50	3.40
Ether extract (%)	4.67	4.70	4.57	4.70	4.67	5.00
Ash (%)	5.43	5.67	5.57	5.57	5.43	5.80

Note: T1: No additive; T2: 100 mg/kg oxytetracycline; T3: 25 mg/kg *Y. schidigera*; T4: 50 mg/kg *Y. schidigera*; T5: 75 mg/kg *Y. schidigera*; T6: 100 mg/kg *Y. schidigera* in diet.

Leucocyte profiling

In order to manually calculate the estimated total white blood cell (TWBC) and differential white blood cell (WBC) counts, peripheral blood smears were stained with Wright Stain. The blood smears were observed under Novex Microscope and CMEX, CMOS camera which linked to Image Focus v3.0 software to visualize the leucocyte images. The types of leucocytes observed were heterophil, lymphocyte, monocyte, basophil, and eosinophil. By counting the WBC in 10 separate fields of the slide at a 40× objective, the TWBC was counted, and the average of

the number of WBC was computed. To calculate an estimated TWBC count per microliter, the average value was multiplied by 2000. Formula: Estimated TWBC/l = (Mean of 10 views) × 2000 was used to perform the computations (Carisch *et al*., 2019). Additionally, 200 WBC were manually counted and identified at a magnification of 100× using emulsion oil to provide a sharper view of the white blood cells for the differential WBC counts. After then, the data were transformed into percentages (Chung *et al*., 2020). On top of that, the heterophil and lymphocyte (H/L) ratio was determined based on the formula: Total heterophil/Total lymphocyte.

Immunoglobulins and cytokines concentrations

Using commercial enzyme-linked immunosorbent assay (ELISA) kits (QAYEE-BIO, China), the concentrations of immunoglobulins (Ig-M, Ig-A, and Ig-G) and cytokines (IL-4 and IL-7) in each blood serum sample were determined. The operating stages for all of the ELISA kits utilized are similar; however, each kit has its unique set of standards with known concentrations. All of the stages were carried out in accordance with the manufacturer's instructions. The optical density (OD) was measured at 450 nm using a microplate reader (Bio-Rad Microplate Reader, USA). The standard curve linear regression equation was developed based on the concentration of the standards and the matching OD values, and the sample concentration was generated correspondingly.

Relative telomere length

The innuPREP blood DNA mini kit (analytic Jena) was used in extracting the DNA from whole blood samples according to manufacturer instructions. The yield and purity of extracted DNA were analyzed with Multiskan Go (Thermo Scientific) (260/280 ratio). The relative telomere length was determined using qPCR for the $2^{-\Delta\Delta Ct}$ method. The master mix solution using SYBR green (SensiFAST™ SYBR No-ROX Kit) was prepared as described by O'Callaghan and Fenech (2011) and was run in triplicate. The qPCR cycling conditions and primers sequences were as outlined previously (Badmus *et al*., 2021).

Statistical analysis

Using the Statistical Analysis System (SAS, 2012), Chi-Square Test was used to test the relationship between *Y. schidigera* saponins and the mortality rate in broilers. Then, data obtained were submitted to a one-way analysis of variance (ANOVA) based on the Completely Randomized Design model. The Tukey Post-Hoc Test was used to establish the significant differences among treatment groups. The data were considered significant at $P < 0.05$.

Results

Clinical signs and mortality rate

The clinical signs and mortality rate of broilers supplemented with *Y. schidigera* saponins are reported in Table 3. There was a single leg problem observed in T5 for both the starter and finisher phases, and 2 cases in T6 only during the finisher phase. Meanwhile, diarrhea was observed in T2 during the starter phase and in T6 during the finisher phase. Collectively, a total of 4 and 22 mortalities were reported in the starter and finisher phases respectively. Nonetheless, there was no significant difference ($P > 0.05$) in the mortality rate, which reflects that supplementing broilers with different fractions of *Y. schidigera* causes no detrimental effects on the clinical signs and mortality. Besides, depression, ruffled feathers, and coughing were not observed throughout the study.

Table 3. Effect of *Y. schidigera* saponins supplementation on the clinical observation of broilers on days 21 and 42

Clinical Observation	Treatments						*P*-Value
	T1	T2	T3	T4	T5	T6	
Starter phase (days 1-21)							
Mortality	0	1	0	1	1	1	-
Leg problem	0	0	0	0	1	0	-
Diarrhoea	0	1	0	0	0	0	-
Finisher Phase (days 22-42)							
Mortality	5	3	4	3	4	3	0.71
Leg problem	0	0	0	0	1	2	-
Diarrhoea	0	0	0	0	0	1	-

Note: Significantly different at $P < 0.05$, by using Chi-Square test. T1: No additive; T2: 100 mg/kg oxytetracycline; T3: 25 mg/kg *Y. schidigera*; T4: 50 mg/kg *Y. schidigera*; T5: 75 mg/kg *Y. schidigera*; T6: 100 mg/kg *Y. schidigera* in diet.

Leucocyte profiling

Table 4 shows the result of the leucocyte profile of broilers supplemented with *Y. schidigera* saponins on day 42. All leucocyte profiles showed significant results ($P < 0.05$) during the finisher phase except for the eosinophil count. T6 broilers demonstrated the highest total WBC count, monocytes, and basophil count. However, the H/L ratio of T2 and T6 broilers was the lowest compared to the other treatments indicating less stress. Similar to the use of antibiotics in T2 broilers, 100 mg/kg of *Y. schidigera* saponins was found to be a good enhancer for leucocyte production, while reducing stress in broilers.

Table 4. Effect of *Y. schidigera* saponins supplementation on the leucocyte profile of broilers on day 42

White Blood Cell	Treatments						*P*-Value
	T1	T2	T3	T4	T5	T6	
Total WBC count	199.40± 13.52^{c}	294.80±17.23^{b}	181.60±2.91^{c}	205.60±7.92^{c}	211.00± 9.56^{c}	348.20±7.61^{a}	0.01
Heterophil (%)	30.40±1.60^{a}	20.00±0.63^{b}	31.60±1.50^{a}	23.80±1.77^{b}	21.60±2.01^{b}	19.60±0.68^{b}	0.02
Lymphocyte (%)	66.20±1.74^{b}	76.40±0.870^{a}	63.20±0.73^{b}	73.00±1.52^{a}	74.80±2.63^{a}	73.80±0.86^{a}	0.01
H/L Ratio	0.46 ±0.03^{a}	0.26±0.01^{b}	0.50±0.03^{a}	0.33±0.03^{ab}	0.29±0.04^{b}	0.27±0.01^{b}	0.01
Monocytes (%)	1.80±0.37^{bc}	1.60±0.24^{c}	1.60±0.24^{c}	1.80±0.20^{bc}	2.60±0.24^{ab}	3.40±0.40^{a}	0.02
Eosinophil (%)	0.20±0.20	0.60±0.24	0.40±0.24	0.60±0.24	0.00±0.00	0.60±0.24	0.27
Basophil (%)	1.40±0.24^{b}	3.20±0.66^{a}	2.60±0.40^{ab}	1.20±0.58^{b}	2.80±0.37^{ab}	3.20±0.80^{a}	0.04

Note: All values were expressed as mean ± SE (n = 10); $^{a, b, c}$ values with superscript within a row are significantly different at $P < 0.05$. H/L: heterophil and lymphocyte ratio. T1: No additive; T2: 100 mg/kg oxytetracycline; T3: 25 mg/kg *Y. schidigera*; T4: 50 mg/kg *Y. schidigera*; T5: 75 mg/kg *Y. schidigera*; T6: 100 mg/kg *Y. schidigera* in diet.

Table 5. Effect of *Y. schidigera* saponins supplementation on the blood biomarkers of broiler on day 42

Parameter	Treatments						*P*-Value
	T1	T2	T3	T4	T5	T6	
Ig-G (ng/mL)	1153±0.48^{b}	1296±1.20^{ab}	1315±0.64^{b}	1208±1.13^{b}	1212±0.69^{b}	1372±0.95^{a}	0.004
Ig-A (ng/mL)	799±0.72^{b}	970±0.18^{ab}	857±0.44^{b}	800±0.53^{b}	897±0.60^{b}	1123±0.75^{a}	0.004
Ig-M (mg/mL)	242±0.08^{bc}	287±0.23^{ab}	228±0.07^{c}	275±0.09^{abc}	265±0.03^{abc}	311±0.26^{a}	0.0249
IL-4 (pg/mL)	2938±1.85^{c}	4242±1.11^{a}	3651±1.14^{b}	2934±1.14^{c}	3539±0.49^{b}	4356±0.96^{a}	<0.0001
IL-7 (pg/mL)	646±0.68^{b}	1614±2.41^{a}	620±0.31^{b}	1245±1.38^{ab}	1327±2.91^{a}	1371±2.43^{a}	0.0098

Note: All values were expressed as mean ± SE (n = 10); $^{a, b, c}$ values with superscript within a row are significantly different at $P < 0.05$. Ig: immunoglobulin; IL: interleukin. T1: No additive; T2: 100 mg/kg oxytetracycline; T3: 25 mg/kg *Y. schidigera*; T4: 50 mg/kg *Y. schidigera*; T5: 75 mg/kg *Y. schidigera*; T6: 100 mg/kg *Y. schidigera* in diet.

Table 6. Effect of *Y. schidigera* saponins supplementation on the relative telomere length of broilers on 42

Parameter	Treatments						*P*-Value
	T1	T2	T3	T4	T5	T6	
Rel. Fold Dif.	1.56±0.15^{b}	1.91±0.45^{b}	1.57±0.19^{b}	1.68±0.15^{b}	1.81±0.10^{b}	2.98±0.26^{a}	0.004

Note: All values were expressed as mean ± SE (n = 10); $^{a, b}$ values with superscript within a row are significantly different at $P < 0.05$. Rel. Fold Dif.: Relative fold difference. T1: No additive; T2: 100 mg/kg oxytetracycline; T3: 25 mg/kg *Y. schidigera*; T4: 50 mg/kg *Y. schidigera*; T5: 75 mg/kg *Y. schidigera*; T6: 100 mg/kg *Y. schidigera* in diet.

Immunoglobulins and cytokines concentrations

The blood biomarkers of broilers supplemented with *Y. schidigera* saponins during the finisher phase are reported in Table 5. There were significant differences ($P < 0.05$) in the concentrations of both antibodies and cytokines parameters among treatments. T6 broilers supplemented with 100 mg/kg of *Y. schidigera* saponins exhibited the highest values of Ig-G, Ig-A, Ig-M, IL-4, and IL-7 followed by T2. While, the lowest expression of Ig-G, Ig-A, Ig-M, IL-4, and IL-7 was significantly observed in T1 broilers not fed with any additives. Hence this study found that the optimum supplementation of 100 mg/kg of *Y. schidigera* saponins was capable of up-regulating the immune status of broilers.

Relative telomere length

Table 6 shows the relative telomere length of broilers supplemented with *Y. schidigera* saponins on day 42. There was a significant difference ($P < 0.05$) in the telomere length expression during the finisher phase. T6 broilers fed with 100 mg/kg of *Y. schidigera* saponins expressed significantly longer relative telomere length as compared to the other treatments indicating that the optimum saponins supplementation was able to mitigate stress effects in broilers under tropical conditions.

Discussion

Clinical sign and mortality rate

Although beneficial, plant-based supplementation needs to be supplied at optimum levels as it may affect growth performances and increase the mortality rate as a result of anti-nutritional factors or toxins present in the plant (Naidoo *et al*., 2008). For example, the properties of saponins increase the worrisome livestock ingestion, especially in ruminants attributable to impairing digestion and absorption leading to bloat (Chung *et al*., 2018; Muniandy *et al*., 2020). Contrariwise, saponins have been proven in some poultry research to reduce mortality rate, improve growth, increase nutrient digestibility, and immune function (Alghirani *et al*., 2021b). Based on the present study, monogastric animals specifically broiler chickens had no issues due to their toxicity adaptability as the mortality rate was insignificant in all treatments. Unlike ruminants, digestion and absorption of feed in chickens are enzyme dependent. Feed is processed by intestinal fluids and digestive enzymes such aminopeptidase, amylase, maltase, and invertase after being ground in the gizzard (Clavijo and Florez, 2018). For that reason, the soapy properties of saponins would not cause any detrimental effect as broilers have no rumen microbes. This finding was supported by previous research that reported the feeding of guar meal saponins in the broiler diets also showed no significant effect on the mortality rate (Hassan *et al.*, 2013). On the other hand, broilers in the present study demonstrated no notable clinical signs during the rearing period except sporadic cases of leg problems and diarrhea, which could be attributed to the broilers' fast-growing genetics and the housing environment (Mack *et al*., 2013).

Leucocyte profiling

The leucocyte profile is a general parameter to understand and indicate the health status of broilers as lymphocytes are important in humoral and act as cell-mediated immunity (Scanes, 2016). According to Ramadan *et al*. (2021), some feed additives can be used effectively to reduce the H/L ratio as well as increase the immune status of broilers. The decrease in heterophil percentage may be related to the modification of some feed additives substances that relieve stressors which is a consequence of the depletion of corticosterone secretion. Hence by sequence, these may increase the immunity responses by increasing the lymphocyte numbers (Ramadan *et al*., 2021). In the present study, T6 broilers fed with 100 mg/kg of *Y. schidigera* saponins demonstrated a decrease in H/L ratio and heterophil percentage as compared to other treatments. The total count of WBC, lymphocytes and basophils percentage were also increased in T6 broilers. All these could be associated with the high steroidal saponins present in *Y. schidigera*. Numerous research found that saponins could improve animal performance, increase antioxidant capacity, anti-tumor, anti-microbial, anti-inflammatory, reduce cholesterol, as well as other useful biological functions, which will then enhance and improve the immune response of those broilers (Su *et al*., 2016; Liu *et al*., 2018). In accordance with Khan *et al*. (2014), phytobiotics like saponins supplemented in broilers reared under heat stress expressed higher leucocytes, lymphocytes, and monocytes count, which indicates that the supplementation helps in improving the immune status and growth performance.

Immunoglobulins and cytokines concentrations

Immunoglobulin is a protein molecule produced by plasma cells that plays an important role in systemic humoral immunity. Avian blood consists of three major Ig namely Ig-A, Ig-M, and Ig-G which are well detected in chickens (Luo *et al*., 2013). Meanwhile, cytokines were primarily responsible for the immune system's function as they produce secretion proteins and signaling proteins for leucocytes and immunoglobulins' biological functioning. IL-4 and IL-7 have become favorable cytokines biomarkers in poultry studies as they are strongly linked to the B-cell and T-cell mechanisms, which are important immune cells in the peripheral immune system (Paul, 2015). According to Iser *et al*. (2016), secondary metabolites such as saponins have the potential to

modulate innate and humoral immune responses. In the present study, broilers supplemented with 100 mg/kg of *Y. schidigera* saponins demonstrated better production of antibodies during the finisher phase. Likewise, IL-4 and IL-7 showed a better production in T6 broilers indicating that the saponins supplementation could assist the stimulation of T-cell and B-cell respectively in producing immunoglobulins. As a result, infections and inflammation were not linked to the elevated levels of Ig-A, Ig-M, and Ig-G found in this study but rather to the diet's supplementation. This is due to the fact that saponins promote the release of cytokines that activate cellular, humoral, and innate immunity (Alghirani *et al*., 2022). As mentioned by Fleck *et al*. (2009), with an optimum level of saponins supplementation, only the immunity would be stimulated and enhanced. This was probably due to the steroidal saponins' ability in assisting the maturation of immune organs in broilers (Su *et al*., 2016). As a result, the matured immune organ will have a better ability in producing higher amounts of cytokines and immunoglobulins, which provide superior immunity for the broilers. Su *et al*. (2016) concur that *Y. schidigera*, ginseng, and tea plant saponins have favorable immunomodulatory capabilities with increased immunoglobulin concentration and favorable impacts on immune organ maturation properties.

Relative telomere length

Telomeres are specialized nucleoprotein end structures on eukaryotic chromosomes that guard against end-to-end fusion, exonuclease destruction, and recombination and maintain genome integrity (Sohn & Subramani, 2014). Telomere length shortening in an individual can be caused by both hereditary and non-genetic causes in addition to aging (Factor-Litvak *et al*., 2017). According to Sohn & Subramani (2014), non-genetic factors include environmental stress such as house system, temperature, humidity, disease, oxidative stress, or stochastic influences that may induce telomere length shortening. Nonetheless, in a report by Sohn *et al*. (2013), broilers supplemented with feed additives were observed to lower the rate of telomere shortening. In the present study, all broilers were subjected to obvious thermal stressors and telomere length shortening as they were reared in an open-sided poultry house. However, broilers supplemented with 100 mg/kg of *Y. schidigera* saponins significantly expressed higher telomere length as compared to other treatments. This is because, the high steroidal saponins presence in *Y. schidigera* has a superior antioxidant property that can help slow down the aging of cells by alleviating telomeric loss and reducing telomere shortening rate (Sohn & Subramani, 2014). Hence, this gave an observable longer telomere length effect on broilers supplemented with *Y. schidigera* saponins, which also indicated improved general health in the present study. The result was consistent with a few studies in which saponins from ginseng improve immune responses while reduce the telomerase activity of lymphocyte nuclei without impairing chicken growth performance as compared to the control groups (Wang *et al*., 2004; Sohn *et al*., 2008).

Conclusion

Supplementing broilers with 100 mg/kg of *Y. schidigera* saponins demonstrated a better health performance as compared to the other fractions. The mortality rate and clinical signs were benevolent and unnoticeable. Moreover, the leucocyte profile indicated less stress as the H/L ratio was the lowest during the finisher phase. In addition, the immunology biomarkers such as cytokines, leucocytes, and immunoglobulins were up-regulated indicating an increased immune system. The relative telomere length was the longest noting that those broilers were healthy and less stressful. Besides antibiotics, this study firmly validated that 100 mg/kg of *Y. schidigera* saponins could be used to enhance the health performances of broilers raised under tropical conditions.

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