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Research article

Blood biochemistry and stress biomarkers of broiler chickens supplemented with different levels of *Yucca schidigera* saponins reared under tropical conditions

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Abstract

The primary goal of this study was to determine the impacts of supplementing varying amounts of Y. schidigera saponins on the blood biochemistry and stress biomarkers of broiler chickens raised in tropical settings. A total of 300 male day-old Ross 308 broilers were randomly assigned to six treatment groups. Treatment 1 broiler chickens were fed commercial diets with no added additives, whereas treatment 2 broiler chickens were offered commercial diets containing 100 mg/kg of the antibiotic oxytetracycline. Treatments 3, 4, 5, and 6 broiler chickens received similar commercial diets supplemented with 25, 50, 75, and 100 mg/kg of powdered *Y. Shidigera* saponins, respectively, without antibiotics. On day 42, six broilers from each treatment were randomly selected, slaughtered, and blood samples were collected for serum lipid profile, liver function, acute phase proteins, hormone, and heat shock protein analyses. There were notable changes ($P < 0.05$) in the serum lipid profile, acute phase proteins, hormone, and heat shock protein among treatments. Broilers treated with 100 mg/kg of *Y. Shidigera* saponins in T6 showed the lowest levels of cholesterol, triglycerides, and low-density lipoprotein cholesterol concentrations while having the highest high-density lipoprotein cholesterol level without affecting the liver parameters. Moreover, the serum amyloid A, alpha-1-acid glycoprotein, corticosterone, and heat shock protein 70 concentrations were also the lowest as compared to the other treatments. In conclusion, supplementing 100 mg/kg of *Y. Shidigera* saponins in broiler diets could improve lipid profiles and act as an anti-stress for commercial broilers raised in tropical regions.

Keywords: Acute phase proteins, Heat shock proteins, Hormones, Lipid profiles, Liver functions, Ross 308, Saponins

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INTRODUCTION

The global poultry sector is expanding at an exponential rate in tandem with the global population growth. Therefore, any disturbances like slow growth and disease outbreaks might possibly hurt supply and demand, which would eventually have an impact on global food security (Chung et al., 2021). The use of antibiotics as growth promoters in the poultry industry has greatly improved growth performance and feed conversion efficiency (Basit et al., 2020). However, administering antibiotics to stimulate growth during the chicken production cycle may also result in antimicrobial resistance in both chickens and people. Developed nations have started to outlaw the use of antibiotics in all animal feed due to concerns about food safety. In order to prevent sickness and encourage a rapid growth rate in poultry, it may therefore be required to investigate additional preventive options. As a result, commercial saponins and other phytobiotics have been employed as growth promoters in poultry feed in place of antibiotics (Alghirani et al., 2022).

Saponins can be found in at least 400 plant types and forage like legumes, lupins, red clover, soybean, fodder plants, Lucerne, and ladino clover (Alghirani et al., 2021a). The commercial saponins that are often used are commonly derived from *Quillaja saponaria* and *Yucca schidigera* (Alghirani et al., 2021b). Saponins have many pharmacological and biological properties like anti-carcinogenic, anti-microbial, anti-inflammatory, hypocholesterolaemic, immunomodulatory, and anti-oxidant effects on poultry. Besides, saponins can play the role of anti-parasitic and anti-fungal effects (Chaudhary et al., 2018). As a result, when compared to synthetic antibiotics, this compound is established to be natural, residue-free, less toxic, and is thought to be an appropriate growth promoter in livestock feed (Hashemi and Davoodi, 2011). In the poultry industry, saponins have been used as feed additives to increase the body weight gain of commercial broilers during all stages of growth with enhanced feed conversion ratio and improved carcass quality (Alghirani et al., 2021a).

Supplementation of phytobiotic in broilers' diet can help increase the high-density lipoprotein cholesterol while reducing the serum concentration of cholesterol, triglycerides, and low-density lipoprotein cholesterol in broilers at both starter and finisher phases (Gilani et al., 2018). For example, supplementing saponins from different sources, in particular, was found to lower serum cholesterol levels despite no significant changes in growth performance in some studies. Saponins can lower blood cholesterol concentrations by two mechanisms: which are by reducing its intestinal absorption through the formation of insoluble complexes with cholesterol, or saponins forming big molecules with bile salts in the gut, hence preventing bile salts reabsorption in the ileum. The latter consequence causes enhanced bile salt production from cholesterol in the liver, leading to serum cholesterol depletion (Tokofai et al., 2021). Saponins have also been shown to reduce the harmful low-density lipoprotein cholesterol specifically in the blood of rats, gerbils, and poultry. Different saponins sources were found to have the potential to lower the serum cholesterol levels in broiler and layer chickens, resulting in healthier meat and eggs for the consumers (Afrose et al., 2010). As indicated by Matsuura (2001), adding purified saponins or extracts containing saponins to poultry diets like *Y. schidigera* could help decrease the plasma and in some cases liver cholesterol concentrations.

Biomarkers such as acute phase proteins (APP), hormones, and heat shock proteins (HSP), on the other hand, are measurable changes in biological substances that are associated with normal or abnormal conditions such as disease incidence and outcome, as well as the repercussions of treatments, interventions, and even unintended exposure to environmental stressors, such as nutrients and heat stress (Strimbu and Tavel, 2010). In the poultry industry, biomarkers play a crucial role in assessing the nutritional impacts on broilers' welfare and growth performance. Heat stress has been shown in several studies to impair poultry growth performance (Imik et al., 2012). Furthermore, many challenges such as transportation, weaning, and housing on slippery floors have been documented to increase serum concentrations of different blood biomarkers via different challenges like inflammation, bacterial infection, endotoxin exposure, tissue injury, and neoplasia (Janmohammadi et al., 2020). Supplementation of phytobiotics in broilers' feed leads to improve growth performance under heat stress due to their antioxidant activity, which can affect the blood biomarkers and serum biochemistry of poultry. Saponins are an example of phytobiotics that might be useful during stressful conditions because of their antioxidant activity, which can affect the blood biomarkers of poultry (Greene et al., 2021).

Despite the widespread use of phytobiotics in livestock feed to promote growth and prevent disease (Boripun et al., 2022; Norkeaw et al., 2022; Reyno et al., 2022), there is still a knowledge gap regarding the potential use and influence of saponins on the blood biochemistry and stress biomarkers of broilers in the tropics. For that reason, this research aimed to elucidate the impacts of supplementing varying amounts of *Y. schidigera* saponins on the serum lipid profile, liver function, APP, hormones, and HSP of broiler chickens raised in hot and humid conditions.

MATERIALS AND METHODS

Broiler chickens 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 randomised design (CRD), 300 day-old male Ross 308 broilers were purchased, weighed, and divided randomly into six dietary regimens, with five repetitions consisting of ten broilers each replication. For 42 days, the broilers were raised on wired flooring battery cages in an open-sided house. For the first three days, anti-stress (VP1000) was added to the drinking water. During rearing, the mean temperature and relative humidity were 29°C and 79%, respectively. All broilers were intraocularly immunised against Newcastle disease and Infectious bronchitis on day 7, and Infectious bursal disease on day 14 (Chung et al., 2021).

Experimental design

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 dietary treatments consist 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. Shidigera saponins. The commercially available Y. shidigera extracted powder purchased from Xi'an Longze Biotechnology Co., Ltd., China, contained 60.60 % of total saponins which were added directly into the commercial diets and were mixed thoroughly before feeding. The nutritional composition of starter and finisher diets supplemented with *Y. schidigera* saponins at various doses is shown in Tables 1 and 2. The nutritional requirements were formulated according to Ross 308 nutritional guidelines. Throughout the 42-day feeding study, feed and fresh water were supplied to the broiler birds *ad-libitum*. From our previous study, T6 broilers treated with 100 mg/kg Y. shidigera 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 blood biochemistry and stress biomarkers of those broilers to support the positive findings of *Y. schidigera* supplementation towards broilers reared under tropical conditions. On day 42, six broilers were chosen at random from each treatment and slaughtered. Blood samples were collected at the time of slaughter to be analysed for lipid profile and liver function, as well as assessment of APP, corticosterone, and HSP concentrations.

Blood biochemistry

For the lipid profile, an automatic analyser (Automatic analyser 902, Hitachi, Germany) was used to determine serum total cholesterol, triglycerides (TG), and high-density lipoprotein cholesterol (HDL), while the Friedewald Equations were used to estimate very low-density lipoprotein cholesterol (VLDL) and low-density lipoprotein cholesterol (LDL): LDL = Total cholesterol - HDL- VLDL; where VLDL = Triglycerides/5.

Alanine transaminase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and aspartate aminotransferase (AST) parameters were determined using a BA400 biochemical and turbidimetry analyser from Spain, (Manual code TEUS00048-07-EN) for liver function.

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
Starter diet (1-21 d)						
Ingredient (%)						
Corn (%)	41.28	41.28	41.28	41.28	41.28	41.28
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
Mono 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
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 (%)	0.10	0.10	0.10	0.10	0.10	0.10
Calculated analysis						
Metabolizable Energy (MJ/kg)	13.01±0.03	12.88±0.10	12.90±0.04	13.10±0.08	12.90±0.28	13.18±0.04
Dry matter (%)	89.77± 0.23	90.77± 0.53	90.33± 0.52	91.33± 0.20	90.57± 0.72	90.43± 0.87
Crude protein (%)	23.10±0.46	23.57±0.15	23.17±0.52	22.90±0.30	23.07±0.27	23.13±0.32
Crude fibre (%)	3.40±0.15	3.40±0.06	3.47±0.09	3.13±0.09	3.23±0.37	2.97±0.27
Ether extract (%)	6.85±0.49	7.20±0.29	7.35±0.20	7.15±0.09	7.20±0.29	7.00±0.00
Ash (%)	5.90±0.10	5.67±0.20	5.77±0.23	5.80±0.10	6.00±0.17	5.80±0.10

Note: All values were expressed as mean ± standard error. Note: T1: Negative control; T2: Positive control; T3: 25 mg/kg; T4: 50 mg/kg; T5: 75 mg/kg; T6: 100 mg/kg

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
Finisher diet (22-42 d)						
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
Mono 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 (MJ/kg)	13.41±0.07	13.67±0.06	13.60±0.06	13.76±0.01	13.61±0.13	13.62±0.19
Dry matter (%)	90.30±0.00	90.20±0.59	89.57±0.43	90.43±0.59	89.97±0.67	90.43±0.30
Crude protein (%)	19.33±0.26	19.77±0.32	19.37±0.19	19.87±0.29	19.00±0.38	19.30±0.32
Crude fibre (%)	3.95±0.20	4.53±0.19	3.70±0.12	3.40±0.38	4.50±1.55	3.40±0.17
Ether extract (%)	4.67±0.20	4.70±0.00	4.57±0.13	4.70±0.00	4.67±0.33	5.00±0.17
Ash (%)	5.43±0.13	5.67±0.20	5.57±0.13	5.57±0.13	5.43±0.13	5.80±0.10

Note: All values were expressed as mean ± standard error. Note: T1: Negative control; T2: Positive control; T3: 25 mg/kg; T4: 50 mg/kg; T5: 75 mg/kg; T6: 100 mg/kg

Stress biomarkers

Using commercial enzyme-linked immunosorbent assay (ELISA) kits (QAYEE-BIO, China), the concentrations of serum amyloid A (SAA), alpha-1-acid glycoprotein (AGP), ceruloplasmin (CP), and heat shock protein 70 (HSP 70) in each blood serum sample were determined. The operating stages for all of the ELISA kits utilised 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.

Statistical analysis

Using the Statistical Analysis System (SAS, 2012), the data obtained were submitted to one-way analysis of variance (ANOVA) based on the Completely Randomized Design model. The Tukey Post-Hoc Test was used to establish whether there was a significant difference between treatment groups. $P < 0.05$ was used to determine significance.

RESULTS

Blood biochemistry

The lipid profile and liver function of broilers supplemented with *Y. schidigera* saponins on day 42 are presented in Table 3. There were significant differences ($P < 0.05$) in the cholesterol, TG, LDL, and HDL levels. Broilers fed with 100 mg/kg *Y. schidigera* saponins in T6 had the lowest levels of cholesterol, TG, and LDL, as well as the highest concentration of HDL when compared to the other treatment groups. The liver function measures showed no significant differences ($P > 0.05$) among treatment broilers.

Stress biomarkers

Table 4 shows the stress biomarkers of broilers supplemented with *Y. schidigera* saponins on day 42. There were notable changes ($P < 0.05$) found in the concentrations of SAA, AGP, corticosterone, and HSP 70 among treatments. Generally, 100 mg/kg of *Y. schidigera* saponins supplemented in T6 broilers demonstrated the lowest levels of stress biomarkers indicating a lower inflammatory reaction and stress response, which can produce a better growth performance. In contrast, there were no noticeable changes ($P > 0.05$) in the CP concentrations among treatments.

Table 3 Effect of *Y. schidigera* saponins supplementation on the blood biochemistry of broilers on day 42.

Parameters	Treatment						*P* value
	T1	T2	T3	T4	T5	T6	
Lipid profile							
Cholesterol (mmol/L)	3.58±0.02^{a}	3.73±0.07^{a}	3.50±0.03^{a}	3.51±0.10^{a}	3.54±0.13^{a}	3.08±0.03^{b}	0.0122
TG (mmol/L)	0.29±0.01^{cd}	0.37±0.01^{ab}	0.39±0.01^{a}	0.33±0.02^{bcd}	0.34±0.02^{abc}	0.27±0.01^{d}	0.0112
LDL (mmol/L)	0.47±0.01^{a}	0.30±0.00^{c}	0.36±0.00^{b}	0.40±0.02^{b}	0.31±0.02^{c}	0.27±0.01^{c}	0.0005
HDL (mmol/L)	2.42±0.14^{ab}	2.54±0.06^{a}	2.42±0.01^{ab}	2.24±0.05^{b}	2.49±0.03^{ab}	2.63±0.10^{a}	0.0118
Liver functions							
ALP (U/L)	4616.52±104.98	4447.10±288.03	4159.48±70.65	4073.01±186.04	3904.41±142.01	4176.40±169.73	0.1055
AST (U/L)	407.87±30.21	300.74±19.01	316.61±52.95	324.07±21.15	380.90±42.61	388.53±33.64	0.2911
GGT (U/L)	21.50±2.50	25.00±3.00	22.00±2.00	28.00±1.00	23.50±3.50	23.00±2.00	0.5210
ALT (U/L)	18.00±2.00	18.50±0.50	19.50±0.50	16.50±2.50	18.50±0.50	15.50±0.50	0.4237

Note: All values were expressed as mean ± SE; a, b, c, d values with superscript within row are significantly different at $P < 0.05$. TG: triglyceride; LDL: low-density lipoprotein cholesterol; HDL: high-density lipoprotein cholesterol; ALP: alkaline phosphatase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; ALT: Alanine transaminase. T1: Negative control; T2: Positive control; T3: 25 mg/kg; T4: 50 mg/kg; T5: 75 mg/kg; T6: 100 mg/kg.

Table 4 Effect of *Y. schidigera* saponins supplementation on the stress biomarkers of broilers on day 42.

Parameters	Treatment						*P* value
	T1	T2	T3	T4	T5	T6	
SAA (ng/ml)	109.85±1.47^{a}	96.97±1.39^{b}	93.90±0.53^{bc}	89.60±1.34^{cd}	98.38±2.60^{b}	87.15±1.36^{d}	<.0001
AGP (ng/ml)	680.84±11.94^{a}	643.75±9.78^{ab}	673.96±16.19^{a}	648.82±11.76^{ab}	645.08±11.81^{ab}	610.90±17.70^{b}	0.0166
CP (µg/ml)	260.38±13.81	261.96±20.21	271.55±12.61	268.95±17.16	253.24±15.62	238.18±16.16	0.7592
Corticosterone (ng/ml)	36.75±2.68^{ab}	31.46±7.52^{b}	51.65±6.04^{a}	36.70±2.75^{ab}	25.15±7.88^{b}	20.10±3.36^{b}	0.0099
HSP 70 (pg/ml)	5791.75±19.59^{a}	5274.67±23.24^{d}	5495.67±20.41^{b}	5362.75±19.36^{c}	5453.92±13.44^{b}	4897.05±12.58^{e}	<.0001

Note: All values were expressed as mean ± SE; a, b, c, d values with superscript within row are significantly different at $P < 0.05$. SAA: Serum Amyloid A; AGP: Alpha-1-acid glycoprotein; CP: Ceruloplasmin; HSP 70: Heat shock protein 70. T1: Negative control; T2: Positive control; T3: 25 mg/kg; T4: 50 mg/kg; T5: 75 mg/kg; T6: 100 mg/kg.

DISCUSSION

Blood biochemistry

T6 broilers treated with 100 mg/kg *Y. schidigera* saponins in the current study showed a substantial decrease in blood cholesterol, TG, and LDL while increasing HDL levels. The drop in those lipid profiles might be ascribed to saponins' cholesterol-lowering effect, which inhibits pancreatic cholesterol esterase, bile acid binding, and lowers cholesterol solubility in micelles, potentially delaying cholesterol absorption in the gut. (Ngamukote et al., 2011). Besides, the reduction in total lipid and cholesterol levels could be incurred by a decrease in the activity of hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase, which is required for cholesterol production in the liver. (Ariana et al., 2011). In accord, the serum levels of total cholesterol, TG, and LDL-cholesterol were significantly decreased in broiler breeders supplemented with 100, 150, and 200 ppm of dietary soapnut (Sapindus mukorossi) shell powder high in saponins (Chaudhary et al., 2018; Bera et al., 2019). On the other hand, Afrose et al. (2010) reported a reduction of 23% blood serum cholesterol and egg yolk cholesterol in laying hens supplemented with 75 mg/kg karaya saponins.

Conversely, supplementation with different levels of Y. shidigera saponins increases HDL levels in the present study. HDL is a lipoprotein that transports lipids from the peripheral to the liver. (Marques et al., 2018). The increase could be related to the properties of saponins, which inhibit cholesterol synthesis via reductase activity and increases bile salt excretion by changing cholesterol to bile salts (Alghirani et al., 2022). As a result, the HDL levels were unregulated, but TG and cholesterol levels were decreased (Ghaedi et al., 2014). The production of micelles inhibits the absorption of cholesterol and bile salts (Haryanto et al., 2016). Marques et al. (2018) further explained that HDL promotes the release of cholesterol from peripheral tissues and transports cholesterol to the liver for catabolism. As further explained by Marques et al. (2018), HDL enhances the release of cholesterol from peripheral tissues and delivers cholesterol to the liver for catabolic activity.

The liver, on the other hand, is one of the biggest and most important organs in animals, playing a vital role in the metabolism, detoxification, and removal of endogenous as well as foreign substances (Paul et al., 2016). Herbs have been reported to have positive effects on serum biochemical attributes as well as liver function regulation (Basit et al., 2020). Despite supplementing with Y. shidigera saponins, no significant alterations were observed in this study indicating that the different concentrations of saponins added were safe and had no deleterious effects on broilers. In accordance, Bera et al. (2019) observed that supplementing 100, 150, and 200 mg/kg of soapnut shell powder to broiler diets as a cheap source of saponins did not influence serum AST or ALT levels, indicating that dietary saponins have no negative effect on liver function. In addition, the serum ALB, ALT, and GGT levels of Ross 308 male broiler chicks supplemented with red ginseng root powder containing saponins at concentrations of 0, 75, 150, and 225 mg/kg also showed no significant changes (He et al., 2021).

Stress biomarkers

The functions of the APP include transport proteins, protease inhibitors, enzymes, coagulation proteins, and modulators of the immune response (Zulkifli et al., 2018; Chung et al., 2019). APPs are utilised as biomarkers in veterinary diagnostics for inflammatory disease monitoring, responding to antibiotic or steroid treatment, and animal production. (Gruys et al., 2005). A key vertebrate APP and the most sensitive protein of the acute phase response in most species (APR) is the SAA. SAA's primary role during an APR is to modulate lipoprotein transport and metabolism. (Humam et al., 2021). During an APR, SAA protects against oxidative tissue damage and can attract immune cells to localised sites of inflammation. On top of that, it is also immunomodulatory, suppressing pyrexia and downregulating pro-inflammatory processes throughout an APR. (Zulkifli et al., 2018). T6 broilers treated with 100 mg/kg of *Y. schidigera* saponins in the current study had lower serum SAA concentration on day 42 than other treatments. Again, saponins' anti-inflammatory and anti-microbial abilities might explain the decrease. Supporting this idea, Abo Ghanima et al. (2021) reported that supplementing 300 mg/kg of tea tree extract (rich in saponins) to the broiler diets improved their growth performance and reduced both serum and liver SAA concentrations.

AGP on the other hand is another major APP in poultry, which is more sensitive to stressful stimuli (Nazifi et al., 2010). Besides its use for measuring poultry well-being, AGP has an important function in homeostasis maintenance by reducing tissue damage caused by the inflammatory response in extrahepatic cells, (Nazifi et al., 2010). Similarly, T6 broilers had a reduced AGP concentration in their serum on day 42. In general, this decrease in AGP level could again be attributed to saponins' anti-inflammatory effects. An earlier study demonstrated that alfalfa, which contains high levels of saponins and is used as a molt diet for hens, was also found to reduce serum AGP levels, suggesting that saponins may reduce stress and inflammation (Dunkley et al., 2007). Additionally, AGP levels were likewise lowered in chicken provided with varying amounts of Macleaya cordata extract at 25, 50, and 100 mg/L, according to Khadem et al. (2014). Besides, the use of dietary supplementation with Allium hookeri at 1% in broilers' diets during immunological stress resulted in lower AGP concentration further supporting the beneficial effects of saponins (Lee et al., 2017).

In the meantime, corticosterone is the main hormone that is related to stress in avian species and has been commonly used to monitor physiological responses to stressors (Najafi et al., 2015). T6 broilers fed with *Y. schidigera* saponins at 100 mg/kg showed a lower concentration of serum corticosterone on day 42. Since it has been established that feeding saponins to broilers have hypocholesterolemic effects, there is a link between serum cholesterol and serum corticosterone. Serum cholesterol is considered a precursor of serum corticosterone, the reduction in serum corticosterone levels could perhaps be linked to the decline in serum cholesterol level. Consequently, the reduction in serum cholesterol level following saponins supplementation may be a reason for the reducing serum corticosterone level (Rokade et al., 2016). A previous saponins study found that supplementing broiler breeders' diet with soapnut shell powder (which contains a high level of saponins) at 150 ppm led to a decline in serum corticosterone levels (Chaudhary et al., 2018).

HSP are examples of stress protein biomarkers, which are a category of proteins found in all cells of all living forms but produced at large quantities when a host is subjected to high or low temperatures or other stressors (Figueiredo et al., 2007). HSP 70 is considered one of the most important protein families and it is a major HSP, so it has been experimented with comprehensively in poultry (Ming et al., 2010). One of HSP 70 most significant purposes is to safeguard cells from the destructive effects of heat while playing a significant part in the oxidative stress response. Typically, as a response in heat-stressed broilers, HSP 70 levels increase in the liver, lungs heart, plus brain, which plays a cytoprotective role in the gastrointestinal tract after injury or stress (Hajati et al., 2015). Since those broilers were raised in an open-sided house in the present study, HSP 70 was included as one of the parameters to determine the influence of saponins on this biomarker under tropical conditions. T6 broilers supplemented with the greatest dose of saponins displayed a lower concentration of serum HSP 70 at the end of the study. The saponins' ability to scavenge radicals, metal chelation, and synergize with other antioxidants may be responsible for the lower HSP 70 concentration (Hajati et al., 2015). The finding was consistent with a study that reported supplementing broilers' diets with ginseng extract, which has a high level of saponins at 135 mg/L, reduced HSP 70 level during heat stress conditions (Sandner et al., 2020).

CONCLUSIONS

In summary, Y. shidigera saponins play a major role in anti-inflammatory, antimicrobial, antioxidant activity, free-radical scavenging properties, and immunological enhancement, all of which contribute to better blood biochemistry and biomarkers findings in broilers. T6 broilers treated with 100 mg/kg of Y. shidigera saponins had the lowest levels of cholesterol, TG, and LDL concentrations, while having the highest HDL level without affecting the liver parameters. Moreover, the SAA, AGP, corticosterone, and HSP 70 concentrations were also the lowest in comparison to the other treatments. The present study conclusively found that 100 mg of *Y. schidigera* saponins per kg of boilers diet can be inferred to be a safe and beneficial additive for improving growth performance in commercial broilers under tropical conditions.

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AUTHOR CONTRIBUTIONS

ELTC, FFAJ, AQS, and TCL postulated the experimental design. MMA, NAK, and YLO performed work associated with this study. MMA and ELTC performed the statistical analysis and prepared the manuscript. All authors reviewed the manuscript upon submission.

REFERENCES

Abo Ghanima, M.M., Swelum, A.A., Shukry, M., Ibrahim, S.A., Abd El-Hack, M.E., Khafaga, A.F., Alhimaidi, A.R., Ammari, A.A., El-Tarabily, K.A., Younis, M.E.M., 2021. Impacts of tea tree or lemongrass essential oils supplementation on growth, immunity, carcass traits, and blood biochemical parameters of broilers reared under different stocking densities. Poult Sci. 100(11), 101443.

Afrose, S., Hossain, M.S., Tsujii, H., 2010. Effect of dietary karaya saponin on serum and egg yolk cholesterol in laying hens. Br. Poult. Sci. 51, 797-804.

Alghirani, M.M., Chung, E.L.T., Jesse, F.F.A., Sazili, A.Q., Loh, T.C., 2022. The potential use of plant-derived saponins as a phytobiotic additive in poultry feed for production and health advancement: a comprehensive review. Poult. Sci. J. 10, 215-226.

Alghirani, M.M., Chung, E.L.T., Jesse, F.F.A., Sazili, A.Q., Loh, T.C., 2021b. Could phytobiotics replace antibiotics as feed additives to stimulate production performance and health status in poultry? An overview. J. Adv. Vet. Res. 11, 254-265.

Alghirani, M.M., Chung, E.L.T., Sabri, D.S.M., Tahir, M., Kassim, N.A., Kamalludin, M.H., Nayan, N., Jesse, F.F.A., Sazili, A.Q., Loh, T.C., 2021a. Can Yucca schidigera be used to enhance the growth performance, nutrient digestibility, gut histomorphology, cecal microflora, carcass characteristic, and meat quality of commercial broilers raised under tropical conditions?. Animals (Basel). 11(8), 2276.

Ariana, M., Samie, A., Edriss, M.A., Jahanian, R., 2011. Effects of powder and extract form of green tea and marigold, and-tocopheryl acetate on performance, egg quality and egg yolk cholesterol levels of laying hens in late phase of production. J. Med. Plant. Res. 5, 2710-2716.

Basit, M.A., Kadir, A.A., Loh, T.C., Abdul Aziz, S., Salleh, A., Zakaria, Z.A., Banke Idris, S., 2020. Comparative efficacy of selected phytobiotics with halquinol and tetracycline on gut morphology, ileal digestibility, cecal microbiota composition and growth performance in broiler chickens. Animals (Basel). 10(11), 2150.

Bera, I., Tyagi, P.K., Mir, N.A., Tyagi, P.K., Dev, K., Sharma, D., Mandal, A.B., 2019. Dietary supplementation of saponins to improve the quality and oxidative stability of broiler chicken meat. J. Food Sci. Technol. 56, 2063-2072.

Boripun, R., Paopradit, P., Prampramote, J., Narinthorn, R., Intongead, S., Sangkanu, S., Narongrit, T., Mitsuwan, W., 2022. Bactericidal activity of Piper betle L. extract against antibiotic resistant Salmonella spp. isolated from pig farms in Southern Thailand. Vet. Integr. Sci. 20, 557-569.

Chaudhary, S.K., Bhar, R., Mandal, A.B., Rokade, J.J., Jadhav, S.E., Kannan, A., Gopi, M., 2018. Effect of dietary soapnut (Sapindus mukorossi) shell powder on selected stress indices, lipid profile and litter quality in broiler breeders. Anim. Nutr. Feed Technol. 18, 311-320.

Chung, E.L.T., Alghirani, M.M., Kamalludin, M.H., Nayan, N., Jesse, F.F.A., Wei, O.T.A., Stephen, M.A.F.M.H., Reduan, M.F.H., Loh, T.C., 2021. Do different vaccination regimes affect the growth performance, immune status, carcase characteristics and meat quality of broilers?. Br. Poult. Sci. 62(1), 32-37.

Chung, E.L.T., Jesse, F.F.A., Marza, A.D., Ibrahim, H.H., Abba, Y., Zamri-Saad, M., Haron, A.W., Lila, M.A.M., Saharee, A.A., Omar, A.R., Bakar, M.Z.A., Norsidin, M.J., 2019. Responses of pro-inflammatory cytokines, acute phase proteins and cytological analysis in serum and cerebrospinal fluid during haemorrhagic septicaemia infection in buffaloes. Trop. Anim. Health Prod. 51, 1773-1782.

Dunkley, C.S., McReynolds, J.L., Dunkley, K.D., Kubena, L.F., Nisbet, D.J., Ricke, S.C., 2007. Molting in Salmonella Enteritidis-challenged laying hens fed alfalfa crumbles. III. Blood plasma metabolite response. Poult. Sci. 86, 2492-2501.

Figueiredo, D., Gertler, A., Cabello, G., Decuypere, E., Buyse, J., Dridi, S., 2007. Leptin downregulates heat shock protein-70 (HSP-70) gene expression in chicken liver and hypothalamus. Cell Tissue Res. 329, 91-101.

Ghaedi, M., Zeinali, N., Ghaedi, A.M., Teimuori, M., Tashkhourian, J., 2014. Artificial neural network-genetic algorithm-based optimization for the adsorption of methylene blue and brilliant green from aqueous solution by graphite oxide nanoparticle. Spectrochim. Acta A: Mol. Biomol. Spectrosc. 125, 264-277.

Gilani, S.M.H., Zehra, S., Galani, S., Ashraf, A., 2018. Effect of natural growth promoters on immunity, and biochemical and haematological parameters of broiler chickens. Trop. J. Pharm. Res. 17, 627-633.

Greene, E.S., Emami, N.K., Dridi, S., 2021. Research Note: Phytobiotics modulate the expression profile of circulating inflammasome and cyto (chemo) kine in whole blood of broilers exposed to cyclic heat stress. Poult. Sci. 100, 100801.

Gruys, E., Toussaint, M.J., Upragarin, N., Ederen, V.A.M., Adewuyi, A.A., Candiani, D., Nguyen, T.K.A., Sabeckiene, J., 2005. Acute phase reactants, challenge in the near future of animal production and veterinary medicine. J. Zhejiang Univ. Sci. B. 6(10), 941-947.

Hajati, H., Hassanabadi, A., Golian, A., Nassiri-Moghaddam, H., Nassiri, M.R., 2015. The effect of grape seed extract and vitamin C feed supplementation on some blood parameters and HSP70 gene expression of broiler chickens suffering from chronic heat stress. Ital. J. Anim. Sci. 14, 3273.

Haryanto, A., Miharja, K., Vijayanti, N., 2016. Ratio and Blood Lipid Profile of Broiler Chickens. Int. J. Poult. Sci. 15, 27-34.

Hashemi, S.R., Davoodi, H., 2011. Herbal plants and their derivatives as growth and health promoters in animal nutrition. Vet. Res. Commun. 35, 169-180.

He, G., Zhao, L., Shishir, M.S.R., Yang, Y., Li, Q., Cheng, L., Guo, A., 2021. Influence of alfalfa meal, as a source of dietary fibre, on growth performance, development, pH of gastrointestinal tract, blood biochemical profile, and meat quality of broilers. J. Appl. Anim. Res. 49, 431-439.

Humam, A.M., Loh, T.C., Foo, H.L., Izuddin, W.I., Zulkifli, I., Samsudin, A.A., Mustapha, N.M., 2021. Supplementation of postbiotic RI11 improves antioxidant enzyme activity, upregulated gut barrier genes, and reduced cytokine, acute phase protein, and heat shock protein 70 gene expression levels in heat-stressed broilers. Poult. Sci. 100, 100908.

Imik, H., Ozlu, H., Gumus, R., Atasever, M.A., Urcar, S., Atasever, M., 2012. Effects of ascorbic acid and α-lipoic acid on performance and meat quality of broilers subjected to heat stress. Br. Poult. Sci. 53(6), 800-808.

Janmohammadi, A., Sheikhi, N., Nazarpak, H.H., Brujeni, N.G., 2020. Effects of vaccination on acute-phase protein response in broiler chicken. Plos one 15, e0229009.

Khadem, A., Soler, L., Everaert, N., Niewold, T.A., 2014. Growth promotion in broilers by both oxytetracycline and Macleaya cordata extract is based on their anti-inflammatory properties. Br. J. Nutr. 112, 1110-1118.

Lee, Y., Lee, S.H., Gadde, U.D., Oh, S.T., Lee, S.J., Lillehoj. H.S., 2017. Dietary Allium hookeri reduces inflammatory response and increases expression of intestinal tight junction proteins in LPS-induced young broiler chicken. Res. Vet. Sci. 112, 149-155.

Marques, L.R., Diniz, T.A., Antunes, B.M., Rossi, F.E., Caperuto, E.C., Lira, F.S., Goncalves, D.C., 2018. Reverse cholesterol transport: molecular mechanisms and the non-medical approach to enhance HDL cholesterol. Front. Physiol. 9, 526.

Matsuura, H., 2001. Saponins in garlic as modifiers of the risk of cardiovascular disease. Nutr. J. 131, 1000-1005.

Ming, J., Xie, J., Xu, P., Liu, W., Ge, X., Liu, B., He, Y., Cheng, Y., Zhou, Q., Pan, L., 2010. Molecular cloning and expression of two HSP70 genes in the Wuchang bream (Megalobrama amblycephala Yih). Fish Shellfish Immunol. 28, 407-418.

Najafi, P., Zulkifli, I., Jajuli, A.N., Farjam, A.S., Ramiah, S.K., Amir, A.A., O'Reilly, E.L., Eckersall, D., 2015. Environmental temperature and stocking density effects on acute phase proteins, heat shock protein 70, circulating corticosterone and performance in broiler chickens. Int. J. Biometeorol. 59, 1577-1583.

Nazifi, S., Dadras, H., Hoseinian, S.A., Ansari-Lari, M., Masoudian, M., 2010. Measuring acute phase proteins (haptoglobin, ceruloplasmin, serum amyloid A, and fibrinogen) in healthy and infectious bursal disease virus-infected chicks. Comp. Clin. Path. 19, 283-286.

Ngamukote, S., Makynen, K., Thilawech, T., Adisakwattana, S., 2011. Cholesterol-lowering activity of the major polyphenols in grape seed. Molecules. 16, 5054-5061.

Norkeaw, R., Arjin, C., Sartsook, A., Hnokeaw, P., Thongkham, M., Detruengsri, B., Chaiwang, N., Mekchay, S., Yano, T., Sringarm, K., 2022. Effect of dietary green tea extract supplementation in growing-finishing pigs on growth performance, meat quality, and oxidative stability of pork. Vet. Integr. Sci. 20, 571-583.

Paul, S., Islam, M., Tanvir, E.M., Ahmed, R., Das, S., Rumpa, N.E., Hossen, M.S., Parvez, M., Gan, S.H., Khalil, M., 2016. Satkara (Citrus macroptera) fruit protects against acetaminophen-induced hepatorenal toxicity in rats. Evid. Based Complement Alternat. Med. 2016, 9470954.

Reyno, S.R.T., Gonzaga, L.J.A., Gonzaga, E.A., 2022. Anthelmintic efficacy of mango seed extract on porcine gastrointestinal nematodes. Vet. Integr. Sci. 20, 507-515.

Rokade, J.J., Bhanja, S.K., Shinde, A.S., Sajad, S., Kapgate, M., Mandal, A.B., 2016. Evaluation of mannan-oligosaccharides (MOS) in broiler chicken during hot humid summer using zoo technical, molecular and physio-biochemical tools. Indian J. Anim. Sci. 86(4), 460-467.

Sandner, G., Mueller, A.S., Zhou, X., Stadlbauer, V., Schwarzinger, B., Schwarzinger, C., Wenzel, U., Maenner, K., Klis, J.D.V., Hirtenlehner, S., Aumiller, T., Weghuber, J. 2020. Ginseng extract ameliorates the negative physiological effects of heat stress by supporting heat shock response and improving intestinal barrier integrity: Evidence from studies with heat-stressed Caco-2 cells, C. elegans and growing broilers. Molecules. 25, 835.

Strimbu, K., Tavel, J.A., 2010. What are biomarkers?. Curr. Opin. HIV. AIDS. 5(6), 463-466. Tokofai, B.M., Idoh, K., Oke, O.E., Agbonon, A., 2021. Hepatoprotective effects of Vernonia amygdalina (Astereaceae) extract on CCI(4)-induced liver injury in broiler chickens. Animals (Basel). 11(12), 3371.

Zulkifli, I., Akmal, A.F., Soleimani, A.F., Hossain, M.A., Awad, E.A., 2018. Effects of low-protein diets on acute phase proteins and heat shock protein 70 responses, and growth performance in broiler chickens under heat stress condition. Poult. Sci. 97(4), 1306-1314.

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