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RESEARCH ARTICLE

Effects of Dietary Palm Oil Inclusion Levels on Zootechnical and Biochemical Performance of Pullets

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Abstract

Objective: This study aimed to improve the production performance of *ISA Brown* laying hens by incorporating palm oil into their diet.

Materials and Methods: Six hundred ten-week-old birds were randomly allocated to four groups (H0, H1, H2 and H3) and reared for a period of ten weeks. Each group consisted of five replicates of 30 birds each. Groups H0, H1, H2 and H3 were fed diets containing 0, 1, 2 and 3% palm oil, respectively. Body weight, feed intake, average daily gain and feed conversion ratio were measured weekly for all birds. At 21 weeks of age, 10 birds were randomly selected from each group for blood sample collection and subsequent biochemical analysis.

Results: The results indicated no significant differences among the four groups in mean body weight, mean daily gain, feed conversion ratio, or serum gamma-GT concentration ($p>0.05$). However, feed intake was significantly higher only in group H1 compared with groups H0, H2 and H3 ($p<0.05$). Groups H2 and H3 exhibited significantly elevated total protein and glucose levels ($p<0.0001$) compared to groups H0 and H1. In contrast, ALT concentration was significantly higher in group H3 than in groups H0, H1 and H2 ($p<0.0001$). Triglyceride levels were also significantly higher ($p<0.0001$) in the treated groups compared with group H0. Moreover, AST and total cholesterol concentrations were markedly elevated in group H3.

Conclusion: Based on these results, the incorporation of 2% palm oil into pullet diets is recommended to improve production performance.

INTRODUCTION

In poultry farming, feed is of paramount importance as it supports physiological functions and optimizes production performance and egg quality in relation to the animal's genetic potential[1]. In chicken feed formulation, feed ingredients are generally classified according to their nutritional functions as energy sources, protein sources, calcium and phosphorus sources and vitamin, macro mineral and trace mineral supplements[2]. To meet the energy

requirements of chickens, cereals such as maize, sorghum, millet, rice and wheat are mainly used, which provide 70 to 90% of poultry energy needs and 35 to 50% of their nitrogen intake[3]. In addition to wheat, maize is one of the most widely used cereals in human and animal nutrition and is the preferred grain in poultry feed[4].

Globally, maize- and soybean-based diets constitute the foundation of conventional poultry nutrition. In Togo, grain maize is the most commonly used cereal in poultry production due to its high energy content. However, increasing human demand for maize driven by population growth, combined with limited production in tropical African countries (4-10 t per hectare), is insufficient to satisfy both human and animal requirements. This situation was further aggravated by the COVID-19 pandemic, which affected Africa, the Caribbean, Asia and South America, leading to economic losses for smallholder farmers due to rising commodity prices caused by lockdown measures and logistical constraints[5-8]. Consequently, the continuous increase in the cost of conventional feed ingredients has encouraged poultry nutritionists to explore alternative energy sources to partially or fully replace maize in poultry diets, including vegetable and animal oils.

With this in mind, Halle[9] and Harms, Russell[10] conducted studies on laying hens and reported an increase in feed intake as the level of added fat increased. The same authors also reported that the inclusion of 6% oil in the diet increased energy consumption by 8.4% over 8 weeks. At the same energy level, energy intake increased by 2.8% with 4% fat inclusion[11]. Gab-we[12] reported that incorporating peanut oil at a level of 4% in the diet of 6-week-old broiler chickens resulted in improved growth performance. Antoniou *et al.*[13] found that replacing soybean oil with tallow reduced feed conversion efficiency. Machin *et al.*[14] and Frttsche *et al.*[15] observed changes in the lipid composition of chicken tissues when birds were fed fish oil. Ravindran *et al.*[16] suggested that vegetable fats are more efficiently utilized and absorbed than animal fats because they are rich in unsaturated fatty acids essential for chickens, such as linoleic, linolenic, and arachidonic acids. Similarly, Pan *et al.*[17] reported optimal growth when dietary lipids were supplied by palm oil, soybean oil and coconut oil.

Palm oil is a vegetable oil extracted from the fruit of the oil palm (Elaeis guineensis). It is currently the most widely consumed vegetable oil worldwide[18]. Global production of palm oil has increased substantially, rising from 20 million tons in 2000 to 64.495 million tons in 2017. Palm oil consists mainly of triglycerides (95-99%)[19]. In addition to these major components, crude palm oil contains minor bioactive compounds, including vitamin E (tocopherols and tocotrienols), carotenoids, phytosterols, squalene and phenolic compounds[19,20].

Despite its relatively high saturated fatty acid content, palm oil does not increase blood cholesterol levels[21]. In animal studies, tocotrienols have been reported to exhibit anticancer effects[22], as well as anti-angiogenic[23], anti-inflammatory[24], antioxidant[25], anti-diabetic[26] and neuroprotective[27] properties. In laying hens, dietary inclusion of palm oil has been shown to modify the egg fatty acid profile, reduce cholesterol and lipoprotein levels in yolk and cause only slight changes in taste[28].

Although many studies on palm oil supplementation in poultry have focused on laying hens, most have been conducted during the production phase. Several zootechnical and physiological effects have been reported; however, to the best of our knowledge, limited research has investigated the use of palm oil in poultry diets, particularly in pullets. This highlights the relevance of the present study, which aims to improve the production performance of laying hens through dietary incorporation of palm oil.

MATERIALS AND METHODS

Ethical statement: The experiment was conducted in strict accordance with the guidelines outlined in the Guide for the Care and Use of Experimental Animals (008/2021/BC-BPA/FDS-UL) of the University of Lomé, Togo.

Study setting: The experiment was conducted at Ayodele Farm in Badja, Avé Prefecture, a village located 47 km northwest of Lomé. The farm is situated at 6°22'15.65"N and 0°58'07.35"E and is one of the poultry farms partnered with the Regional Center of Excellence for Avian Science at the University of Lomé (CERSA/UL). The experimental period lasted 10 weeks.

Birds, experimental design and management: One-day-old ISA Brown chicks were imported from Incubel, Belgium and transported to the experimental station of the Regional Center of Excellence for Avian Science (CERSA) in Badja. During the starter phase (weeks 1 to 8) and the early growth phase (weeks 8 to 10), the chicks were housed in floor cages with ad libitum access to feed and water. The rearing temperature was maintained at 33°C on the first day and gradually reduced to 25°C by the end of the starter phase. An intermediate lighting program was also implemented throughout this period.

A total of 600 ISA Brown laying pullets at 10 weeks of age were used in the experiment. The birds were randomly allocated to four treatment groups (H0, H1, H2 and H3), each consisting of 150 birds. Group H0 received the control diet (0% palm oil), whereas groups H1, H2 and H3 received diets supplemented with 1, 2 and 3% palm oil, respectively. Each treatment group comprised five replicates of 30 hens and was arranged according to a completely randomized design. The production parameters of each group were recorded before the start of the trial.

During the starter phase, all birds received their respective chick diets from two weeks of age until the end of the phase. Thereafter, the corresponding grower diets (H0, H1, H2 and H3) were provided until the onset of egg production. Feed was weighed and distributed daily throughout the experimental period and feed refusals were recorded weekly to determine feed intake. Birds were weighed weekly by group to determine average body weight, average daily gain and feed conversion ratio. At 21 weeks of age, 10 birds from each treatment group were selected for blood collection and serum samples were obtained for biochemical analyses.

The experimental diets were formulated by the Poultry Production Techniques Laboratory of CERSA-UL to meet the nutritional requirements of pullets while maintaining a constant energy-to-protein ratio. Palm oil was purchased in the Maritime Region, specifically from the canton of Badja. In its crude form, the oil contained 9,370 kcal/kg of metabolizable energy, 0% protein and 99.7% fat.

Four isocaloric and isonitrogenous diets were formulated for the pullet phase (weeks 10-21) according to the nutritional requirements of pullets (Table 1). These included a control diet (H0) without palm oil supplementation and three experimental diets (H1, H2 and H3) supplemented with 1, 2 and 3% palm oil, respectively.

Data collection

Growth performance: Body weight was measured at 10 weeks of age and subsequently at weekly intervals until week 21 using an electronic scale (Camry EK3250; accuracy±1 g). Feed intake was recorded weekly and the Feed Conversion Ratio (FCR) was calculated as the ratio of feed consumed (g) to body weight gain (g).

Serum biochemical parameters: At 21 weeks of age, 10 pullets from each treatment group were selected for blood collection. Blood samples were obtained from the jugular vein using tubes without anticoagulant and were centrifuged at 3,000 rpm and 4°C for 15 min. The resulting serum was stored at −20° C until analysis. Serum concentrations of triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT) and total cholesterol were determined using a colorimetric method with a HOSPITEX Master T analyzer (Master T-Semi Auto Biochemistry Analyzer, Inscon Medical Solutions Pvt. Ltd., Kochi).

Serum glucose concentration was measured using the GPSL-0497 reagent (ELITech Clinical Systems, France). Briefly, 300 µL of reagent was added to 3 µL of serum. The glucose oxidase and peroxidase enzymes present in the reagent catalyzed the enzymatic oxidation of glucose. Following incubation at 37°C for 11 min and 30 sec, the absorbance of the reaction mixture was measured at 550 nm. The results are expressed as mg/dL.

Serum total protein concentration was determined using the PROB-0600 reagent (ELITech Clinical Systems, France) according to the Biuret method. This method is based on the formation of a colored complex between serum proteins and a copper salt in an alkaline medium. For the assay, 300 µL of PROB-0600 reagent was added to a tube containing 3 µL of

Table 1: Chemical composition of the different feed rations according to the treatments

	Experimental feeds			
Ingredients (%)	H0	H1	H2	H3
Corn	56.00	53.32	50.32	48.50
wheat bran	12.00	13.90	15.59	16.50
Fishmeal	6.00	6.00	6.00	7.00
Roasted soybeans seed	13.00	11.14	9.45	6.00
Soybean meal	4.00	5.64	7.64	10.00
Meat concentrate	4.00	4.00	4.00	4.00
Shell	5.00	5.00	5.00	5.00
Palm oil	0.00	1.00	2.00	3.00
Total	100.00	100.00	100.00	100.00
Nutrient composition calculated				
ME (kcal/kg DM)	2762.00	2762.00	2762.00	2762.00
CP (%)	17.62	17.56	17.67	17.54
Ca (%)	2.22	2.22	2.22	2.22
CF (%)	4.81	4.88	4.95	4.93

ME: Metabolizable energy, CP: Crude protein, Ca: Calcium and CF: Crude fiber

serum sample. After incubation at 37°C for 11 min and 30 sec, the absorbance of the reaction mixture was measured at 546 nm. The results are expressed as g/dL.

Statistical analysis: Data were analyzed using GraphPad Prism version 5.00 software. One-way analysis of variance (ANOVA), followed by Tukey's post hoc test, was used to analyze the experimental data. Differences were considered statistically significant at $p<0.05$. The results are presented as mean±standard error of the mean (SEM).

RESULTS

Physiological parameters of pullets: Figures 1-4 present the results for feed intake (FI), body weight (BW), Average Daily Gain (ADG) and feed conversion ratio (FCR), respectively. Pullets in group H1, between 11 and 21 weeks of age, exhibited higher FI than those in groups H0, H2 and H3 at the same age (Fig. 1, $p<0.05$). However, no significant difference in FI was observed among groups H0, H2 and H3 (Fig. 1, $p>0.05$).

Figure 2 illustrates the BW of the experimental pullets throughout the study period. Overall, the growth curves showed a similar pattern among all treatment groups, indicating that palm oil supplementation did not affect the average body weight of the pullets ($p>0.05$). At 21 weeks of age, the average body weights were 928.7±85.06 g, 949.7±85.86 g, 951.6±82.06 g and 964.1±87.38 g for groups H3, H1, H0 and H2, respectively.

The ADG results showed that the inclusion of palm oil in the diet had no significant effect on growth performance (Fig. 3, $p>0.05$). No significant differences were observed among the treatment groups, with ADG values of 11.40±0.53 g for H0, 11.50±0.90 g for H1, 11.80±1.07 g for H2 and 11.87±0.54 g for H3.

Similarly, the FCR results indicated that palm oil supplementation did not influence feed efficiency in the pullets (Fig. 4, $p>0.05$). No significant differences were detected among the treatment groups. The FCR values were 3.91±0.26 for H2, 4.05±0.27 for H1, 4.09±0.28 for H3 and 4.15±0.16 for H0.

Serum biochemical parameters of pullets: The serum biochemical results showed that total protein and glucose concentrations were higher in pullets from groups H2 and H3 than in those from groups H0 and H1 (Table 2, $p<0.0001$). However, no significant differences were observed between groups H0 and H1 or between groups H2 and H3 (Table 2, $p>0.05$).

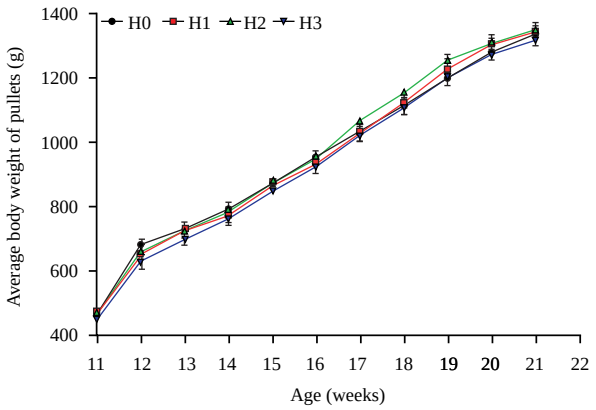

Fig. 1: Daily feed intake of pullets as a function of the palm oil content in their diet
Histograms not sharing the same letter are significantly different ($p<0.05$). SEM: Pooled Standard Error of Means (n = 5)

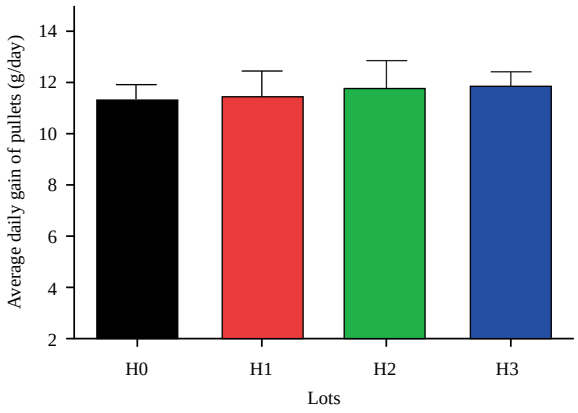

Fig. 2: Average body weight of pullets according to their age and treatments
SEM: Pooled Standard Error of Means (n = 5)

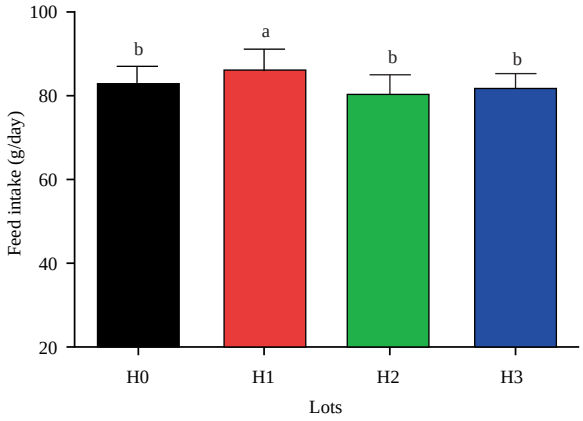

Fig. 3: Average daily gain of pullets as a function of the rate of incorporation of palm oil in their ration
SEM: Pooled Standard Error of Means (n = 5)

Table 2: Influence of pullet feeding treatments on serum concentrations of total protein and glucose

	Lots				
Item	H0	H1	H2	H3	P value
Total protein (g/dL)	5.30±0.11^{b}	4.98±0.16^{b}	6.26±0.25^{a}	6.44±0.24^{a}	P < 0.0001
Glucose (mg/dL)	306.70±5.77^{b}	303.90±6.98^{b}	340.90±4.86^{a}	352.80±6.15^{a}	P < 0.0001

Abbreviation: H0, 0% palm oil; H1, 1% palm oil, H2, 2% palm oil, H3, 3% palm oil, Values not sharing the same letter in the row are significantly different (p<0.0001) and SEM: Pooled Standard Error of Means (n = 10)

Table 3: Exploration of liver function according to treatment of pullets

	Lots				
Item	H0	H1	H2	H3	p-value
AST (UI/L)	214.70±4.90^{ab}	214.4±4.49 ^{ab}	211.40±1.52^{b}	235.40±6.68^{a}	0.019
ALT (UI/L)	4.59±0.43^{b}	3.35±0.11 ^{b}	3.22±0.14^{b}	8.54±1.95^{a}	0.001
Gamma-GT (UI/L)	22.80±1.02	23.75±0.48	22.40±0.95	25.64±1.80	0.205

H0, 0% palm oil; H1, 1% palm oil; H2, 2% palm oil; H3, 3% palm oil, Values not sharing the same letter in the row are significantly different (p<0.05), SEM: Pooled standard error of means (n = 10)

Table 4 Influence of pullet feeding treatments on serum concentrations of triglycerides and total cholesterol

	Lots				
Item	H0	H1	H2	H3	P value
Triglycerides (mg/dL)	120.40±11.92^{b}	466.00±55.03^{a}	566.30±92^{a}	543.70±37.3^{a}	p<0.0001
Total Cholesterol (g/L)	1.44±0.04^{ab}	1.27±0.02^{b}	1.25±0.02 ^{b}	1.63±0.09^{a}	p<0.0001

H0: 0% palm oil. H1: 1% palm oil, H2: 2% palm oil, H3, 3% palm oil, Values not sharing the same letter in the row are significantly different (p<0.05) and SEM: Pooled Standard error of means (n = 10)

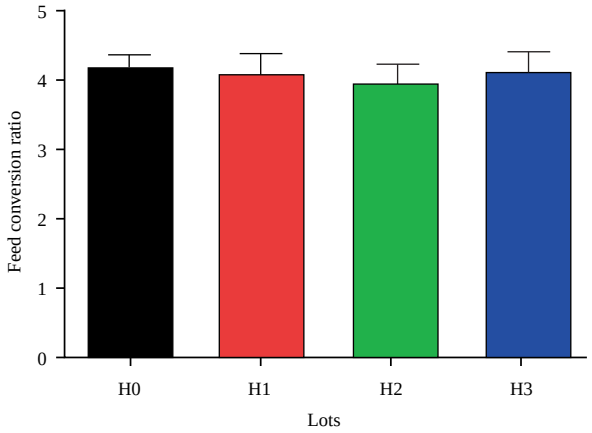

Fig. 4: Feed conversion ratio of pullets as a function of the rate of palm oil incorporation in their ration
SEM: Pooled Standard Error of Means (n = 5)

Table 3 presents the serum concentrations of transaminases and gamma-glutamyl transferase (GGT) according to treatment. Serum GGT concentrations were similar across all four groups. In contrast, ALT concentrations were higher in group H3 than in the other treatment groups (p<0.05). No significant differences in ALT concentration were observed among groups H0, H1 and H2 (p>0.05). In addition, AST concentrations were lower in H2 pullets than in H3 pullets (p<0.05).

The results for serum triglyceride (TG) and total cholesterol (TCHO) concentrations are presented in Table 4. TG concentrations were higher in pullets from groups H1, H2 and H3 than in those from group H0 (p<0.0001). However, TG concentrations did not differ significantly among groups H1, H2 and H3. In contrast, TCHO concentrations were lower in groups H1 and H2 than in group H3 (Table 4, p<0.0001).

DISCUSSION

It is common practice to supplement poultry diets with moderate amounts of fat, particularly in broiler production. Dietary fat increases the energy concentration of feed by 2 to 10% and improves feed conversion ratios[29]. Several studies have reported that the inclusion of fat in poultry diets enhances growth performance in broiler chickens. However, the optimal dietary fat level required to maximize growth remains controversial among authors. Reported optimal inclusion levels range from 2.5-5% 30 to 15-20%[31], while others recommend levels between 6 and 12%[32-35]. In contrast, some studies have reported no effect of dietary fat on chicken growth[36]. These authors observed that growth performance, feed intake and feed cost remained unchanged despite increasing levels of dietary fat.

In the present study, palm oil supplementation did not affect growth performance, feed conversion ratio, or weight

gain in pullets. These findings are consistent with those reported by Olomu and Offiong[36]. However, feed intake was significantly greater in group H1 than in the other treatment groups. The increase in feed intake observed in birds receiving 1% palm oil may be associated with improved feed palatability. Indeed, lipid supplementation has been reported to improve feed utilization efficiency in poultry[33,37].

The reduction in feed intake observed at palm oil inclusion levels above 1% may be attributed to the fulfillment of the birds' energy requirements. As reported by Coon *et al.*[38], chickens reduce feed intake once their energy needs are satisfied. These authors demonstrated that birds fed low-energy diets increase their feed consumption until their energy requirements are met, after which feed intake decreases. Similarly, Blum and Leclercq[32] reported that feed intake declines when dietary lipid concentrations become excessively high. In general, the regulation of feed intake in poultry is associated with the need to limit the volume of feed consumed while maintaining an adequate supply of energy and nutrients[1].

Feed intake is regulated by several sensory, digestive and metabolic factors[39]. Birds respond to both internal factors, such as hormones, nutrient concentrations and energy reserves and external factors, including feed availability, feed characteristics, photoperiod and environmental temperature, to maintain energy balance. The hypothalamus serves as the central regulatory site for energy homeostasis[1,40].

The absence of differences in weight gain among pullets receiving different levels of dietary fat may be related to the limited efficiency of fat utilization in growing pullets[41]. Cheng, *et al.*[42] reported that increasing dietary energy content by +215 kcal/kg from 0 to 6 weeks and +357 kcal/kg from 6-18 weeks did not affect live body weight at 20 weeks of age but increased body fat deposition by 7%. Likewise, Crespo and Esteve-Garcia[43] demonstrated that dietary fat and oil supplementation not only provide additional calories but also influence carcass composition. Beyond its caloric contribution, oil can slow the rate of gastrointestinal transit, thereby enhancing nutrient absorption[44]. In the treated pullets, these nutrients may have been utilized for the synthesis of tissue proteins[45,46] and adipose tissue[47]. This may explain the similar body weight and weight gain observed between the palm oil-supplemented groups and the control group.

With respect to the biochemical parameters, the observed responses varied considerably among treatment groups. Overall, pullets in group H3 exhibited elevated values for all measured biochemical parameters. Total serum proteins comprise proteins associated with nutritional status, inflammation, immune function, transport processes and enzymatic activities[48]. The increase in total protein concentration observed in pullets fed diets containing 2% or 3% palm oil may be indicative of hyperproteinemia resulting from inflammatory or infectious conditions that stimulate gamma globulin production, or it may be associated with dehydration. However, total protein concentration alone lacks sufficient specificity. Therefore, it is often necessary to assess albumin concentration simultaneously to determine whether the observed changes in total protein levels are attributable to alterations in albumin, globulin, or both[49].

On the other hand, the increase in glucose levels in pullets fed diets containing 2% or 3% palm oil may be attributed to diabetes mellitus. According to Harrison and Harrison[50], this condition can induce hyperglycemia in several poultry species. During glycolysis, glucose is metabolized to glycerol and pyruvate. Pyruvate contributes to the formation of acetyl-CoA within the Krebs cycle. Acetyl-CoA represents the basic dicarbon backbone required for fatty acid synthesis[51]. When blood glucose levels are elevated and glucose is not utilized for energy production, it is stored as glycogen through glycogenesis, mainly in the liver and also in muscle tissue. Once the hepatic glycogen storage capacity is reached, excess glucose is converted into lipids via lipogenesis in the liver and adipose tissues. This mechanism may explain the high cholesterol concentrations observed in groups H0 and H3.

Liver damage is generally associated with elevated levels of transaminases and gamma-glutamyl transferase (GGT), which are widely used as biomarkers due to the liver's central role in metabolic and detoxification processes. Hepatic injury may result from malnutrition, viral, parasitic and bacterial infections, poisoning and hepatomegaly[49,52]. GGT is primarily of hepatic origin, ALT is predominantly associated with the liver, whereas AST is mainly found in muscles and the heart[52]. In the present study, liver function assessment showed similar gamma-GT levels across all four groups. However, AST and ALT levels were significantly higher in group H3. The elevated ALT and AST values in group H3 may indicate hepatocellular damage[53]. However, given the similarity in gamma-GT levels among all groups, it is unlikely that the observed increase in transaminases reflects pathological liver dysfunction. Instead, it may originate from extrahepatic tissues, since these enzymes are also present in various organs and their activity can increase with metabolic stimulation.

Lipid profile analysis revealed a significant increase in triglycerides and total cholesterol in treated groups, particularly in group H3. The elevation in triglycerides is closely related to both lipid and carbohydrate metabolism, as triglycerides can be synthesized from carbohydrates, amino

acids, or fatty acids[54]. Triglycerides represent a major source of stored fatty acids and their circulating levels are positively associated with body fat deposition[55]. Excess triglycerides are stored in adipocytes, contributing to abdominal fat accumulation. Palm oil is rich in saturated fatty acids and provides an additional dietary source of these fatty acids when included in the feed. Consequently, increasing levels of palm oil supplementation increase the intake of saturated fatty acids. Overall, saturated fatty acids are considered hypercholesterolemic, as they increase total cholesterol by elevating both LDL and HDL fractions[56]. This may explain the increased cholesterol levels observed in group H3, which received the highest inclusion level of red palm oil. These findings are consistent with those of Onibi *et al.*[57], who also reported increased cholesterol levels in broiler chickens fed diets with increasing levels of palm oil.

CONCLUSION

The findings of this study indicate that the inclusion of palm oil in the diets of 10-week-old pullets had no significant effect on body weight, average daily gain, or feed conversion ratio compared with the control group. However, feed intake was significantly affected only in group H1. Palm oil supplementation also influenced certain biochemical parameters, including increased triglyceride and total cholesterol concentrations. In addition, elevated levels of transaminases and gamma-glutamyl transferase (GGT) were observed in relation to the level of palm oil inclusion in the diet. The feed conversion ratio and average daily gain observed in pullets receiving 1, 2 and 3% palm oil suggest that dietary inclusion at the 2% level may potentially optimize production performance. Further studies are required to evaluate hormonal profiles and egg quality in pullets fed a 2% palm oil-supplemented diet compared with those receiving a standard control diet.

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