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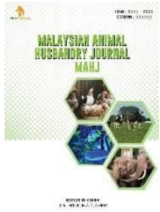

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RESEARCH ARTICLE

SUPPLEMENTATION *OF FICUS THONNINGII* AND VITAMIN C: EFFECTS ON ANTIOXIDANT STATUS, LEPTIN, T3, INSULIN, AND HSP 70 IN BROILER CHICKENS

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ABSTRACT

The effect of supplementing two strains of broiler chickens under tropical settings with *Ficus thonningii* leaf powder (FTLP) and vitamin C on brain and meat oxidative enzymes as well as serum hormonal biomarkers is examined in this study. Three hundred thirty-six one-day-old chicks (168 Cobb 500 and 168 Arbor Acres) in total, weighing 37.40±0.45g, were split into eight groups and given seven duplicates of each experimental food (6 birds per replicate; 42 birds per treatment). Vitamin C (200 mg/kg of basal diet) was added to diets 3 and 4, whereas diets 1 and 2 functioned as controls without supplementation. Brain catalase and glutathione peroxidase levels were raised by vitamin C and FTLP treatment; the Cobb 500 strain showed greater values. Furthermore, meat glutathione peroxidase and catalase were increased, and meat lipid oxidation was decreased by vitamin C and FTLP administration. Overall, the antioxidant capacity of the brain and the quality of the meat in grill chickens were enhanced by dietary supplementation with 200 mg/kg of vitamin C and 1% FTLP. The levels of T3, insulin, and leptin were considerably ($P<0.05$) impacted by the combined administration of 1% FTLP and vitamin C, whereas the level of HSP 70 was dramatically ($P<0.05$) decreased. These results demonstrate how supplementing with both *Ficus thonningii* and vitamin C can strengthen antioxidant defenses and lessen oxidative stress in systems used in the production of broilers. As a result, these supplements taken together could have complementary effects.

KEYWORDS

Brain catalase, antioxidant defenses, endocrine functions, Heat Shock Protein, oxidative stress

1. INTRODUCTION

Recent years have seen a significant amount of study on dietary supplements aimed at enhancing livestock health and productivity, particularly with regard to the diets of broiler chickens (Osowe et al., 2022). African fig, or *Ficus thonningii*, and vitamin C are two of the many natural additions that have been the subject of much research. When combined, they provide a thorough approach to improving broiler production systems' physiological resilience, nutrient utilization, and antioxidant status (Osowe et al., 2023). Particularly in fast-growing broiler chickens maintained under high stress circumstances, antioxidants are crucial for maintaining cellular integrity and minimizing damage brought on by oxidative stress (Surai et al., 2019).

With a strong antioxidant profile, *Ficus thonningii* is a prospective food source of phytochemicals such carotenoids, flavonoids, and polyphenols that have the ability to chelate metals and scavenge free radicals (Muhammad and Oluwaniyi, 2022). Comparably, vitamin C, a well-known water-soluble antioxidant, enhances cellular antioxidant defenses and regenerates additional antioxidants to complement these effects (Liu et al., 2020). Numerous research have shown how supplementing with vitamin C and *Ficus thonningii* can improve the antioxidant status of broiler chickens. It has been demonstrated that adding *Ficus thonningii* extract to the diet increases the activity of antioxidant enzymes including catalase (CAT) and glutathione (GSH) while lowering the levels of lipid peroxidation in broiler tissues (Ijoma et al., 2023).

Similarly, in broiler chicks exposed to heat stress and high stocking densities, vitamin C supplementation has been linked to increased plasma antioxidant capacity and decreased oxidative stress indicators (Hieu et al., 2022). The body uses leptin to control how much food is consumed and how much energy is expended. It does this mainly by acting as a marker of the central nervous system's long-term energy reserves (Flier and Maratos-Flier, 2017). In chickens, the thyroid hormone triiodothyronine (T3) is essential for controlling growth, nutritional utilization, and metabolic rate (Wasti et al., 2020). *Ficus thonningii* phytochemicals have been demonstrated to have regulatory effects on T3 receptor expression and thyroid hormone metabolism, which may have an impact on broiler chickens' metabolic rate and energy use (Dangarembizi et al., 2014).

Vitamin C has also been shown to influence the synthesis and secretion of thyroid hormones; research has shown that it helps lambs produce more T3 and express thyroid hormone receptors (Omidi et al., 2015). In broiler chickens, insulin is a major regulator of glucose metabolism and is essential for energy balance and food partitioning (Rahman et al., 2021). Insulin signaling pathway dysregulation might hinder the body's ability to utilize nutrients, making birds more vulnerable to metabolic diseases like diabetes and obesity (Wen et al., 2022). Furthermore, a major obstacle to contemporary broiler production is heat stress, which impairs performance by upsetting metabolic homeostasis (Nawaz et al., 2021). *Ficus thonningii Phytochemical* have insulin-sensitizing qualities that improve the absorption of glucose and insulin signaling in the tissues of broilers. thereby improving metabolic efficiency and reducing the risk of metabolic disorders (Muhammad and Oluwaniyi, 2022).

Additionally, it has been demonstrated that vitamin C supplementation can lessen the effects of heat stress on insulin sensitivity and glucose metabolism in broiler chickens, in part because of its anti-inflammatory

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and antioxidant properties (Attia et al., 2022). Furthermore, studies have shown that heat shock protein 70 (HSP70), a molecular chaperone, is upregulated in response to dietary supplementation with *Ficus thonningii and vitamin C*, and plays a critical role in cellular stress response and thermotolerance in chicken (Dangarembizi et al., 2014). In order to clarify any potential synergistic effects and provide practical implications for poultry nutrition, this paper will investigate the effects of *Ficus thonningii* and vitamin C supplementation on antioxidant status, leptin, triiodothyronine (T3), insulin, and heat shock protein 70 (HSP70) expression in two different strains of broiler chickens.

2. MATERIALS AND METHODS

2.1 Ethical Approval: Collection, Processing, and Analysis of Phytogens

The Research and Ethics Committee of the Department of Animal Production and Health, Federal University of Technology, Akure, Nigeria, approved the animal and animal protocol requirements (FUTA/APH/2023/12). The techniques for collecting *Ficus thonningii* leaves, processing them into *Ficus thonningii* Leaf Powder (FTLP), and analyzing FTLP for phytochemical content, proximate components, antioxidants, and mineral composition were described and published by Osowe *et al*. (2021).

2.2 Management and Arrangement of Experimental Birds

The feeding trial took place in February and March 2021 at the Federal University of Technology's Teaching and Research Farm in Akure, Nigeria. Based on the description provided by Jimoh *et al*. (2017), the thermo-hygrometer was used to construct the temperature-humidity index (THI). THI = Ta − [(0.31 − 0.31 × RH) (Ta − 14.4)] was the formula that was applied, where Ta stood for ambient temperature and RH for relative humidity/100. THI categorizes heat stress into four categories: mild (< 27.8 °C), moderate (27.8–28.9 °C), severe (28.9–30 °C), and very severe (> 30 °C). The THI was determined to be 28.57, with the average temperature and relative humidity being 29.79 °C and 74.5%, respectively.

A 2 × 2 × 2 Factorial arrangement with two strains of broiler chickens: Cobb 500 (CO) and Arbor Acres (AB), two doses of vitamin C (0 and 200 mg/kg), and two levels of FTLP (0 and 1%) comprised the completely randomized experimental design. For the starter and finisher phases of broiler chicken rearing, a baseline diet was developed and divided into eight equal pieces (Table 1). The appropriate supplements were added to each meal. Vitamin C (200 mg/kg of basal diet) was supplemented in diets 3 and 4, but not in diets 1 or 2. Diets 7 and 8 combined 1g FTLP/kg of basal diet with 200 mg of vitamin C, while diets 5 and 6 got 1g FTLP/kg of supplementation each. 336 day-old chicks (168 CO and 168 AB breeds) weighing 37.40±0.45g at a day old were divided into 8 experimental feeding groups, each consisting of 7 duplicates (42 birds per treatment; 6 birds per replicate).

Table 1: Composition and nutrient contents of experimental basal diets

Ingredients (%)	**Starter (1 to 21 days)**	**Finisher (22 to 42 days)**
Maize	50.20	52.70
Wheat offal	1.50	8.00
Soybean meal	35.60	28.90
Fish meal	3.50	3.00
Vegetable oil	3.50	4.00
Bone meal	1.60	1.40
Dicalcium phosphate	1.10	1.00
Premix	0.50	0.50
Methionine	0.20	0.20
Lysine	0.10	0.10
Salt	0.20	0.20
Total	100	100
Calculated Nutrients (% DM)		
Metabolizable energy (kcal/kg)	3139.69	3170.93
Crude protein	23.00	20.00
Calcium	1.23	1.12
Available Phosphorus	0.59	0.56
Methionine	0.56	0.53
Lysine	1.35	1.22

Diets 1, 3, 5, and 7 were assigned to Arbor Acre, and diets 2, 4, 6, and 8 were assigned to CO. The birds were kept in 2 m × 2 m experimental pens filled with 3 cm of dry wood shavings. Management techniques like sufficient stocking density, appropriate pen ventilation, and upkeep of dry litter by avoiding water spillage were implemented to avoid the build-up of litter moisture and ammonia levels. For the first week, the temperature of the experimental home was kept at 31±2°C; for the next two weeks, it was lowered by 2°C, and for the remaining weeks of the raising period (week four to six), the birds were reared at the natural ambient temperature. Throughout the first day of rearing, lighting was available for 24 hours, followed by 23 hours on days 1 through 7, and 18 hours throughout the remaining days. For the six weeks of the trial, all birds were fed ad-libitum.

2.3 Total Protein and Antioxidant Enzymes of the Brain and Lipid Oxidation and Antioxidant Enzyme Levels of Meat and Brain

Following slaughter, seven birds—one bird per treatment group—were chosen for analysis (1 bird per replication) in order to measure the levels of cholesterol, antioxidant enzymes, and meat lipid oxidation as well as brain total protein. Using a high-speed homogenizer, the whole brains of broiler chickens were removed and homogenized in cold saline 0.9% in a 1:10 (w/v) ratio. After centrifuging the homogenate samples for 20 minutes at 2000 revolutions per minute, they were divided into 1.0 ml aliquots and stored at -18 °C until needed. The total protein was calculated using the UV technique (Zaia et al., 2000). Additionally, the activities of glutathione peroxidase and catalase were measured (Khan et al., 2012).

After dressing, a sample of the birds' breast flesh was removed, placed in an oxygen-permeable bag aerobically, and frozen for 20 days at -18 °C. The amount of lipid oxidation in the meat was measured using the thiobarbituric acid (TBA) assay technique (Tokur et al., 2006). The activities of catalase and glutathione peroxidase were also measured (Muhlisin et al., 2016; Cichoski et al., 2012). According to de Almeida et al. (2006), commercial kits (Asan Pharm. Co., Ltd., Seoul) were used to quantify the cholesterol levels in meat using spectrophotometry.

2.4 Serum Hormonal Markers Determination

After being drawn, whole blood samples were left to clot for 15 to 30 minutes at room temperature. In a chilled centrifuge, the clot was extracted by centrifuging at 1,000–2000 x g for ten minutes. The blood serum that resulted from this process was called supernatant, and it was frozen at -20°C prior to serum hormonal tests. Using commercially available Enzyme-linked Immunosorbent Assay (ELISA) kits, the levels of serum triiodothyronine (T3), insulin, leptin, and heat shock protein 70 were examined. The Triiodothyronine (T3) ELISA kit T3225T was used to analyze the concentration of serum T3, the Insulin ELISA kit (Cat. No. IS130D) was used to determine insulin, the Rat Leptin ELISA kit (Cat. No.

E0561 Ra) was used to determine leptin, and the Rat Heat Shock Protein 70 ELISA kit (Cat. No. E0522 Ra) was used to measure HSP 70.

2.5 Statistical Data Evaluation

SPSS version 20 was used to do the statistical analysis. Using the general linear model (Yijk = + Fi + Vj + ST + eijk), data were analyzed in a 2x 2x 2 factorial arrangement with 2 levels of FTLP, 2 levels of vitamin C, and 2 strains of broiler chickens. The variables included = mean, Fi = effect of FTLP, Vj = effect of vitamin C, ST = effect of strain, and eijk = random error. Duncan's multiple range analysis was employed at a significance level of P < 0.05 to evaluate the variations in the diets' means.

3. Results

3.1 Brain Antioxidant Status

Table 2 shows the brain antioxidant level for the two breeds of broiler chickens fed meals supplemented with vitamin C and FTLP. Birds fed diets 7 and 8 had brain glutathione and catalase levels that were greater and statistically comparable to those found in the birds fed the other diets. In comparison to the AB strain, the CO strain exhibited considerably greater (P<0.05) levels of brain glutathione and catalase. While the interactions between vitamin C and FTLP were not significant, their inclusion was significant (P<0.05).

Table 2: Brain antioxidant status of two breeds of broiler chickens fed *Ficus thonningii* leaf powder and vitamin C supplemented diets

Diet	BRD	VC mg/kg	FTLP %	BTP (mg/g)	BCAT (Ku)	BGSH (µmol/mg)
1	AB	0	0	0.63	27.35^{d}	24.40^{f}
2	CO	0	0	0.54	41.21^{c}	43.10^{e}
3	AB	200	0	0.64	47.68^{bc}	45.63^{de}
4	CO	200	0	0.65	53.77^{b}	54.76^{cd}
5	AB	0	1	0.63	52.11^{b}	57.78^{bc}
6	CO	0	1	0.58	65.02^{a}	66.37^{ab}
7	AB	200	1	0.65	68.52^{a}	72.52^{a}
8	CO	200	1	0.57	73.07^{a}	76.60^{a}
SEM				0.01	3.07	3.52
P-value				0.65	0.01	0.01
	AB			0.64	48.92^{b}	50.08^{b}
	CO			0.58	58.27^{a}	60.21^{a}
	SEM			0.02	1.42	1.79
	P-value			0.13	0.01	0.01
		0		0.59	46.42^{b}	47.91^{b}
		200		0.62	60.76^{a}	62.38^{a}
		SEM		0.02	1.42	1.79
		P value		0.38	0.01	0.01
			0	0.61	42.50^{b}	41.97^{b}
			1	0.60	64.68^{a}	68.32^{a}
			SEM	0.02	1.42	1.79
			P value	0.84	0.01	0.01
	Interactions *P-value*					
	BRD x VC			0.62	0.06	0.18
	BRD x FTLP			0.72	0.76	0.15
	VC x FTLP			0.40	0.31	0.44
	BRD x VC x FTLP			0.41	0.94	0.62

Means with different superscripts in the same column are significantly (P<0.05) different; BRD: Breeds; VC: Vitamin C; FTLP: *Ficus thonningii leaf powder*; BTP: Brain Total Protein; BCAT: Brain Catalase; BGSH: Brain Glutathione; AB: Arbor acres; CO: Cobb 500; SEM: Standard error of the means.

3.2 Meat Antioxidative Status

Table 3 shows the meat antioxidant status of the two breeds of grill chickens fed diets supplemented with vitamin C and FTLP. Meat glutathione, meat catalase, and lipid oxidation were significant (P<0.05). The control diet 1 had considerably higher (P<0.05) levels of lipid oxidation than the other diets, according to the data. Meat glutathione levels showed that, in comparison to the other diets, diets 5 and 6 had significantly greater amounts (P<0.05). The levels of meat catalase in diets 3, 4, 5, 6, 7, and 8 were considerably greater (P<0.05) than in diets 1 and 2, but they were not significantly different from one another (P>0.05). The interaction between vitamin C and FTLP was significant (P<0.05) for lipid oxidation and glutathione, while the interactive effect of strain, vitamin C, and FTLP was significant for lipid oxidation.

Table 3: Meat antioxidant status of two breeds of broiler chickens fed *Ficus thonningii* leaf powder and vitamin C supplemented diets

Diet	BRD	VC mg/kg	FTLP %	LPDOX (mgMDA/g)	CHOL (mg/dl)	GSH (µmol/mg)	CATLS (Ku)
1	AB	0	0	5.03^{a}	121.55	28.30^{b}	34.75^{b}
2	CO	0	0	4.09^{b}	115.01	29.78^{b}	38.71^{b}
3	AB	200	0	3.26^{bc}	118.63	49.25^{a}	53.22^{a}
4	CO	200	0	3.08^{cd}	114.22	52.67^{a}	53.71^{a}
5	AB	0	1	2.15^{e}	111.61	45.36^{a}	52.37^{a}
6	CO	0	1	2.67^{cde}	110.73	53.20^{a}	55.53^{a}
7	AB	200	1	3.14^{cd}	117.56	54.20^{a}	55.60^{a}
8	CO	200	1	2.30^{de}	115.13	55.30^{a}	50.02^{a}
SEM				0.20	4.07	2.47	2.33
P-value				0.01	0.99	0.01	0.09
	AB			3.39	117.34	44.28^{b}	48.99^{b}
	CO			3.04	113.77	47.74^{a}	51.05^{a}

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Table 3 (Cont.): Meat antioxidant status of two breeds of broiler chickens fed *Ficus thonningii* leaf powder and vitamin C supplemented diets

	SEM			0.14	6.80	2.12	2.81
	P-value			0.09	0.71	0.26	0.01
		0		3.49^{a}	114.73	39.16^{b}	45.34^{b}
		200		2.94^{b}	116.38	52.85^{a}	54.70^{a}
		SEM		0.14	6.80	2.12	2.81
		P value		0.01	0.86	0.01	0.03
			0	3.87^{a}	117.35	40.00^{b}	45.10^{b}
			1	2.56^{b}	113.76	52.01^{a}	54.94^{a}
			SEM	0.14	6.80	2.12	2.81
			P value	0.01	0.71	0.01	0.02
	Interactions *P-value*						
	BRD x VC			0.45	0.98	0.69	0.71
	BRD x FTLP			0.33	0.84	0.74	0.97
	VC x FTLP			0.01	0.72	0.01	0.08
	BRD x VC x FTLP			0.01	0.92	0.48	0.95

Means with different superscripts in the same column are significantly (P<0.05) different; BRD: Breeds; VC: Vitamin C; FTLP: *Ficus thonningii leaf powder*; LPDOX: Lipid oxidation; CHOL: Cholesterol; GSH: Glutathione; CATLS: Catalase; AB: Arbor acres; CO: Cobb 500; SEM: Standard error of the means.

3.3 Serum Hormonal Marker Levels

Table 4 shows the impact of vitamin C supplemented meals and *Ficus thonningii* leaf powder (FTLP) on hormonal indicators in two strains of broiler chickens. Diets 6 and 8 were significantly (P<0.05) higher than the other diets, but not significantly different from one another, according to T3 levels. T3 levels in Cobb 500 were notably higher than those in Arbor Acres (P<0.05). Vitamin C and FTLP supplementation was significant (P<0.05), as were the interactions between FTLP and strain, FTLP and vitamin C, and the combined effect of FTLP, vitamin C, and strain.

Table 4: Effects of *Ficus thonningii* leaf powder and vitamin C supplemented diets on hormonal biomarkers of two breeds of broiler chickens

Diet	BRD	VC mg/kg	FTLP %	T3 (ng/dL)	INSULIN (U/mL)	LEPTIN (ng/mL)	HSP70 (AU/mL)
1	AB	0	0	0.65^{d}	3.61^{c}	1.63^{bc}	0.68^{a}
2	CO	0	0	1.34^{b}	3.72^{bc}	0.47^{f}	0.62^{b}
3	AB	200	0	1.46^{b}	3.18^{d}	0.68^{ef}	0.58^{c}
4	CO	200	0	0.94^{cd}	4.06^{b}	0.79^{e}	0.51^{d}
5	AB	0	1	1.00^{c}	3.51^{cd}	1.81^{b}	0.47^{e}
6	CO	0	1	2.48^{a}	3.86^{b}	1.19^{d}	0.42^{f}
7	AB	200	1	1.56^{b}	5.65^{a}	1.47^{cd}	0.43^{f}
8	CO	200	1	2.59^{a}	5.94^{a}	2.26^{a}	0.41^{f}
SEM				0.14	0.20	0.14	0.05
P-value				0.01	0.01	0.01	0.01
	AB			1.17^{b}	3.64^{b}	1.01^{b}	0.54^{a}
	CO			1.84^{a}	4.74^{a}	1.65^{a}	0.49^{b}
	SEM			0.05	0.06	0.05	0.03
	P-value			0.01	0.01	0.01	0.01
		0		1.37^{b}	4.08^{b}	1.52^{a}	0.50^{b}
		200		1.64^{a}	4.29^{a}	1.14^{b}	0.46^{a}
		SEM		0.05	0.06	0.05	0.03
		P value		0.01	0.04	0.01	0.01
			0	1.10^{b}	3.98^{b}	1.14^{b}	0.45^{a}
			1	1.91^{a}	4.40^{a}	1.52^{a}	0.41^{b}
			SEM	0.05	0.06	0.05	0.03
			P value	0.01	0.01	0.01	0.01
	Interactions *P-value*						
	BRD x VC			0.01	0.07	0.01	0.42
	BRD x FTLP			0.01	0.35	0.01	0.01
	VC x FTLP			0.38	0.01	0.01	0.01
	BRD x VC x FTLP			0.01	0.01	0.01	0.01

Means with different superscripts in the same column are significantly (P<0.05) different; BRD: Breeds; VC: Vitamin C; FTLP: *Ficus thonningii leaf powder*; T3: Triiodothyronine; INSULIN; LEPTIN; HSP70: Heat Shock Protein 70; AB: Arbor acres; CO: Cobb 500; SEM: Standard error of the means.

Diets 7 and 8 did not differ significantly from one another, however they did significantly (P0.05) raise insulin levels compared to the other diets. Compared to Arbor Acres, the Cobb 500 strain displayed much higher (P<0.05) levels. Significant impacts (P<0.05) were observed with the addition of vitamin C and FTLP supplementation, as well as the interactions between vitamin C and FTLP and the combined effect of strain, vitamin C, and FTLP.

Compared to the other diets, diet 8 had considerably (P<0.05) greater levels of leptin. Compared to Arbor Acres, the Cobb 500 strain exhibited significantly (P<0.05) greater levels. Vitamin C inclusion had no significant (P<0.05) effect, however FTLP supplementation had a significant (P<0.05) effect. Significant (P<0.05) interactions were seen between strain and vitamin C, strain and FTLP, vitamin C and FTLP, and the combined effect of strain, vitamin C, and FTLP.

Diets 6, 7, and 8 had the least significant (P<0.05) HSP 70 levels, whereas diet 1 had significantly (P<0.05) greater levels than the other diets. Compared to Cobb 500, the Arbor Acres strain exhibited significantly (P<0.05) greater levels. Supplementing with FTLP was the least significant (P<0.05), but vitamin C inclusion was significant (P<0.05). Significant (P<0.05) interactions were seen between strain and FTLP, as well as between vitamin C and FTLP and the three of them together.

4. Discussion

All organisms exposed to oxygen include the antioxidant enzyme catalase, which helps with the following processes: it breaks down hydrogen peroxide (H_2O_2) into water, gets rid of organic hydro peroxides, and uses H_2O_2 to oxidise poisons like alcohols, formic acid, phenols, and formaldehyde (Singh and Kumar, 2019). According to a study, the brain's microglial cells have high concentrations of glutathione and catalase, which gives them a powerful antioxidant capacity to shield them from oxidative damage (Dringen, 2005). This is important for the brain's microglial cells' defensive and reparative roles. According to a study, the high antioxidant levels in birds given FTLP and diets enriched with vitamin C demonstrate the leaf powder's potent antioxidative qualities (Osowe et al., 2021).

It is well known that the oxidative stability of poultry meat can be improved by herbal antioxidants. Antioxidants found in herbs and their products can stop cholesterol and unsaturated fatty acids (UFA) in animal products from oxidising, increasing the animal's total antioxidant capacity (Jachimowicz et al., 2022). According to recent research, food changes made during animal production can alter the fatty acid content of meat to better conform to dietary recommendations (Jachimowicz et al., 2022). According to study conducted by a group researcher, supplemented diets had considerably lower levels of lipid peroxidation than the control diet, indicating that FTLP's antioxidant qualities may enhance the quality of broiler meat (Osowe et al., 2021).

Muscle oxidation rate after death depends on the animal's ability to combat oxidative stress. Endogenous antioxidative enzymes such as Glutathione Peroxidase (GPx), Catalase, and Superoxide Dismutase (SOD) can all delay the oxidation of muscle components. According to a study, these enzymes work well to stop oxidation reactions, improving the quality of meat and prolonging the shelf life of animal products (Chen et al., 2012). Because broiler diets containing FTLP have significant quantities of these antioxidants, it is possible that the leaf powder will increase the broilers' natural antioxidative state, enhancing muscle quality and shelf life. This validates the results of *Ficus thonningii* antioxidant status (Osowe et al., 2021).

Under the effect of both internal and external stimuli, thyroid hormones (T3 and T4) control homeostasis and nutrition digestion (Garasto et al., 2017). Energy metabolism in chickens depends on synchronizing thyroid gland activity, including T3 and T4 concentrations, to maintain body temperature and homeostasis (Jiang et al., 2020). Because thyroid hormones are crucial for regulating a bird's metabolic rate throughout growth and egg production phases, the thyroid gland is important for adapting to high ambient temperatures (Melesse et al., 2011). According to a study, high ambient temperature disrupts the neuroendocrine system and activates the hypothalamic-pituitary-adrenal (HPA) axis by increasing plasma corticosterone and decreasing circulating thyroid hormones such as T3 and T4.

As a result, there is less weight gain and compromised metabolic function (Kumari et al., 2018). Due to the addition of vitamin C and FTLP, diets 6 and 8 had greater T3 levels than diets 1 and 2, indicating that the herbal supplementation may be able to lessen the harmful effects of heat stress on the birds. This is consistent with the results that broilers exposed to extreme heat stress at different temperatures—such as 36°C for four to six hours a day from days 22 to 42—had lower T3 levels (Ahmad et al., 2022). In contrast, some researcher found that broilers exposed to acute heat stress for one hour at 50°C at five days of age had lower blood serum T3 and T4 levels (Bowen and Washburn, 1985). Conversely, a group of researcher found that serum T3 and T4 levels in broilers under thermal stress at 41°C for 4 to 6 hours on day 4 remained unaffected (May et al., 1986).

In quails raised under acute heat stress (38°C for 24 hours), blood serum T3 levels were reduced compared to those in thermoneutral conditions (25°C) (Del Vasco et al., 2017). Reduced protein production due to decreased thyroxine levels lead to reduced body weight and daily gain under heat stress. Nutritionally, loss of appetite and decreased feed intake are attributed to reduced growth in birds under high ambient temperatures (Infante et al., 2011). These reports show that thyroid hormones in broilers are negatively affected by heat stress, which varies by several factors, including stress intensity, duration, and bird age. The increased serum concentrations in this study confirm the antioxidant properties of *Ficus thonningii* reported by (Osowe et al., 2021).

The main purpose of insulin is to aid in the storage of lipids, proteins, and carbs and to stop their breakdown (Cefalu, 2001). Additionally, it helps cells use potassium while limiting glucose synthesis from non-sugar molecules, reduces protein degradation, lowers liver ketones, minimizes fat breakdown, and improves cells' ability to synthesize and use amino acids. The increased levels of insulin hormone in this study as a result of FTLP supplementation are consistent with the findings of who found that birds' ability to fully utilize high-energy foods is impeded by low insulin levels. According to a study, heat stress raises insulin levels, maybe as a coping strategy to preserve glucose tolerance (Wang et al., 2021). The minimization of carbohydrate and lipid metabolism alterations, resulting in metabolic disorders due to heat stress as reported in previous studies was achieved in this study by the insulin hormone levels, which helped the supplemented birds maintain normal metabolic processes (Lu et al., 2007). This supports the antioxidant properties of FTLP reported by (Osowe et al., 2021).

Among mammals, leptin, a 16-kDa protein that is produced in adipose tissue-containing organs, is the most potent inducer of feed consumption. Enough adipocytes produce and supply leptin, which is mostly associated with obesity and, to a lesser extent, with total body weight (Zhang et al., 1994). Adipocyte-produced leptin and adiponectin are essential for controlling feed intake (Zhang et al., 1994). During a negative energy balance, leptin increases food intake and conserves energy through neuroendocrine, behavioral, and metabolic adjustments (Chan et al., 2003). Leptin modulates central thermoregulatory processes, which in turn controls body temperature and immunological responses. Brain leptin signaling regulates glucose homeostasis independently of its effect on adiposity, as well as various behaviors and brain functions (Pelleymounter et al., 1995; Coppari et al., 2005).

Leptin replacement in leptin-deficient animals restores energy balance, glucose homeostasis, neuroendocrine and cognitive functions (Paz-Filho et al., 2008). In this study, diets supplemented with FTLP showed higher leptin levels than control diets, indicating maintained physiological, homeostasis, and metabolic activities for optimal performance. A group of researcher reported that 5% powdered *Phyllanthus buxifolius* leaf reduced leptin levels, body weight, carcass weight, intracellular lipid accumulation, abdominal fat weight, and cholesterol in broilers (Wardah et al., 2012). However, in this study, 1% FTLP in broiler diets enhanced performance and other physiological functions, affirming the antioxidant properties of *Ficus thonningii* as reported by (Osowe et al., 2021).

All live cells include stress proteins called heat shock proteins (HSPs). Cells react to high ambient temperatures by activating certain proteins in a process known as the "heat shock response," which shields them from environmental stresses like heat (Frier and Locke, 2007). By synthesizing other proteins, HSPs preserve cellular order. In times of stress, they also draw immune cells to the site of damage (Shankar and Mehendale, 2014). Moreover, HSPs control translocation, folding and unfolding, and protein synthesis and degradation. When faced with stressors like high temperatures, all organisms release HSPs (De Maio and Vazquez, 2013). According to the HSP family, which is expressed in the muscles, liver, heart, kidney, and blood vessels, heals damaged cells in broilers following acute stress (Zhang et al., 2014).

The function of HSPs in heat-stressed living organisms is well established; they induce HSP mRNA and protein concentrations. Different HSP isoforms react differently to heat stress (Surai et al., 2019). By regulating oxidative stress and apoptotic activity, HSP isoforms stabilize cytoskeletal proteins (Concannon et al., 2003). Under heat stress, excessive ROS production causes cytoskeletal protein breakdown and lipid peroxidation, which harms cells (Kim and Kim, 2018; Arslan et al., 2019; Hu et al., 2019). As reported in a study, they demonstrated that FTLP supplementation decreased HSP70 concentrations in broiler serum, indicating the antioxidative benefits of *Ficus thonningii* during heat stress (Osowe et al., 2021).

5. Conclusion

These findings demonstrate the potential advantages of supplementing with both vitamin C and *Ficus thonningii* to strengthen antioxidant defenses and lessen oxidative stress in broiler producing systems. Thus, *Ficus thonningii* and vitamin C used together may have synergistic effects on thyroid and leptin function, improving feed efficiency and growth performance in broiler chickens.

Credit Authorship Contribution Statement

Clement O. Osowe: experimental design, original draft preparation, validation, supervision, project administration, methodology, investigation, formal analysis, data curation. **Olugbenga D. Oloruntola:** Review and editing of writing; validation; supervision; formal analysis; resources; methodology; conceptualization. **Funmilayo T. Azeez:** validation, supervision, methodology, data curation. **Olufemi A. Adu:** review and editing of writing; validation; resources; visualization; data curation. **Clifford A. Chineke:** Review and editing of writing, validation, resources.

Declaration of Generative Ai and Ai-Assisted Technologies in the Writing Process

During the preparation of this work, the authors utilized GPT-3.5 and Quillbot for paraphrasing and to reduce the similarity index of the initial draft. The authors subsequently reviewed and edited the content as necessary and took full responsibility for the content of the publication.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon request.

Ethical Approval

The study was conducted with the approval of the institutional ethics committee for the care and use of animals for research at the host institution. Ethics Reference No.: FUTA/APH/2023/12.

Conflict of Interest

The authors declare no competing interests.

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