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Original Research Article

Growth Performance and Cholesterolemic Level of Broilers Chicken fed with *Penicillium camemberti*-fermented Sweet Orange Peels-based Diet

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ABSTRACT
A forty-two (42)-days feeding trial was conducted with thirty (30) day-old broiler chicks' weighing 34.45 g average and grouped into three groups (n =10). Groups 2 and 3 were fed with feeds substituted with 10% and 20% of 7 days of *Penicillium camemberti*-fermented orange peels (PCFOP) as a replacement for maize. Meanwhile, Group 1 was fed 0% PCFOP. Subsequently, we assessed the growth performance, including body weight, feed intake, and feed conversion ratio, as well as the lipid profile of several tissues (serum, breast, thigh, and drumstick). The study showed that the formulated diets improved broiler chicken growth performance, lowered cholesterol, triglycerides, and low-density lipoprotein, and increased ($p<0.05$) the high-density lipoprotein level in broiler tissue. This study, therefore, proved the novelty of using PCFOP as a replacement for scarce maize grain in the formulation of chicken diets for quality broiler production. However, 20% of PCFOP inclusion proved to be better.
Keywords: Lipid content; Fermentation; Fungi; Carcas; Feeds

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Introduction

The global mortality rate due to excessive fat and cholesterol is increasing annually. Increased cholesterol consumption reportedly causes 2.6 million deaths annually (Moreira et al., 2019). According to Wang et al. (2014), hypercholesterolemia is a known risk factor for cardiac diseases such as atherosclerosis; a condition where arteries narrow due to plaque deposition as a result of higher cholesterol levels, thus restricting blood flow, causes obesity, hypertension, cardiovascular diseases, and diabetes (Santika, 2016). Cholesterol is a fatty substance in food such as beef and chicken (Mahardika, 2017).

The poultry sector plays a substantial role in nourishing the human population. Sahraoui et al. (2019) state that cheap protein-rich meat fulfils man's demand. According to the Food and Agriculture Organization of the United Nations (2018), broiler chickens are specially raised due to meat production, fast growth, and high feed efficiency. Over 90 billion chickens are raised annually for meat production, most of which are broiler chickens (Food and Agriculture Organization of the United Nations, 2018). Broiler production is a major agricultural industry that produces a significant source of meat protein for human consumption. Unfortunately, broiler chickens have some flaws; according to Regar (2019), meat is high in fat and cholesterol, which can cause many diseases. The occurrence of heightened fat and cholesterol quantities in broiler chickens can be ascribed to tactics instigated at increasing the chickens' body weight within a short period. Owning to the high cholesterol content of broiler meat when excessively consumed, particularly with the skin. Cardiovascular diseases like atherosclerosis are linked to high LDL cholesterol. Fruits and vegetables include phenols that regulate cholesterol ester production, lowering heart disease risk. (Ito, 2021).

Agro-industrial by-products like citrus peel have produced animal diets instead of cereals (Oluremi *et al*., 2018). This is largely due to their richness in molasses, flavonoids, pectin, saponin, tannin, limonene, and oxalate (Ademosun et al. 2018). Previous studies on orange peel indicate its richness in calories and protein relative to maize and have ascertained its hypocholesterolemic activity via inhibition of HMG CoA reductase, an enzyme involved in cholesterol synthesis (Oyeyemi et al., 2018; Ademosun et al., 2018; Ashraf et al., 2019), antihypertensive (Oguntuase et al., 2022) and cognitive properties (Oyeleye et al., 2021). Its antinutrient composition, including phytate, has been reported to be improved by fermentation (Oluremi et al., 2018).

Several factors limit the use or high incorporation of non-conventional feedstuffs in livestock feed, including low protein content, high fiber, amino acid imbalance, and the presence of anti-nutritional factors. (Mourao *et al.,* 2008). To eliminate the anti-nutrients and pathogens present within the citrus peels, the peels can be subjected to different processing, including drying and fermentation (Ghanem *et al*., 2012). Fermentation is a metabolic process where microbes, like fungi, biologically transform sugars into other products, influencing nutrition, agriculture, and industry. According to Oluremi et al., (2018), fermentation is done on citrus peels using Microfungi to halt spoilage, remove harmful compounds, and improve the nutrient and digestibility of the by-product. Previous nutritional research on monogastric animals revealed that incorporating fermented peel products into broiler diets as a novel replacement for up to 20% of maize does not negatively alter bird performance (Oboh et al., 2009). In this study, fermentation was carried out on the orange peels to enhance their digestibility and make micronutrients present using *Penicillium camemberti*, and further determine the effectiveness of *Penicillium camemberti* fermented orange peels as a substitute in the broiler feed, and also to its effect on growth performance (body weight, feed intake, and feed conversion ratio), and tissue (serum breast, thigh, and drumstick) lipid profile in the serum and tissues of broiler chickens.

Materials and Method

Collection and processing of orange peels

Fresh orange peels were collected from orange fruit vendors within the Campus. The peels were further subjected to drying using the oven drying technique. Upon drying, the peels were milled into powder using a locally fabricated grinding machine for easy handling, storage, and analysis. Thereafter, 2 kg of the dried milled peels were measured, each in five (5) fermenter boxes provided.

Solid-state fermentation was carried out on the dried milled orange peels to improve their digestibility and nutrients and to break down the cellulose components. *Penicillium camemberti* was obtained from the Department of Microbiology, FUTA. The nutrient solution was prepared by measuring the appropriate mass of the following reagents: urea, $MgSO_4.7H_2O$, KH_2PO_4, and citric acid. The measured reagents were dissolved into 1000 ml of distilled water. *Penicillium camemberti* in 370 ml nutrient solution [containing urea (8.0g), $MgSO_4.7H_2O$ (7.0g), KH_2PO_4 (1.3 g), and citric acid (2.0 g)] was carefully mixed with the peels to produce a solid matrix (Fig. 1). This was done on three fermenter boxes fermented for 7 days. The atmospheric temperature and humidity were kept at 30 ℃ and 90-93%, respectively. (Oboh 2006). The fermentation was terminated after seven days and removed from the fermenter boxes to compound the diet.

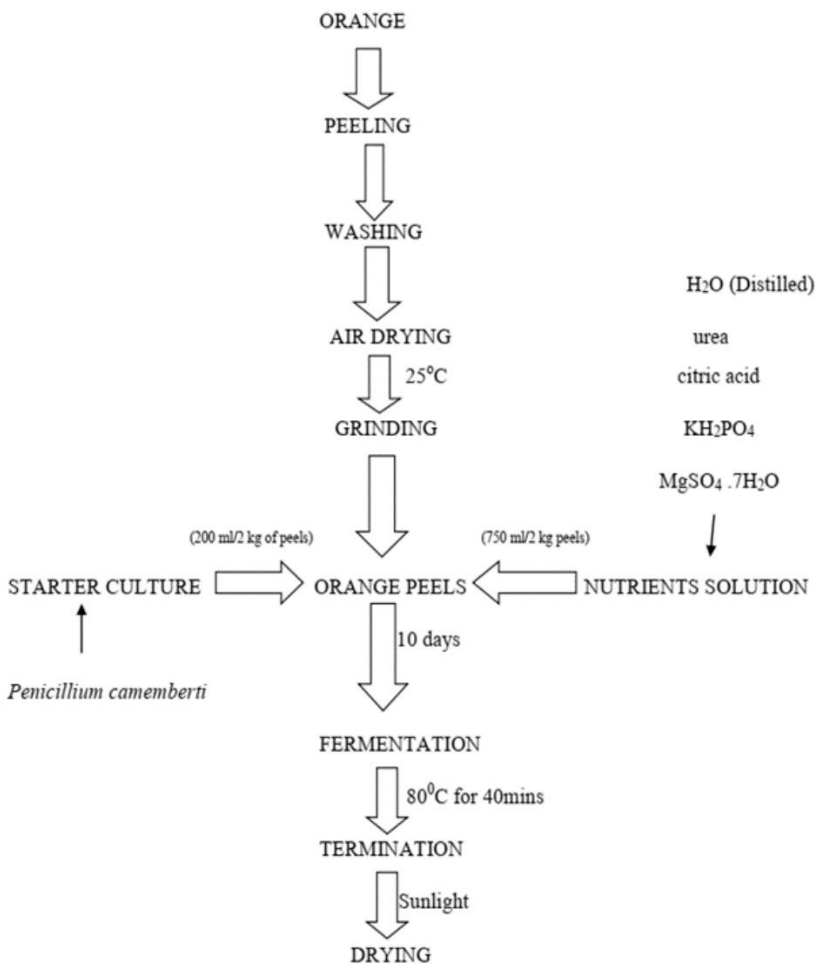

Figure 1: Solid substrate Fermentation flowchart

Experimental Diets Formulation

The basal Diet was formulated for the broilers (1 to 42 days of age) phase, as shown in (Table 3.1) to meet the National Research Council (NRC) requirement. The diets were supplemented with the Microfungi fermented orange peels and thoroughly mixed, with 70% of each diet pelletised using a Hobart pelleting Machine (Hobart Model 200 CA, USA) to get uniform-size pellets (2mm) to form the basal diet. The pelleted feeds were dried for 3days. The basal diet without orange peels serves as the control. There were 3 diets for the feeding trial, and they consisted of Diet 1 = 0% PCFOP, Diet 2 = (10% PCFOP), Diet 3 = (20% PCFOP)

Table 1: The Experimental Feed Formulation

Feed for broiler (in grams)	0% PCFOP	10% PCFOP	20% PCFOP
Fish meal	900	900	900
Soya bean	16200	16200	16200
Maize	28545	25690.5	22836
Fermented peel	___	2854.5	5709
Wheat ova	1835	1835	1835
Palm oil	450	450	450
Bone meal	1125	1125	1125
Limestone	225	225	225
Methionine	270	270	270
Premix	225	225	225
Salt	225	225	225
Total	50000	50000	50000

Experimental Design

Thirty-day-old Arbor broiler chickens were taken from the hatchery and randomly assigned to three (3) treatments, with the first being the control. The three (3) treatments were divided into two groups with a stocking density of 5 birds each.

Site Preparation

For this experiment, a deep litter system was used. Six pens were thoroughly cleaned, covered with white nylon for brooding, and disinfected with Izaal solution and detergents. Dry wood shavings obtained from sawmills were poured on the floor to serve as litter for the chicks. Also, feeders and drinkers were cleaned and disinfected before the birds arrived.

Management and Handling

Upon the arrival of the birds, water containing glucose was given to them to relieve them of the stress. The birds were randomly allotted to 3 different dietary treatments, with each treatment having ten birds each. Each treatment was replicated, with each replicate having a stock density of 5 birds each. The brooding continued 24 hours a day for two weeks, but the heating intensity was reduced as the birds grew older. Adequate ventilation was provided for the birds to eliminate heat stress and ammonia buildup. Flat plastic trays and chick drinkers were used for the first few weeks before it was changed to bigger steel feeders and bowl drinkers. Birds of the same treatment but of different replicates were placed on the same diet, and leftover portions of the ration for each replicate were collected and weighed at the end of each week. Birds were carefully separated per replicate so they could neither eat nor cross over from one dietary treatment to another. They were managed under intensive care, and hygienic measures were implemented. Water drinkers were cleaned daily and filled with clean, cool water.

Growth Performance

The birds were weighed on the first day they were received and continuously every seven days until the sixth week to evaluate their growth performance. Body weight was evaluated on the sacrifice day.

Feed Conversion Ratio (FCR) and Feed Intake

The FCR measures feed intake per body weight gain and measures the efficiency of feed utilisation in broiler chicken (Chen et al., 2019). A lower FCR indicates better feed efficiency and improved growth performance (Pesti et al., 2018). FCR was evaluated by being weighed weekly and measuring the feed before it was given to the birds. The weight difference between provided and eaten food was computed. This figure was calculated by subtracting the whole feed ration from the weekly feed ration during feeder cleaning.

Tissue Collection and Homogenization

On the sacrifice day, three birds were taken from each treatment and slaughtered. The blood, breast, thigh, and drumstick were taken from each bird and labelled concerning their group. The blood was centrifuged to obtain the serum, while the gizzard, breast, thigh, and drumstick were homogenised using a Teflon glass homogeniser. The resulting homogenates were centrifuged at 5000 × g for 10 minutes, and the supernatants from the homogenate and the serum were stored in a freezer and used for subsequent biochemical analyses.

Biochemical Assays

Chemicals and Reagents

The kits used were from FORTRESS Diagnostics Laboratories Limited. Urea, Na_2CO_3, $NaHSO_4$, $MgSO_4.7H_2O$, KH_2PO_4, and citric acid were from BDH Chemicals Ltd. The water used was glass distilled.

Determination of Lipid Profile in Chicken Tissue Samples

The lipid profile encompassing Total cholesterol (TC), Triglycerides (TG), Low-density lipoprotein (LDL), and High-density lipoprotein (HDL) of both the homogenates and serum were determined using the Fortress diagnostic kits from Fortress Laboratories Limited, UK.

Determination of Low-density Lipoprotein (LDL) Concentration

The LDL-cholesterol concentration of the samples was determined according to the methods reported by Oyeleye et al. (2019).

LDL cholesterol (mg/dl) = Total cholesterol – Triglycerides/5 – HDL cholesterol.

Data Analysis

The results in replicates were analysed using GraphPad PRISM and were pooled and expressed as mean and standard deviation (SD) values. One-way analysis of Variance (ANOVA) was used to analyse the results with levels of significance accepted at $p<0.05$, $p<0.01$, and $p<001$.

Result and Discussion

Hypercholesterolemia is defined as a heightened serum cholesterol level. According to Hu et al (2020), hypercholesterolemia is a major risk factor for atherosclerosis and coronary artery disease. Hence, serum and tissue cholesterol levels must be controlled to prevent it. According to Farzaneh and Carvalho (2015), recent research has examined the medicinal benefits of side-effect-free plant extracts.

Orange peels are regarded as waste from oranges, and several studies have reported their beneficial effects with hypolipidemic, hypoglycemic anti-inflammatory, and antioxidant properties, making them a promising candidate for regulating serum and tissue lipid levels in a living system. Alzawqari et al. (2016) indicated that integrating dried orange peel into the broiler diets could positively modify the health status and regulate the serum cholesterol level and the antioxidant status in broiler chickens. The hypocholesterolemic effect of citrus peel (Fig. 2) agrees with an earlier report where it was shown to possess some cholesterol-lowering agents (Munakata et al., 2012).

The results in Table 2 and Table 3 revealed that weight, total weight gain, total feed intake, and daily feed intake increase as the level of inclusion increases in the starter and finisher phases compared with the control. Studies revealed that broilers fed with fungi-fermented diets formulated with the inclusion of citrus peels had improved performance relative to those that were fed with non-fermented citrus peels formulated diets. According to the report of Sugiharto (2023), high energy, carbohydrate, and fat contents present in fruit peel meals could be responsible as poultry nutritionists relate the incorporation of fruit peel into poultry meals, especially when the goal is to decrease conventional energy sources. Adekeye et al. (2021) replaced wheat and maize bran with cassava peel meal at 150 kg/ton, improving growth performance and cost savings. Badr et al. (2019) used pear fruit peel powder in broiler feed, enhancing feed conversion ratio, growth rate, biological values, and net protein utilisation. The research above ascribed the improved development performance to the diverse bioactive constituents of the fruit peels. The PCFOP composite feed may have enhanced the functionality and health of the broilers' digestive systems, hence augmenting protein efficiency, growth performance, and nutritional digestibility (Sugiharto, 2023).

Table 2: Growth Performance of Broilers Fed with *Penicillium camemberti* Fermented Orange Peel (PCFOP) Supplemented Diet on the Starter Phase (Weeks 0 to 3)

Parameters	**0% PCFOP**	**10% PCFOP**	**20% PCFOP**
Initial weight (g)	34.40	34.40	34.35
Final weight (g)	610.00^{a}	629.50^{b}	640.00^{c}
Total weight gain (g)	575.60^{a}	595.10^{c}	605.65^{d}
Total feed intake (g)	948.99^{b}	969.99^{d}	991.41^{e}
Daily feed intake (g)	45.19^{b}	46.19^{d}	47.21^{e}
*Feed C. R (g)	1.41^{e}	1.59^{b}	1.56^{c}

Values represent mean ± standard deviation. Feed conversion ratio

Table 3: Growth Performance of Broilers Fed with *Penicillium camemberti* Fermented Orange Peel (PCFOP) Supplemented Diet at the Finisher Phase (Weeks 4 to 6)

Parameters	**0% PCFOP**	**10%** PCFOP	**20%** PCFOP
Initial weight (g)	610.00^{a}	629.50^{b}	640.00^{c}
Final weight (g)	2150.00^{b}	2220.00^{c}	2250.00^{d}
Total weight gain (g)	1540.00^{b}	1590.50^{c}	1610.00^{d}
Total feed intake (g)	3004.00^{a}	3115.00^{c}	3180.00^{d}

Values represent mean ± standard deviation.

These alterations can influence their willingness to consume the feed, making it less appealing to the chickens. However, the broiler's feed conversion, which increases as the level of the *P.* camemberti fermented orange peels increases, could be due to the nutrient composition of the orange peels. If feed components are not in optimal proportion for broiler growth, it can affect feed efficiency. Also, it could be attributed to the nutrient digestibility of the orange peels, which may vary with their diet levels. Palatability, feed intake, and the byproducts of the fermentation could also influence the gut environment. When the groups were compared with the control group, the group that fed 20% *P. camemberti* fermented orange peel supplemented had the best ($p>0.05$) and lowest feed conversion ratio at the starter phase. In comparison, the group fed 10% *Penicillium camemberti* fermented orange peel supplemented was best ($p>0.05$) at the finisher phase. This means that both groups most efficiently converted feeds to meat at their respective phases.

The results from Figures 2-5 showed that *P. camemberti* fermented orange peels caused a significant ($p>0.05$) reduction in triglyceride, total cholesterol, and LDL-cholesterol levels of the serum and the tissues. Conversely, the HDL-C levels in these samples increased significantly compared to the control group. Hence, these results indicate that *Penicillium camemberti* fermented orange peels can effectively prevent hypercholesterolemia in broiler chickens. In citrus fruits, peels are reported to possess the highest amounts of polyethoxylated flavones compared to other edible parts of the fruit (Wang et al., 2014). The citrus flavonoids comprise two categories of compounds: glycosides, notably hesperidin and naringin, and O-methylated aglycones of flavones, such as nobiletin (Li et al., 2014). The presence of abundant phenolics and flavonoids, particularly hesperidin, in orange peels is likely to reduce triglyceride, total cholesterol, and LDL-cholesterol levels while boosting HDL-C levels in serum and tissues. Phytochemicals, such as phenolic and flavonoids, have been discovered to impede the activities of two significant enzymes that play a role in the production and absorption of cholesterol. These enzymes are 3-hydroxyl-3methylglutaryl CoA reductase (HMG-CoA reductase) in the liver, which is primarily responsible for synthesising cholesterol, and intestinal acyl CoA: cholesterol acyltransferase (ACAT), which is essential for converting cholesterol into ester form before it can be absorbed (Nguyen et al., 2012). Several epidemiological studies have shown that citrus peel effectively decreases plasma liver cholesterol, serum triglyceride levels, serum total cholesterol, liver total lipids, and liver cholesterol (Ling et al., 2020).

The study demonstrated that fermentation of orange peels using *P. camemberti* had improved the hypocholesterolemic activity of orange peels incorporated in broiler feed by degrading the fibre contents to aid digestion, boosting its medicinal potentials via microorganism multiplication and secretion of extracellular enzymes and improving the bioavailability of micronutrients (Oboh et al., 2009).

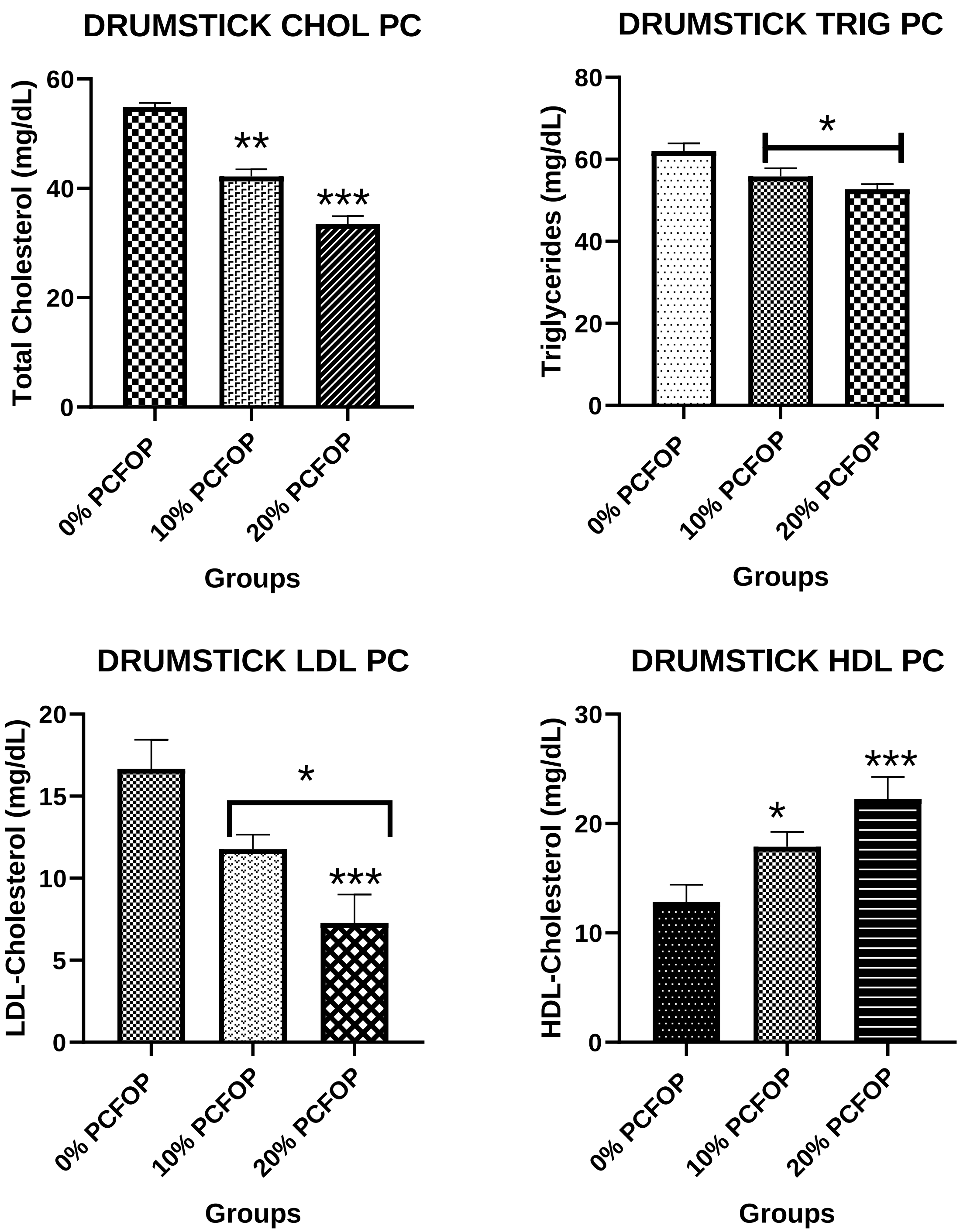

Figure 2: Lipid Profile Levels in the Drumstick Tissues of Chickens Fed with *Penicillium camemberti*-Fermented Orange Peels (PCFOP) of Broiler Chicken.

Keys: Control group fed with 0% PCFOP; P at 10% group fed with 10% PCFOP; P at 20% group fed with 20% PCFOP.

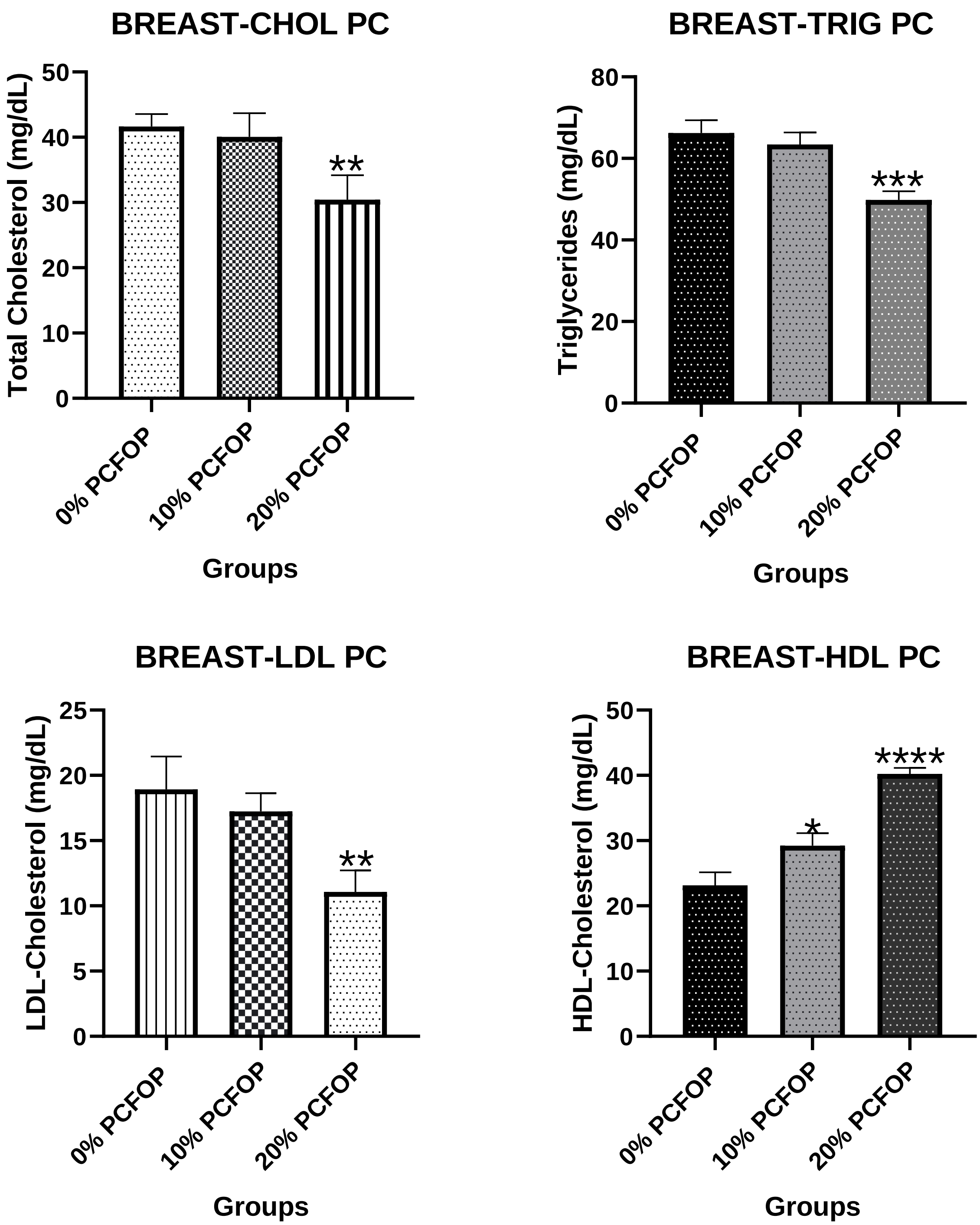

Figure 3: Lipid Profile Levels in the Breast Tissues of Chickens Fed with *Penicillium camemberti*-fermented Orange Peels (PCFOP).
Keys: Control group fed with 0% PCFOP; 10% PCFOP fed group; 20% PCFOP fed group.

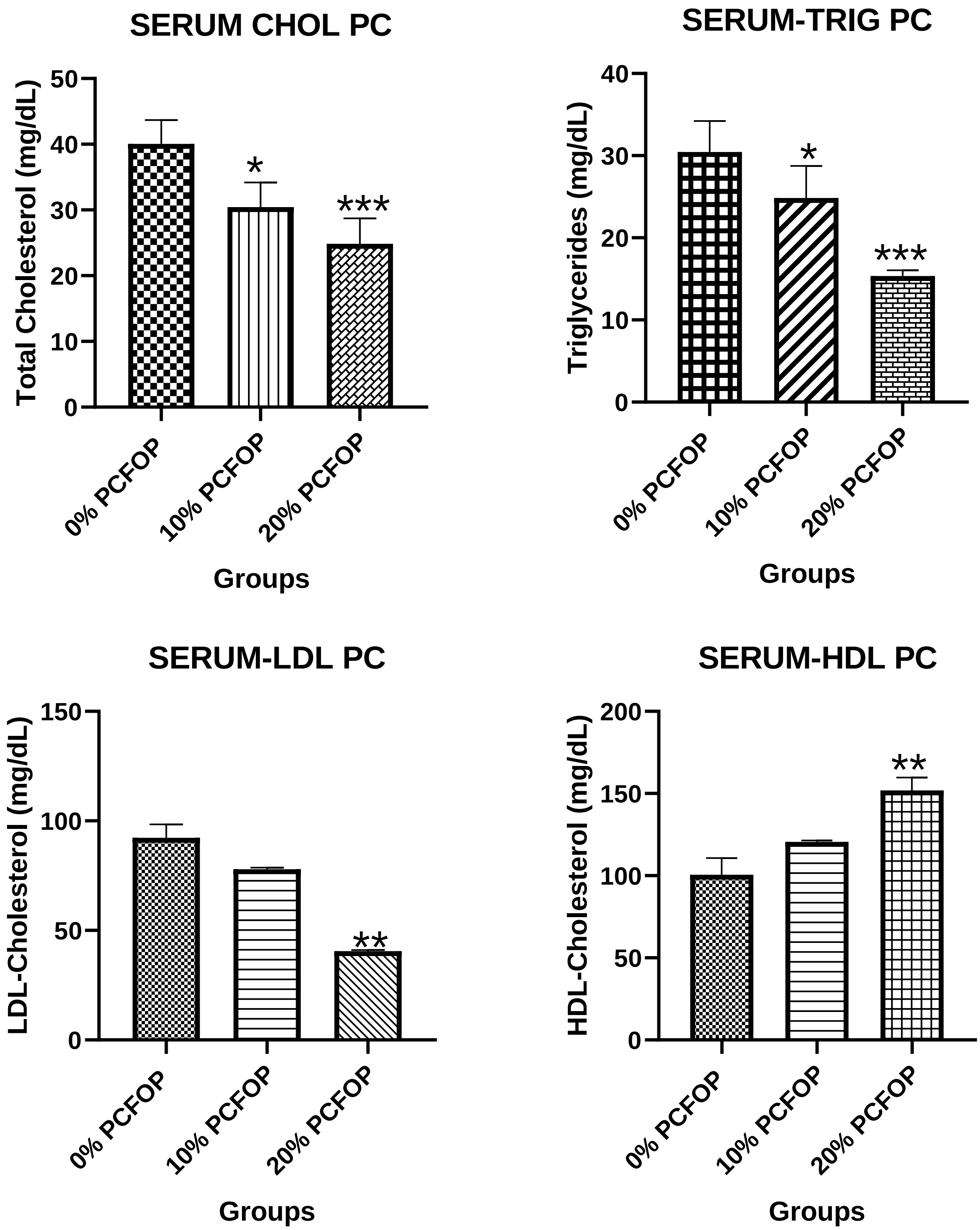

Figure 4: Lipid Profile Levels in the Serum of Chickens Fed with *Penicillium camemberti*-fermented Orange Peels (PCFOP).
Keys: Control group fed with 0% PCFOP; P at 10% group fed with 10% PCFOP; P at 20% group fed with 20% PCFOP.

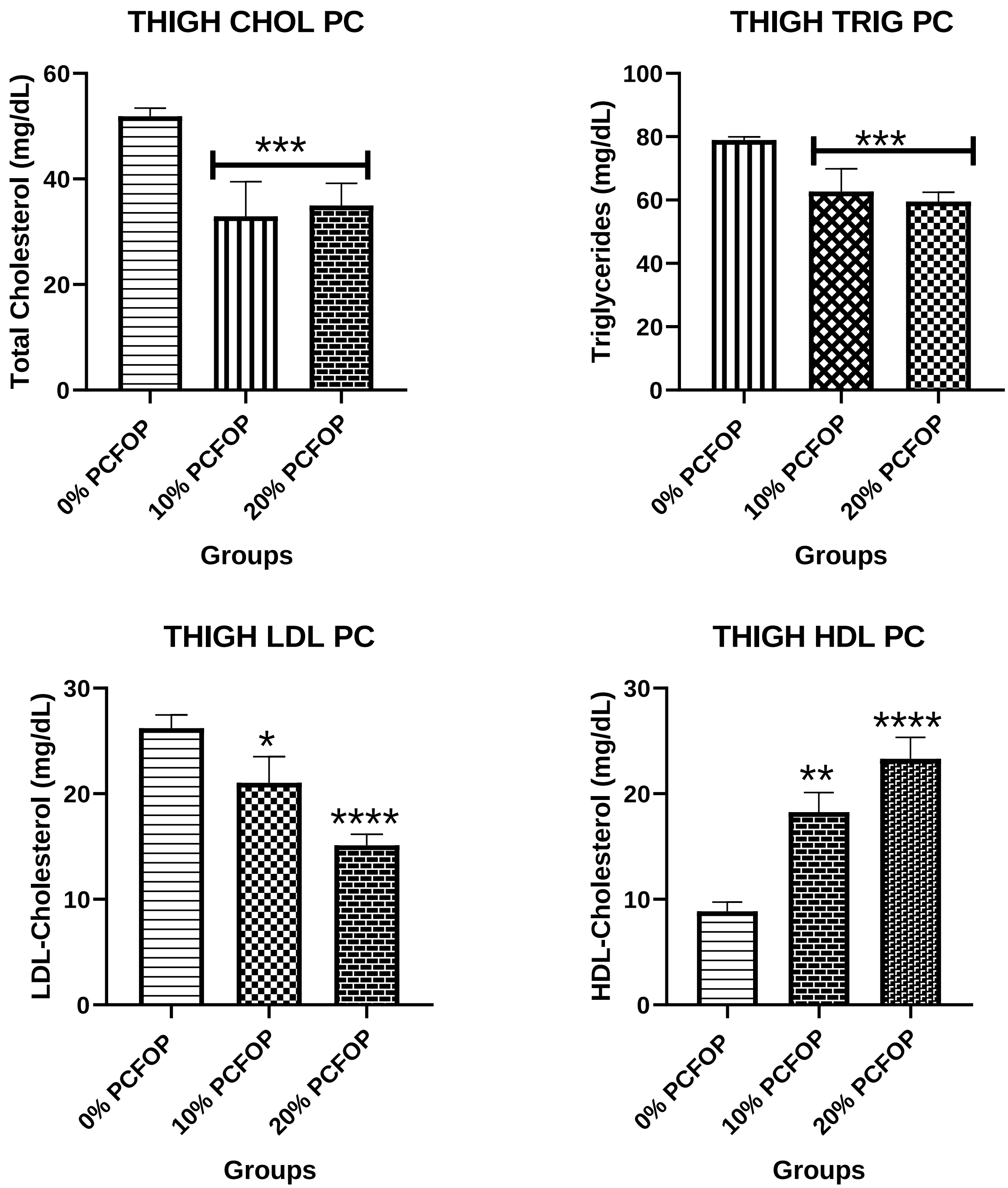

Figure 5: Lipid Profile Levels in the Thigh Tissue of Chickens Fed with *Penicillium camemberti*-fermented Orange Peels (PCFOP).
Keys: Control group fed with 0% PCFOP; P at 10% group fed with 10% PCFOP; P at 20% group fed with 20% PCFOP.

Conclusion

The study findings provide valuable insights into how fermentation can improve the hypocholesterolemic activity of orange peels using *Penicillium camemberti* in broiler birds and an avenue to convert agricultural wastes to wealth. Based on the observed results, dietary *Penicillium camemberti* fermented orange peel up to 20% may be beneficial to regulate the serum and tissue lipids level and improve the HDL level and FCR of the broilers, with no negative effect on the blood biochemistry of broilers. Hence, this study therefore recommends the use of *Penicillium camemberti* fermented orange peel, especially at 20%, as a feed supplement for raising broilers,

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Conflict of Interest,

On behalf of all authors, the corresponding author states that there is no conflict of interest**.**

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Author Contributions

GO, TO, and BA conceived and designed the experiment. AA, OO, LN, SB, OO, and IO generated the data, analysed them, and drafted the manuscript. GO, TO, BA, AA, OO, LN, and OO reviewed the manuscript. All authors read and approved the manuscript.

Ethical Approval

Not applicable

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