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Effects of Palm (*Elaeis Guineensis*) Oil on Performance, Thermotolerance, and Welfare of Broiler Chickens in Heat Stress Condition

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ABSTRACT

Heat stress negatively affects the broiler chickens' productivity and well-being. This study was carried out to assess the effect of dietary palm oil inclusion on the growth performance, thermotolerance, biochemical parameters, and welfare of broiler chickens raised in tropical climates. A total of 500 broiler chickens aged 15 days were divided into four treatments, each consisting of five replicates with 25 chickens per replicate in a randomized design. The control group was fed a standard diet without palm oil (T), and the remaining diets contained palm oil at the inclusion levels of 1% (P_1), 2% (P_2), and 3% (P_3). During the 4 weeks of experimentation, daily temperature and relative humidity in the poultry house were measured by thermo-hygrometers, and growth performance was weekly recorded. At 45 days old, six broiler chickens were slaughtered with measurements taken for carcass compositions and intestinal length. At 42 days of age, blood samples were collected for the Triiodothyronine (T3) and Thyroxine (T4) hormones, biochemical profiles, and Heterophil: lymphocyte (H/L) ratio assessment at the Regional Center of Excellence on Avian Sciences. Gait abnormality and litter quality were assessed at 38 days of age. The results indicated that the incorporation of 1% palm oil improved the growth performance of chickens compared to other groups. Similarly, the concentrations of T4 and T3 were higher in the 1% palm oil group. Triglycerides and total protein concentrations were higher in the broiler chickens of the control group, compared to other treatment groups. The dropping weight and gait score decreased with the increasing rate of palm oil. The results suggest that palm oil can be a beneficial dietary supplement for broiler chickens, particularly under heat-stress conditions. The incorporation of 1% palm oil contributes to the improvement of growth performance and the well-being of broiler chickens in tropical climates. However, it is crucial to consider the appropriate level of palm oil inclusion, as higher levels may have adverse effects, such as increased mortality.

Keywords: Energy, Feeding strategies, Heat stress, Palm oil, Welfare

INTRODUCTION

In recent years, the substantial increase in global environmental temperatures, attributed to global warming, has inflicted significant damage on animal production, notably in broiler farming. This has led to elevated mortality rates and substantial economic losses (Sejian et al., 2018; Kpomasse et al., 2021). The challenging conditions pose a major threat to the broiler industry in hot and humid climates and jeopardize the sector's sustainability (Rahimi et al., 2021). A large proportion of broiler production industries are located in hot and humid areas (Daghir, 2008). The negative effects of heat stress include physiological impacts, metabolic disorders, and impairment of the functionality of the digestive system, which affects the chickens' welfare (Rostagno, 2020; Brugaletta et al., 2022). Apart from genetic and management strategies, nutritional approaches have also been suggested, such as incorporating oil into the diet (Attia et al., 2020).

Vegetable oils have been used to improve the performance of broilers under heat-stress conditions (Rafiei-Tari et al., 2021). Accordingly, the inclusion of vegetable oils in broiler diets enhances their performance and health status (Tari et al., 2020). The African oil palm (*Elaeis guineensis*), originating from West Africa, holds the status of the world's most significant palm species (Murphy et al., 2021). It is extensively cultivated and thrives in the humid tropics of Asia, Africa, and the Americas (Castellanos-Navarrete et al., 2021). Nonetheless, Rafiei-Tari et al. (2021) reported contradicting findings, indicating that adding palm oil had no positive effects on antioxidant activity and lipid attributes in Cobb 500 broiler chickens raised in a hot climate.

Little research has been carried out to improve the performance of broilers in the heat stress context. In addition, there is a need for evaluation of the adequate proportion of palm oil to be incorporated to improve heat-stressed broiler performance and welfare. The purpose of this experiment was to evaluate how adding palm (*Elaeis guineensis*) oil to the diet affected the broiler chickens' growth performance, thermotolerance, biochemical parameters, and overall welfare in tropical climates.

MATERIALS AND METHODS

Ethical approval

The care and handling of the animals were performed in strict accordance with the recommendations of the Guide for the Care and Use of Experimental Animals of the University of Lome, Togo. The protocol was approved by the Ethics of Animal Experimentation Committee of the same University. All efforts were made to minimize discomfort for the chickens (008/2021/BC-BPA/FDS-UL).

Study location

The experiment was carried out at the experimental unit of the Regional Center of Excellence on Avian Sciences (CERSA) in Badja, 41 km west of Lomé, Togo, located in a hot and humid climate. The average temperature was recorded at 28.85°C ± 0.62, while the average relative humidity was recorded at 71.62% ± 1.75. The study was conducted during the dry season in Togo from February to March 2023.

Study design

A total of 500 15-day-old Cobb 500 broiler chickens with 300 g of average weight acquired from the company “Le Poussin” were randomly divided into 4 treatments of 5 replicates of 25 chickens each using a completely randomized design (CRD). The dietary treatments included a control and a standard diet without palm oil (T), while the experimental groups were provided with diets containing 1%, 2%, and 3% palm oil inclusion, denoted as P1, P2, and P3, respectively. The trial lasted 28 days and the chickens were reared on a floor covered with wood shavings in an open poultry house with a density of 10 chickens/m2 and a lighting program of 23 hours of light and 1 hour of darkness. Water and feed were freely available. The chickens were vaccinated according to the following prophylaxis plan (Appendix 1).

Growth performance and carcass composition evaluation

In this trial, four thermo-hygrometers were used to measure the daily temperature and relative humidity in the poultry house. Growth performance parameters were measured during the trial to determine the weight gain and feed conversion ratio (FCR) according to Formula 1.

$$FCR = \frac{feed\ intake\ (g)}{weight\ gain\ (g)} \qquad \text{(Formula 1)}$$

Six chickens of identical weights from each treatment were humanely slaughtered and eviscerated at 42 days old to collect carcass data, such as belly fat, carcass yield, breast yield, thigh yield, the weights of empty gizzard, liver, heart, kidney, and intestines and length of intestines. Individual weights were represented as a percentage of body weight using Formula 2.

$$\text{Carcass yield (\%)} = \frac{\text{Carcass part (g)}}{\text{Body weight (g)}} \times 100 \qquad \text{(Formula 2)}$$

Biochemical profiles and physiological responses to heat stress

At 42 days of age, before slaughtering, approximately 3 mL of blood was collected from the brachial veins of 15 chickens per treatment, discharged into a dry tube without anticoagulant, and immediately centrifuged at 3000 rpm for 15 minutes to obtain serum. Then, the serum obtained was stored in a freezer at -20°C and further used for biochemical parameter analysis, such as total protein, total cholesterol, uric acid, glucose, and triglycerides, as well as physiological parameters, such as Triiodothyronine (T3) and Thyroxine (T4) and Heterophil: lymphocyte (H/L) ratio. Using automated COBAS® (Germany) systems, enzymatic procedures were used to evaluate the concentrations of glucose, triglycerides, total cholesterol, and total protein. The blood concentrations of total protein using the biuret method (Busher, 1990), total cholesterol (Borner and Klose, 1977), serum triglycerides (Wahlefeld, 1974), glucose (Heinz and Beushausen, 1981), and uric acid (Walter, 1990), were measured. The samples were all run in the same essay to prevent variability across essays for every biochemical parameter. Thyroid hormone concentrations T3 and T4 were assessed by the Enzyme-Linked Fluorescence Assay (ELFA) method using Vidas Biomerieux kits (France). The ELFA method was performed on an in-house automated analyzer (Anderson et al., 2017). The H/L ratio was assessed by counting heterophils and lymphocyte cells using a Hemocytometer. The ratio was estimated by the Formula 3.

$$\text{H/L ratio (\%)} = \frac{\text{Total Heterophils}}{\text{Total Lymphocytes)}} \qquad \text{(Formula 3)}$$

Welfare assessment

Gait score

The gait score was determined at 38 days old based on the notation adopted by Garner et al. (2002). In this regard, 0 was for a chicken that walks normally without ambiguity, 1 for a slight but unidentifiable gait impairment, 2 for a visible and identifiable anomaly with little effect on walking ability, 3 for an obvious anomaly affecting movement ability (the chickens are unable to stand for 15 minutes), 4 for a severe abnormality, and a score of 5 was not included since chickens with such gaits could walk and would have been culled previously. During the current study, gait scores 2 and 3 were chosen to represent moderate lameness, while scores 4 and 5 were utilized to represent severe lameness (Buijs et al., 2016). The prevalence of gait disorders was then determined using the Formula 4.

$$\text{Prevalence (\%)} = \frac{\text{Total no.of chickens with scores 3 and 4}}{\text{Number of live chickens at the time of assessment}}\ x\ 100\%$$
(Formula 4)

Litter quality assessment

The assessments were carried out on broiler chickens aged 42 days using the WQ (2009) procedure and Tuyttens et al. (2015). The litter score, litter depth, and dropping weight were all calculated. At the start of the experiment, the same amount of wood shavings (5 kg/m2) was put on the floor to guarantee that the whole floor area of each enclosure was covered. It was removed once every 4 weeks after weighing the litter and evaluating its depth and texture. The amount of droppings was calculated by subtracting the initial weight of the litter from the weight of the litter at the time of evaluation. The wood shavings were disinfected and spread on the floor of each cage at a density of 5kg per m^2. Each cage was separated into four zones for the different inspections to assess litter depth and texture. The average value of the four zones was the depth of the litter (measured using a metal ruler) and texture score (based on eye assessment) of the cage. Bouassi et al. (2016) used a five-point scale to assess litter texture including dry and friable litter, friable and slightly wet, friable but crusty in some places, crusty at the surface but friable by digging and designating a completely caked or wet litter.

Statistical analysis

The general linear model (GLM) procedure of GraphPad Prism 8 v.8.02. (GraphPad Software, San Diego, CA, USA) was used for statistical analysis. The growth performance parameters, slaughtering performance (carcass characteristics, abdominal fat weight, digestive organ weights, small intestine length, and weight), biochemical parameters, litter depth, and droppings weight were subjected to a one-way analysis of variance (ANOVA). When the difference was significant, further analyses were performed using Tukey's test (Benjamini and Braun, 2001). Mortality was analyzed with a $\chi 2$ test. The Kruskall-Wallis test followed by the Mann-Whitney U test was used for abnormal scores and litter quality which were ordinal variables. For each tested parameter, the difference was significant when the p-value was less than 5%. Data are presented as the mean ± standard deviation

RESULTS

Growth performance

As indicated in Table 2, daily feed intake was higher in P1, P2, and P3 chickens, compared to those of T ($p < 0.05$). The body weight and weight gain of chickens fed a diet containing palm oil especially those of P1 group were significantly higher ($p < 0.05$) than those of the control group (Table 2). The feed conversion ratio (FCR) of the broiler chickens in P1 was significantly lower than the P2, P3, and T groups ($p < 0.05$). Moreover, in the T and P3 groups, chickens recorded significantly higher mortality compared to those in the other treatment groups ($p < 0.05$).

Carcass' parts yield

The effect of palm oil on meat yield and abdominal fat is presented in Table 3. No significant differences were observed in thigh and breast weights between the control and other treatment groups ($p > 0.05$). However, the carcass yield of chickens in treatment P1 was significantly higher than that of the other groups ($p < 0.05$). Additionally, abdominal fat was reduced in all treatment groups compared to the control group, howver this reduction was significantly recorded ($p < 0.05$) in broiler chickens of treatments P1, and P2 compared to the control group.

Internal organ weights

The weights of the kidney, bile, gizzard, heart, lung, and pancreas were not significanty different among all treatment groups ($p > 0.05$). However, Table 4 shows that the liver weight of P2 broilers was lower than that of P1 broilers and significantly higher than that of P3 broilers ($p < 0.05$).

The weight and length of different segments of the small intestine

The length of the small intestine was impacted by the dietary inclusion of palm oil (Table 5). The duodenum length of chickens in the P1 and P2 was significantly higher ($p < 0.05$), compared to that of the T group. The broiler chickens in the P1 group showed an increase in jejunum length ($p < 0.05$), compared to those of the other treatment groups, while P1 and P2 presented higher ileum length than T and P3 chickens ($p < 0.05$). No significant differences were indicated in duodenum, jejunum, and ileum weights among all the treatment groups ($p > 0.05$, Table 5).

Palm oil on gait score and litter quality

The effects of supplementing palm oil in the diet on gait score and litter quality are shown in Table 6. When palm oil was added to the diet, the dropping weight and litter depth decreased significantly, and also litter score significantly improved compared to those of the control group ($p < 0.05$). Compared to the control group, adding palm oil to broiler feed decreased significantly the prevalence of abnormal gait and its incidence ($p < 0.05$).

Blood biochemical profile

As indicated in Table 7, no difference was observed in glucose and cholesterol levels among all treatment groups ($p > 0.05$). Serum protein level was reduced by the inclusion of palm oil in the diet ($p < 0.05$). Similarly, serum uric acid level was reduced for chickens in P1, P2, and P3 compared to the control group ($p < 0.05$). The control group had the highest triglyceride level and it had a significant difference compared to P1 and P2 ($p < 0.05$).

Immune organ weights, thyroid hormone contents, and heterophil: Lymphocyte ratio

The impacts of adding palm oil to the diet on the thymus, spleen, and bursa of Fabricius weights, as well as the physiological reactions of broiler chickens, are shown in Table 8. The mean weights of the spleen and bursa of Fabricius did not differ significantly in all treatment groups ($p > 0.05$). However, the thymus weights of P1 and P3 chickens were higher than that of the chickens in the T group ($p < 0.05$). The level of T4 was not significantly different ($p > 0.05$) in T and P3 groups, but it was higher in P1 and P2 than in T and P3 broiler chickens ($p < 0.05$). Blood T3 content was higher in P1 and P2 than in T and P3 ($p < 0.05$). Lastly, the broiler chickens in the T group had a higher heterophil/lymphocyte (H/L) ratio than that of the other groups.

Table 1. Composition of experimental diets for broiler chickens during the dry season in Togo from February to March 2023

Ingredients (kg) / Experimental diets	T	P_1	P_2	P_3
Maize	53.6	46.3	35.9	25.5
Wheat	14.6	20.4	29.8	39.2
Soybean	19	19.5	19.5	19.5
Oyster shell	1.5	1.5	1.5	1.5
Salt	0.3	0.3	0.3	0.3
Broiler concentrate (5%)[1]	5	5	5	5
Dried brewers grains	6	6	6	6
Palm oil (%)	0	1	2	3
Total	100	100	100	100
Diet chemical composition				
Metabolizable energy (Kcal/kg)	3067.38	3102.66	3121.60	3140.55
Crude protein (%)	18.05	18.26	18.37	18.26
ME/CP	169.93	169.93	169.93	169.93

Chickens fed standard diets without palm oil (T) and broiler chickens fed diets containing palm oil inclusion: 1% (P_1), 2% (P_2), and 3% (P_3); ME/CP: Metabolizable energy (Kcal/kg) to crude protein ratio [1]:Composition Soybean meal, rapeseed meal, sunflower seed meal, corn gluten feed, vinasse, soybean oil, palm fatty acids, sodium chloride. Vitamin A: 12000 IU, Vitamin E, dl-a-tocophervl acetate: 20 mg, menadione: 2.3 mg, Vitamin D3: 2200 ICU. Riboflavin: 5.5mg, Calcium pantothenate: 12 mg, Nicotinı C acid: 50 mg, Choline: 250 mg. Vitamin B12: 10 ug Vitamin B6: 3mg, Thiamine: 3 mg, Folic acid: l mg, d-biotin: 0.05 mg. Trace mineral (mg/kg of diet): Mn: 80, Zn: 60, Fe: 35, Cu; 8, Selenium 0.1 mg

Table 2. Effects of dietary inclusion of palm oil on feed intake of 15 days old broiler chickens during the dry season in Togo from February to March 2023

Parameters \ Treatments	T + SD	P_1 +SD	P_2 + SD	P_3 + SD	P-value
Daily feed intake (g)	65.39 ± 2.46^c	86.17± 1.91^a	73.24 ± 3.31^b	73.05 ± 1.76^b	0.001
Body weight (g)	1583.00 ± 15.53^d	1835.00 ± 13.40^a	1697.00 ± 13.01^c	1757.00 ± 13.80^b	<0.001
Weight gain (g)	36.09 ± 1.46^c	53.91± 4.24^a	38.93 ± 4.05^c	41.85 ± 2.90^b	0.002
Feed conversion ratio	1.88 ± 0.05^a	1.62 ±0.06^b	1.80 ± 0.04^a	1.75 ± 0.16^a	0.076
Mortality (%)	4.00 ± 0.000^a	0.00 ± 0.00^b	0.00 ± 0.00^b	4.00 ± 0.00^a	0.04

Chickens fed standard diets without palm oil (T) and broiler chickens fed diets containing palm oil inclusion at levels of 1% (P_1), 2% (P_2), and 3% (P_3). SD: Standard deviation. $^{a-c}$ Different superscript letters in the same row varied significantly differences ($p < 0.05$).

Table 3. Effects of dietary inclusion of palm oil on meat yield and abdominal fat of 15 days old broiler chickens during the dry season in Togo from February to March 2023

Parameters \ Treatments	T + SD	P_1 +SD	P_2 + SD	P_3 + SD	P-value
Carcass yield (%)	62.70 ± 0.80^b	75.20 ± 2.40^a	64.50 ± 2.57^b	62.72 ± 1.97^b	0.011
Thigh yield (%)	4.23 ± 0.04	4.30 ± 0.25	4.27 ± 0.15	4.55 ± 0 .23	0.123
Breast yield (%)	12.24 ± 0.91	12.93 ± 1.07	13.96 ± 0.13	14.73 ± 1.20	0.053
Abdominal fat (%)	1.60 ± 1.38^a	1.22 ± 0.27^b	1.24 ± 0.10^b	1.47 ± 0.28^{ab}	0.041

Chickens fed standard diets without palm oil (T) and broiler chickens fed diets containing palm oil inclusion at levels of 1% (P_1), 2% (P_2), and 3% (P_3), SD: Standard deviation. $^{a-c}$ Different superscript letters in the same row varied significantly differences ($p < 0.05$).

Table 4. Effects of dietary inclusion of palm oil on the physiological organs of 15 days old broiler chickens during the dry season in Togo from February to March 2023

Parameters \ Treatments	T + SD	P_1 +SD	P_2 + SD	P_3 + SD	P-value
Liver (%)	2.24 ± 0.21^b	2.50 ± 0.40^a	2.1 ± 0.11^b	1.94 ± 0.02^c	0.0245
Kidney (%)	0.42 ± 0.07	0.49 ± 0.32	0.43 ± 0.14	0.49 ± 0.05	0.132
Bile (%)	0.17 ± 0.09	0.19 ± 0.06	0.21 ± 0.13	0.21 ± 0.11	0.071
Gizzard (%)	3.19 ± 0.24	3.29 ± 0.34	3.86 ± 0.61	3.68 ± 0.68	0.057
Heart (%)	0.42 ± 0.07	0.46 ± 0.06	0.47 ± 0.06	0.46 ± 0.07	0.097
Lung (%)	0.50 ± 0.05	0.55 ± 0.07	0.60 ± 0.12	0.47 ± 0.04	0.510
Pancreas (%)	0.27 ± 0.06	0.31 ± 0.03	0.26 ± 0.02	0.26 ±0.02	0.062

Chickens fed standard diets without palm oil (T) and broiler chickens fed diets containing palm oil inclusion at levels of 1% (P_1), 2% (P_2), and 3% (P_3). SD: Standard deviation. $^{a-c}$ Different superscript letters in the same row varied significantly differences ($p < 0.05$).

Table 5. Effects of dietary inclusion of palm oil on small intestinal segments' weight and length of 15 days old broiler chickens during the dry season in Togo from February to March 2023

Parameters \ Treatments	T+ SD	P_1+ SD	P_2+ SD	P_3+ SD	P-value
Duodenum length (cm)	32.67 ± 1.45^b	36.00 ± 0.99^a	35.00 ± 0.97^a	34.67 ± 1.47^{ab}	0.045
Jejunum length (cm)	70.03 ± 2.30^c	83.00 ± 0.64^a	79.17 ± 1.05^b	73.33 ± 2.30^c	0.003
Ileum length (cm)	70.00 ± 2.88^b	75.67 ± 2.07^a	74.33 ± 1.46^a	71.03 ± 2.00^b	0.004
Duodenum weight (%)	1.15 ± 0.24	1.26 ± 0.42	1.19 ± 0.32	1.19 ± 0.18	0.083
Jejunum weight (%)	2.16 ± 0.26	3.06 ± 0.36	3.04 ± 1.00	2.52 ± 0.07	0.171
Ileum weight (%)	5.05 ± 0.39	5.36 ± 0.61	6.92 ± 1.12	5.19 ± 0.41	0.075

Chickens fed standard diets without palm oil (T) and broiler chickens fed diets containing palm oil inclusion at levels of 1% (P_1), 2% (P_2), and 3% (P_3). SD: Standard deviation. $^{a-c}$ Different superscript letters in the same row varied significantly differences ($p < 0.05$).

Table 6. Effects of dietary inclusion of palm oil on gait score and litter quality of 15 days old broiler chickens during the dry season in Togo from February to March 2023

Parameters \ Treatments	T+ SD	P_1+ SD	P_2+ SD	P_3+ SD	P-value
Droppings weight (g/chicken)	2.22 ± 0.08^a	1.86 ± 0.03^b	1.52 ± 0.02b^c	1.33 ± 0.12^c	0.005
Litter depth (cm)	2.30 ± 0.26^a	2.06 ± 0.31^b	1.76 ± 0.57^c	1.68 ± 0.73^c	0.004
Litter score	2.00 ± 0.000^a	1.4 ± 0.57^b	1.2 ± 0.333^b	1.3 ± 0.02^b	0.003
Abnormal gait prevalence (%)	6.62 ± 0.02^a	3.36 ± 0.13^b	3.34 ± 0.03^b	0.00 ± 0.000^c	<0.001

Chickens fed standard diets without palm oil (T) and broiler chickens fed diets containing palm oil inclusion at levels of 1% (P_1), 2% (P_2), and 3% (P_3). SD: Standard deviation. $^{a-c}$ Different superscript letters in the same row varied significantly differences ($p < 0.05$).

Table 7. Effects of dietary inclusion of palm oil on biochemical parameters of 15 days old broiler chickens during the dry season in Togo from February to March 2023

Parameters \ Treatments	T+ SD	P_1+ SD	P_2+ SD	P_3+ SD	P-value
Glucose (g/l)	2.37 ± 0.11	2.28 ± 0.06	2.30 ± 0.09	2.46 ± 0.01	0.068
Uric acid (g/l)	51.93 ± 1.78[a]	34.37 ± 1.42[b]	32.73 ± 1.56[b]	29.19 ± 1.29[b]	0.001
Triglycerides (g/l)	0.51 ± 0.03[a]	0.41 ± 0.02[b]	0.41 ± 0.04[b]	0.46 ± 0.02[ab]	0.016
Cholesterol (g/l)	1.38 ± 0.11	1.33 ± 0.09	1.37 ± 0.08	1.00 ± 0.04	0,124
Protein (g/l)	39.03 ± 1.13[a]	36.54 ± 2.77[b]	36.98 ± 1.80[b]	31.24 ± 1.35[c]	0.007

Chickens fed standard diets without palm oil (T) and broiler chickens fed diets containing palm oil inclusion at levels of 1% (P_1), 2% (P_2), and 3% (P_3). SD: Standard deviation. [a-c] Different superscript letters in the same row varied significantly differences ($p < 0.05$).

Table 8. Effects of dietary inclusion of palm oil on physiological parameters and immune organ weights of 15 days old broiler chickens during the dry season in Togo from February to March 2023

Parameters \ Treatments	T+ SD	P_1+ SD	P_2+ SD	P_3+ SD	P-value
T3	6.33 ± 0.28[b]	8.22 ± 1.05[a]	8.12 ± 0.24[a]	6.51 ± 0.87[b]	0.004
T4	8.60 ± 1.01[b]	10.82 ± 1.01[a]	10.35 ± 0.51[a]	9.16 ± 0.85[b]	0.024
H/L	1.82 ± 0.54[a]	1.16 ± 0.12[c]	1.17 ± 0.05[c]	1.34 ± 0.19[b]	0.030
Thymus (%)	0.24 ± 0.21[b]	0.32 ± 0.02[a]	0.29 ± 0.04[b]	0.31 ± 0.02[a]	0.035
Spleen (%)	0.17 ± 0.1	0.15 ± 0.02	0.18 ± 0.07	0.13 ± 0.05	0.052
Bursa of Fabricius (%)	0.07 ± 0.06	0.07 ± 0.03	0.12 ± 0.06	0.10 ± 0.08	0.078

Chickens fed standard diets without palm oil (T) and broiler chickens fed diets containing palm oil inclusion at levels of 1% (P_1), 2% (P_2), and 3% (P_3). SD: Standard deviation, T3: Triiodothyronine, T4: Thyroxine. [a-c] Different superscript letters in the same row varied significantly differences ($p < 0.05$).

Appendix 1. Vaccination program

Age (days)	Diseases	Vaccines
1	Marek's disease at the hatchery	PREVEXXION RN
3	Newcastle disease and infectious bronchitis	B_1H_{120}
6	Gumboro disease	Gumbo L
7	Newcastle disease and infectious bronchitis	B_1H_{120}
10	Gumboro disease	IBDL

DISCUSSION

The thermal challenge due to global warming is one of the most important environmental concerns in broiler production that negatively affects their optimum productivity, requiring mitigation strategies such as feeding strategies (Kpomasse et al., 2021; Olgun et al., 2021). The results showed that broiler chickens fed diets containing palm oil recorded a higher feed intake and consequently gained more weight than the control ones. In practice, under heat stress conditions, chickens reduce their feed intake to regulate and maintain their body temperature within the range of 40.6-41.0°C (Olgun et al., 2021). The incorporation of palm oil helped broiler chickens mitigate the effect of heat stress, resulting in improved feed consumption. Therefore, an increased feed intake of chickens led to the supply of more nutrients and, thus, an increase in body weight (Abdollahi et al., 2018).

The improved feed efficiency recorded for the 1% inclusion level of the palm oil suggests that low levels of dietary palm oil could be effective in improving the chickens' performance. This was evident as a higher mortality rate of chickens was recorded in the P3 group. In the same line, Wang et al. (2003) observed that the incorporation of 2% of fish oil in broiler diets could improve their performance. On the contrary, Jimenez-Moya et al. (2021) observed a positive impact on growth performance by including as much as 6% of palm oil in boiler chicken's diet. The differences observed can be explained by the environment, since in the present study, chickens were thermally challenged. Kang et al. (2001) reported the presence of polyunsaturated fatty acids in palm oil. This has been shown to improve both the immune status and nutrient digestibility in broilers and can also explain the low mortality of P1 and P2 chickens (López-Ferrer et al., 2001). Another compelling reason could be linked to an increase in palmitic acid in the palm oil used (44% of total fats) (Carta et al., 2017), which could affect some cells (Korbecki and Bajdak-Rusinek, 2019), including pancreatic cells, muscle cells, adipocytes

(Fat Cells), liver cells, and cardiovascular cells. Seifert et al. (2010) reported an adverse impact on mitochondria by increasing the generation of reactive oxygen species (ROS), which is harmful to cell development and chicken growth. Excessive ROS accumulation could be responsible for cell damage, death, or metabolism disturbance (Fedyaeva et al., 2014). These metabolic disturbances might also explain the lower feed intake and lower growth performance of P2 and P3 chickens.

The thigh, breast, kidney, bile, gizzard, heart, lung, and pancreas weights were not different among all the treatment groups in this present study. However, carcass weight increased in P1 broiler chickens. This could be linked to the increase in feed efficiency leading to reduced abdominal fat in these chickens. Since palmitic acid could impair myogenesis and negatively affect skeletal muscle (da Paixão et al., 2021), the inclusion of palm oil above a certain limit in thermally challenged chickens could potentially lead to a decline in muscle weight and growth performance in broiler chickens (Kpomasse et al., 2021). Furthermore, a diet high in fat may have increased bile secretion, which in turn may have caused liver hyperactivity and a subsequent drop in liver weight (Fouad and El-Senousey, 2014). Excessive fat deposition affects the consumers' acceptance of the meat (Schumacher et al., 2022). When the dietary energy is not fully used by the chickens, the liver converts the excess into fat (triglycerides) stored in adipose tissues, which leads to a loss of dietary energy (Hermier, 1997). This might explain the low blood triglyceride content recorded.

Regarding the different segments of the small intestine, the duodenum length of broiler chickens that received the palm oil was longer, compared to that of the control group, while 1% presented an increase in jejunum and ileum length in this present study. This suggests a morphological and histological alteration of the features of the gastrointestinal tract, which might affect partly the efficiency of the utilization of nutrients in broiler chickens (Swatson et al., 2002; Simon et al., 2019). An increasing length of intestinal segments leading to improved surface area available for nutrient absorption would have enhanced nutrient utilization in chickens (Ravindran et al., 2006).

The inclusion of palm oil in the diet decreased the weight of the droppings, and litter depth, with an improvement in litter score in the chickens that were given the oil palm. Also, abnormal gait and the prevalence of abnormal gait were considerably reduced by including palm oil in the broiler chickens' diets. Chickens that are thermally challenged produce a large amount of urine and wet droppings, which affect litter quality (Dayyani and Bakhtiari, 2013). Consequently, heat stress induces disturbances in bone metabolism, affecting the gait of broiler chickens (Dayyani and Bakhtiare, 2013). According to Dunlop et al. (2016), adding dietary palm oil to broiler chickens' diets under heat stress might have enhanced their metabolism by limiting disturbances and enhancing their welfare. However, an improvement in welfare status was not consistent with the higher mortality of P3 chickens. This supports the hypothesis developed concerning the 3% inclusion rate of dietary palm oil.

The incorporation of palm oil in the diet of broiler chickens did not affect glucose and cholesterol levels in this present study. However, serum protein and serum uric acid content were reduced by the inclusion of palm oil in the diet. The increase in blood protein content reflected a situation of inflammation, which is an infectious phenomenon stimulating gamma globulin production or dehydration caused by thermal challenge (Ansar and Ghosh, 2016). This occurs particularly in the liver and intestinal tracts (Quinteiro-Filho et al., 2012; Liu et al., 2022); Such infectious phenomena adversely affect the growth performance of broiler chickens (Remus et al., 2014). Protein degradation releases uric acid, which produces nitrogen, carbohydrates through gluconeogenesis, and lipids through lipogenesis, as well as carbon dioxide and energy (Gherghina et al., 2022). This might explain the relationship between protein and uric acid levels noticed in the present study.

The inclusion of palm oil did not affect the spleen or bursa of Fabricius. Nevertheless, thymus weight was higher and the heterophil/lymphocyte (H/L) ratio was lower in the experimental groups with increased blood T3 and T4 concentrations in the 1 and 2% palm oil diets. The thymus is a lymphoid organ involved in nonspecific (nonadaptive) and specific (adaptive) immune responses in poultry (Reese et al., 2006). More humoral antibody production is linked to a higher thymus weight (Igwe et al., 2020). The thymus produces the T lymphocytes that produce cellular antibodies or immunity (Davison et al., 2008). The inclusion of dietary palm oil in broiler chickens' diets could enhance immunity by fostering lymphocyte proliferation, consequently resulting in positive immune responses, including a transient decrease in the neutrophil/lymphocyte ratio and activation of leukocyte migration to infection sites (Dohms and Metz, 1991). Heat stress leads to a drop in Triiodothyronine and Thyroxin (Gonzalez-Rivas et al., 2019). The activity of antioxidant enzymes is regulated by thyroid hormones (Kpomasse et al., 2023). This implies that the improved concentrations of the hormones T3 and T4 could be linked to the enhanced antioxidant status of broiler chickens. A heterophil to lymphocyte ratio (H/L ratio) in broiler chickens exposed to environmental challenges is the ratio of heterophils (a type of white blood cell involved in the immune system's reaction to stress and infection) to lymphocytes (another type of white blood cell involved in the immune system's response to specific pathogens). It serves as a stress resilience selection criterion (Al-Murrani et al., 2006; Gil et al., 2023). Thermal challenges may result in an increased H/L ratio (Bartlett and Smith, 2003; Mashaly et al., 2004). The body triggers stress responses during heat stress in broiler chickens, and these responses can change the immune system. A series of physiological reactions, including the release of stress hormones like corticosterone, are brought on by heat stress (Kyrou and Tsigos, 2009). Through affecting white blood cell production and function, this hormone can impact the immune system Gombart et al. (2020). In particular, heat stress tends to reduce the number of lymphocytes in the blood and increase the number of heterophils in circulation. This shift in the white blood cell count leads to an elevated H/L ratio. In the present study, results revealed that broiler chickens fed diets containing palm oil at levels of 1 and 2% showed a decreased H/L ratio compared to P1 and T treatments. Therefore, P1 broiler chickens expressed more health stress compared to P2 and P3 broiler chickens. This could be explained by a metabolic disruption caused by the increased consumption of palmitic acid, which makes up 44% of total fats in palm oil. This disruption might have impacted the type functions of certain cells (Carta et al., 2017). The higher H/L ratio in comparison to the broiler chickens in the T group indicates a change in the immune response toward a state that is less infection-fighting and more stress-related. It is a sign that the immune system of the chickens is stressed instead of actively fighting off infections (La Rosa et al., 2021)

CONCLUSION

The results of the study imply that broiler chickens' physiology and performance may be affected by feeding palm oil. Particularly under heat stress circumstances, it causes increased feed intake and weight gain. The low levels of palm oil inclusion (1%), which may boost the chickens' immune systems and nutritional digestibility, seem to be more beneficial for enhancing feed efficiency and growth performance. The effectiveness of palm oil, at higher concentrations (3%) can be linked to higher mortality rates. The findings also highlight the potential of palm oil to influence organ weights, gut morphology, and overall broiler chicken welfare. The specific effects of palm oil on internal organ physiology and gut function could be further investigated to optimize the inclusion of palm oil in broiler chicken diets.

DECLARATIONS

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Authors' contributions

Yarkoa Tchablémane Songuine Tchabltien, Pitala Wéré and Kpomasse Cocou Claude participated in the study conceptualization and performed the statistical analysis. Yarkoa Tchablémane, Parobali Tchilabalo, and Songuine Tchablitien carried out the design, methodology, and biochemical analysis. Karou Damintoti Simplice, and Pitala have contributed equally to this work. They conceived the study, participated in its design and administration, and helped draft the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

There are no nucleic acid sequences, protein sequences, or atomic coordinates in the present study. The data are available upon request from the corresponding author.

Competing interests

The authors declare no conflict of interest.

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Ethical considerations

In order for this article to be published with scientific research standards in the Journal of the World's Poultry Research, all authors have ruled and agreed on ethical issues, including fabrication of data, double publication and submission, redundancy, plagiarism, consent to publication, and misconduct.

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