



Serum Biochemistry and Antioxidant Enzyme Response of Local Laying Hens Fed Jackfruit (*Artocarpus Heterophyllus*) Leaf Meal

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ABSTRACT

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This study, that was carried out for 8 weeks, evaluated the serum biochemistry and antioxidant enzyme responses of seventy-two local laying hens fed different levels of jackfruit leaf meal (*Artocarpus heterophyllus*; JLM). The hens were randomly distributed to four different treatment (T) groups in a completely randomized design. Each treatment containing 18 hens were replicated 3 times, with each replicate containing 6 laying hens. JLM was prepared by plucking the fresh leaves which were air -dried at room temperature until crispy. The dried leaves were milled into powder and stored in an air- tight container. The JLM was included in the basal feed at 0 g/kg, 20 g/kg, 40 g/kg, and 60 g /kg of feed, representing T1, T2, T3, and T4. Results showed highly significant differences ($p < 0.01$) in AST among the treatment groups. Hens fed 60 g JLM /kg diet exhibited significantly ($p < 0.01$) lower AST (54.02 IU/l) and MDA (1.67 mg/dl) compared to those of control birds (59.67 IU/l and 4.15 mg/dl). ALT and ALP did not vary significantly ($p > 0.01$) across the treatment groups. SOD and CAT levels increased as the inclusion level of JLM increased across the treatment groups with the highest mean value observed in hens that received 40 g (8.01 and 0.62 mg/dl) and 60 g JLM inclusion (8.30 and 0.78 mg/dl), while GPx was not significantly affected ($p > 0.01$). The study concluded that local laying hens under tropical conditions should be fed 40 g to 60 g JLM/ kg diet for optimal metabolic health and antioxidant capacity.

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INTRODUCTION

The native chicken of Nigeria is a great source of protein for the people of Nigeria (Manyelo et al., 2020). In Nigeria, the native chickens significantly improve household food security and is the most preferred choice for consumers over commercial chicken as a result of its distinct sensory qualities such as its flavor, texture and little chemical contamination (Odunitan-Wayas et al., 2018). It provides stability and investment, particularly for the impoverished in rural areas where it is mostly utilized as a supply of meat and eggs for both revenue and consumption (Mujiyambere et al., 2022). However, the problem of diseases, high cost and scarcity of essential feedstuff have limited the Nigerian native chicken from attaining its full potential thus limiting the animal protein supply (Abadula et al., 2022). The native chicken grows slowly and because feed is expensive, there is little need to feed them high amount of protein and energy to increase their growth rate and egg laying performance (Afolabi, 2013). Nevertheless, it is crucial to find ways to enhance the general health and strengthen their immune system so that production can increase without needlessly increasing costs.

Jackfruit (*Artocarpus heterophyllus*) is a tropical tree fruit indigenous to southwest India and is a member of the Moraceae plant family. Jackfruit leaves are readily available in different parts of Nigeria and have not been fully exploited as feedstuff for animal (Eburuaja et al., 2019). Every part of the plant and its fruit is utilized for human consumption, animal feed, and as a source of wood for making furniture. While jackfruit is the primary fruit of the tree, the wood is valued for furniture because of its attractive texture and color (Khan et al., 2021).

The jackfruits have been utilized in herbal remedies due to the presence of pharmacological properties such as anti-bacterial, anti-diabetic, anti-oxidant, anti-inflammatory and anti-helminthic (Vazquez-Gonzalez et al., 2020; Jishma and Sreelakshmi, 2021; de Sousa et al., 2022). Studies have indicated that the leaves of jackfruit may offer various health advantages, including being an excellent provider of vitamin C and abundant in other vital minerals like nitrogen, phosphorus, potassium, calcium, magnesium, zinc, copper etc. (Afotey et al., 2024). The health benefit associated with jackfruit can be ascribed to the presence of bioactive substance such as flavonoids, stilbenoids, phenolics, carotenoids and steriods (Vázquez-González et al., 2020).

Jackfruit leave meal (JLM) in feeds of poultry can boost the immune system, enhance liver functions and antioxidative enzymes of local poultry enhancing productivity as it is rich with bioactive compounds, vitamins and minerals which are necessary for both animal and human existence (Dixit et al., 2023). Several studies have reported the crucial role antioxidants play in maintaining cellular integrity and function, particularly in the liver, which is pivotal for egg production (Obianwuna et al., 2022;

Wang and Xu, 2022; Anene et al., 2023; Han et al., 2023). The liver synthesizes lipids and proteins necessary for yolk formation, making its health directly correlated with egg quality and quantity (Wang et al., 2017; Heinzl, 2021; Gu et al., 2021; Anene et al., 2023). The micronutrients in JLM are necessary for animals' regular metabolism and regular physiological processes like growth, development, maintenance, and reproduction, in addition to optimal health (Shastak and Pelletier, 2024). Incorporating JLM into the diet of local hens could improve their metabolic health by providing essential antioxidants that combat oxidative stress (Khan et al., 2021; Afotey et al., 2024). The majority of these bioactive compounds in poultry, must come from the diet because the poultry birds cannot synthesis them in large enough quantities to meet physiological needs. They can be taken from the diet during digestion and are found in small concentrations in a variety of feedstuffs. Certain deficiency disorders or syndromes can be brought on by micronutrient deficiencies or by inadequate absorption or utilization in the diet leading to malfunctioning of major organ such as the liver which is crucial in egg laying (Shastak and Pelletier, 2024).

The production of the native chicken is currently more challenged than ever to increase efficiency in order to reach its full potential in the cost-driven economy of today. The local hens with low performing ability and poor feed conversation efficiency can be improved to a high laying performance, optimal feed conversion capacities, and favorable health traits, if the birds' diet, can be enriched with unconventional feedstuff that promote liver function and reduce oxidative stress (Bryden et al., 2021). Their supply can be maximized to reach the genetic potential of the local laying hens, meeting the increasing demand of consumers and contribute to the source of revenue of the rural poor who rear indigenous chickens. To authenticate this postulation, it becomes crucial to assess the blood profile of native laying hen to ascertain the level of improvement to the overall animal health. The function of the liver is crucial in egg production as its malfunction can lead to health disorders, poor egg quality and low performance (Saraswati et al., 2013). It becomes important to monitor the liver health especially in laying hen. Evaluating the serum levels of liver enzymes such as alanine aminotransferase and aspartate aminotransferase are critical in evaluating liver health, as their elevated levels can indicate liver damage or dysfunction, which could adversely affect egg production in the hens (Heinzl, 2021; Anene et al., 2023). Furthermore, understanding how jackfruit leaves influence antioxidant enzyme activities (such as superoxide dismutase and glutathione peroxidase) can provide insights into their protective effects against oxidative stress. This is particularly important given that aging hens often experience increased oxidative damage, leading to reduced productivity (Gu et al., 2021).

In Nigeria, there is little information related to the leaves of jackfruit on local hens; factors such as age, sex, breed, diet, and environment can influence the serology and oxidative enzymes hence this research was executed to examine the serum

biochemistry and oxidative enzyme response of local laying hens fed Jackfruit leaf meal.

MATERIAL AND METHOD

Ethical Approval

The research procedure was reviewed and accepted by the Animal Welfare and Ethic Committee of the Department of Animal Science, University of Nigeria, Nsukka (UNN), on the use of animals for experiment. The approval code is No: UNN/C076ANS14.06.07.2024.

Location and Duration of The Study

The trial was conducted at the Avian unit of the Department of Animal Science, Teaching and Research Farm, UNN, in Enugu State, South Eastern Nigeria. Nsukka is located at the latitude 6°51'28.19" N and longitude 7°23'44.77" E at an altitude of 430 m above sea level (Onyenucheya and Nnamchi, 2018). The average temperature is 24.90 C / 76.80 F and annual rainfall is 1579 mm/62.2 inches (Ihinegbu, 2019). The experiment took 8 weeks to complete.

Sourcing and Handling of Jackfruit Leaf Meal

Fresh leaves of jackfruit were plucked during the flowering and fruiting stage from the Crop Science Department, UNN. Dirt was removed from leaves by washing them, afterwards, air-dried at room temperature 28 °C until the leaves are crispy to touch. The dried leaves are milled using the miller and subsequently sieved to obtain powder. The powder was stored in air- tight containers.

Experimental Animal, Design and Management

A total of seventy- two local laying hens of 34 weeks old were used for this study. The local hens were obtained from the Avain Unit, of the Department of Animal Science, Teaching and Research Farm, UNN. The experiment was conducted in a completely randomized design (CRD) with four treatments: control, 0 g of JLM /kg of feed; 20 g of JLM/kg of feed, 40 g of JLM/kg and 60 g of JLM/kg of feed, representing T1, T2, T3 and T4, respectively (Kusmartono, 2007). The laying hens were uniformly distributed to the 4 treatment groups, each with three replicates, comprising 6 hens each. They were housed in deep litter pens with adequate space, ventilation and lighting. Wood flakes were used as the main bedding material. Hens were fed ad libitum with basal diets and safe drinking water. The chickens were properly administered routine vaccinations and prophylactic treatments, and were closely monitored for signs of ill health throughout the study. A standard diet that meets the nutritional requirements

of laying hens in the tropical environment by the National Research Council (1994) was fed to the hens. The ingredients and nutrient compositions of the experimental diets are presented on dry matter basis in Table 1 while Proximate composition, phytochemical and antibacterial activity of JLM are presented in Table 2. The nutrient compositions were calculated from standard values (Aduku, 2004), while proximate compositions were determined following the official methods of analysis outlined by the Association of Official Analytical Chemists (2023). Alkaloid content was assessed using the method described by Daniel et al. (2020) and further quantified according to Nilakandhi et al. (2023). Phenolic compounds were separated by High Performance Liquid Chromatography (HPLC) as per the procedure of Dhibi et al. (2022). Additionally, the antioxidant activities of the leaf meal were evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity assay following the protocol specified by Murali et al. (2021).

Table 1. Ingredients and nutrient compositions of the experimental diets

Ingredients (g)	Control	20 g JLM	40 g JLM	60 g JLM
Maize	530.00	512.00	494.00	488.00
Soybean meal	250.00	248.00	246.00	232.00
Jackfruit leaf meal (JFL)	0.00	20.00	40.00	60.00
Wheat offal	200.00	200.00	200.00	20.00
Limestone	10.00	10.00	10.00	10.00
Di-calcium phosphate	5.00	5.00	5.00	5.00
Salt	2.50	2.50	2.50	2.50
Vitamin and mineral premix*	2.50	2.50	2.50	2.50
Total diet weight (g)	1000.00	1000.00	1000.00	1000.00
<i>Calculated nutrients (%)</i>				
Crude protein	18.50	18.70	19.00	19.30
Crude fiber	6.50	6.80	7.10	7.40
Crude fat	4.00	4.10	4.30	4.50
Calcium	2.50	2.50	2.50	2.50
Phosphorus	1.60	1.60	1.60	1.60
Metabolizable energy (kcal/kg)	3264.00	3253.00	3243.00	3252.00
<i>Proximate composition (%)</i>				
Dry matter	90.00	89.50	89.00	88.50
Crude protein	20.60	20.90	21.30	26.30
Crude fibre	7.20	7.50	7.80	8.11
Ether extract	4.40	4.50	4.70	4.90
Ash	6.70	6.80	7.00	7.20
Nitrogen-free extract	51.11	50.30	49.20	48.60

*composition per kg diet: vit A, 6000.00 IU; vit D₃, 2000.00 IU; vit E, 10.00 mg kg⁻¹; vit K₃, 1.60 mg kg⁻¹; vit B₁, 1.40 mg kg⁻¹; vit B₂, 4.00 mg kg⁻¹; vit B₆, 2.00 mg kg⁻¹; vit B₁₂, 0.01 mg kg⁻¹; niacin, 30.00 mg kg⁻¹; folic acid, 11.00 mg kg⁻¹; choline, 0.24 g kg⁻¹; zinc, 50.00 mg kg⁻¹; Fe, 50.00 mg kg⁻¹; Mn, 50.00 mg kg⁻¹; Cu, 100.00 mg kg⁻¹; iodine, 1.20 mg kg⁻¹; Se, 0.20 mg kg⁻¹.

Table 2. Proximate composition, phytochemical and antibacterial activity of JLM

Proximate parameters	Percentage (%)
Moisture	5.80
Ash	4.11
Fibre	4.64
Protein	20.89
Energy (kg/KJ)	12353
Total phenol (g/100g)	9.72
Flavonoids (g/100g)	7.78
Alkoloids (g/100g)	5.76
DPPH radical scavenging activity (IC ₁₀₀ (µg/mL)	8.91

Data Collection

Upon conclusion of the trials, blood (3 ml) was drawn from the wing vein of six hens in each replicate using sterilized syringes and needles and transferred into sample tubes without ethylene diamine tetra acetic acid for serum biochemistry analysis. Following collection, the blood samples were left to clot for 30 minutes, after which it was centrifuged at 3000 revolution per minutes for 10 minutes in order for the serum to be separated from the clot. Serum was carefully collected after centrifugation and transferred into a clean sample bottle. Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) and Alanine aminotransferase (ALT) and oxidative stress indicators such as catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione peroxidase (GSH-Px) were determined calorimetrically according to the method of Young (2001).

Statistical Analysis

The data were subjected to one-way ANOVA analysis using SPSS software (version 22.0) to analyze experimental data for significant differences by comparing means of the different treatments. Significant differences among means were identified using Duncan's new multiple range test.

RESULTS AND DISCUSSION

Serum Biochemistry of Local Laying Hen

The findings of the effect of JLM on serum biochemistry of local laying hens are presented in Table 3. Results showed that AST was highly significant ($P < 0.01$) while ALP and ALT were not significant ($P > 0.05$). AST values decreased from T2 –T4 (58.00 to 54.00). The control recorded the highest mean value (56.67) while T4 recorded the lowest mean value (54.00).

Table 3. Effect of JLM on the serum biochemistry of local laying hens

Parameters (IU/L)	T1	T2	T3	T4	SEM	P- value
AST	59.67 ^a	58.00 ^{ab}	56.33 ^{bc}	54.00 ^c	0.76	0.01**
ALT	23.67	23.67	23.33	24.33	0.82	0.88 NS
ALP	52.00	52.00	49.33	53.33	1.23	0.27 NS

a, b, c: means in the same row with different superscripts are significant at (** P<0.01); SEM, standard error of the mean NS; not significant; AST: Aspartatase aminotransferase; ALT: Alanine aminotransferase; ALP: alkaline Phosphatase, T1 = control, T2 = 20 g/kg of feed, T3 =40 g/kg of feed, T4 = 60 g/kg of feed

AST, ALP and ALT are liver enzymes and are crucial in assessing the normal operation of the liver (Odunitan-Wayas et al., 2018). An increment in the values of these enzymes may indicate damaged or diseased cells which signify the condition of the liver function. The results of this experiment indicate values that are consistent with the established range for AST: 40.20- 41.25 U/L; ALT: 22.50-41.25 and ALP: 23.0-287.9 reported by some authors (Atansuyi et al., 2019; Okpogba et al., 2019; Alum et al., 2020). Specific range was not reported in literature for ALT, but the values are usually lower than AST. ALP is reported to vary widely with few literatures reporting values up to 287.98 U/L in Nigeria indigenous chickens. This variation in ALP may be due to environmental factors and specific population that was studied. The values got in this study are higher and not consistent with that stated by Adewole et al. (2021), and are lesser than the values reported by Odunitan-Wayas et al. (2018). From the result, ALP and ALT were not affected by the treatment. This may indicate that the liver and kidney of the local hens were functioning normal within the range of the inclusion level of JLM used.

This result implies that the liver was functioning well and was not compromised. The improved liver health may be attributed to the hepatoprotective and anti-inflammatory properties of jackfruit leaves. These properties help prevent the formation of harmful radicals in the liver and enhance the liver's detoxification process, allowing it to effectively remove toxins and harmful substances. As a result, the hens are protected from liver toxins and infections, which supports liver cell regeneration and promotes their overall well-being (Han et al., 2023; Anene et al., 2023). The JLM is rich in antioxidant properties which includes flavonoids, phenolic compounds, alkaloids, and vitamin C. These bioactive compounds may have worked together to elevate the immune system, decreasing oxidative stress in the liver (Khan et al., 2021; Ajbe et al., 2023), improving liver function and the synthesis of important compounds such as protein and lipids essential for yolk and egg white promoting quality of eggs and its production rate in the local hens (Wang and Xu, 2022; Anene et al., 2023). From the result, the liver parameters showed healthy liver function of the hens fed JLM. This implies that there will be an increase in the metabolism of fat-soluble vitamin of the treatment hens which include vitamin A, D, E and K. These fat-

soluble vitamins are vital in formation of egg shell, calcium metabolism and egg shell strength (Luo et al., 2022).

Antioxidant Enzyme of Local Laying Hens

The result of the effect of JLM on the antioxidant enzyme of local laying hen is shown in Table 4. The result showed that SOD, MDA and CAT were significantly ($P < 0.01$) affected by the JLM while GPx was not significantly ($P > 0.01$) affected. The mean values as represented in the table revealed that the hens in T4 recorded the highest ($P < 0.01$) SOD (8.30), and CAT (0.78). while the control recorded the highest mean value for MDA (4.15), the birds on T4 recorded the lowest mean value (1.67)

Table 4. Effect of JLM on antioxidant enzyme of local laying hens

Parameters (mg/dl)	T1	T2	T3	T4	SEM	P-value
SOD	2.72 ^c	5.41 ^b	8.01 ^a	8.30 ^a	0.42	0.00**
MDA	4.15 ^a	2.14 ^b	2.04 ^b	1.67 ^b	0.33	0.00**
CAT	0.51 ^c	0.67 ^b	0.62 ^b	0.78 ^a	0.02	0.00**
GPx	5.70	4.78	4.12	4.39	0.24	0.16 NS

a, b, c: means in the same row with different superscripts are significant at (** $P < 0.01$); NS; not significant; SOD superoxide dismutase; MDA: malondialdehyde; CAT: catalase; GPx glutathione peroxidase, T1 = control, T2 = 20 g / kg of feed, T3 = 40 g / kg of feed, T4 = 60 g / kg of feed

From the results on Table 4, as the inclusion level of JLM increased, the activity of SOD and CAT in the local laying hens increased, while MDA decreased across the treatment group, GPx was not affected ($P > 0.01$). The highest values of SOD, and CAT were recorded for hens in T4 (60 g/kg). The control recorded the least values in SOD and CAT, while MDA recorded highest in T1. This suggests that higher levels of JLM inclusion enhance the antioxidant defense mechanisms in these hens ($p < 0.01$). Monitoring the antioxidant enzymes of laying hens is important in sustaining the health and egg laying performance of the local laying hens (Radwan et al., 2008). The leaves of jackfruit is an excellent source of antioxidants with its active ingredient one of which is isoquercitrin from flavonoids of jackfruit leaves, that possess tough antioxidant capacity that can neutralize superoxide, hydrogen peroxide by removing free radical or by elevating CAT and SOD production (Utari and Warly, 2021). The exact reference range for oxidative enzyme for Nigerian local hens were not stated in literature, however, the values assayed in the current study are within range reported by some authors for normal physiology and health of tropical indigenous layers (Odunitan-Wayas et al., 2018; Atansuyi et al., 2019; Alum et al., 2020; Adewole et al., 2021). The present study observed that hens fed diets with 60 g of JLM/kg diets exhibited significantly higher SOD activity compared to control birds. This aligns with Surai (2020), who noted that dietary antioxidants boost poultry's antioxidant defense by increasing SOD and CAT activities. The increased antioxidant enzyme activity observed in this study, supports the concept that natural antioxidants help reduce oxidative stress in poultry. The present study reported significantly lower serum MDA

levels in hens fed JLM compared to control birds, indicating reduced oxidative stress. This finding corroborates research by Attia et al. (2022), which showed that dietary supplementation with antioxidants significantly decreased MDA levels in broiler chickens, suggesting a protective effect against lipid peroxidation. The higher CAT activity observed in hens fed the 60 g JLM diet aligns with findings from literature, where dietary antioxidants were shown to improve CAT activity, thereby enhancing the birds' ability to detoxify reactive oxygen species (Surai, 2020). Nonetheless, results from this present study indicated that GPx were not significantly different from control levels. This contrasts with findings from the study by Attia et al. (2022), where GPx levels were significantly increased with higher dietary antioxidant supplementation, suggesting that different sources or types of antioxidants may yield varying effects on GPx activity. Furthermore, while the present study found lower AST levels in hens fed the 60 g diet, contrasting results have been reported in other studies where high AST levels were associated with increased oxidative stress due to poor dietary management or environmental factors (Ekeocha et al., 2021). These discrepancies may arise from differences in dietary formulations or the specific conditions under which the studies were conducted. Moreover, the nutritional response to JFL supplementation observed in the current study may differ from findings reported by Ijarotimi and Keshinro (2013), where alternative feed sources did not significantly enhance antioxidant parameters or serum enzyme levels in local laying birds. Such variations could be attributed to differences in feed composition, environmental conditions, or genetic factors affecting the poultry populations studied. However, findings from this study contribute to the growing body of literature supporting the use of natural antioxidants like Jackfruit leaves in poultry diets to enhance health and productivity. While the present results align with previous research highlighting the benefits of antioxidant supplementation, the contrasting findings underscore the need for further investigation into how different dietary components interact and affect various physiological parameters in local laying hens under tropical conditions.

CONCLUSION and RECOMMENDATION

In conclusion, adding jackfruit leaf meal (JLM) to the diet had a substantial impact on the antioxidant enzyme and serum biochemical parameters studied in local laying hens. There may be a protective effect on liver function given the notable decrease in AST levels that was seen when JLM levels rose. The birds' improved antioxidant defense and decreased oxidative stress are indicated by the rise in antioxidant enzymes like SOD and CAT and the notable decrease in MDA levels, even if ALT and ALP did not change significantly. The GPx remains unaltered, indicating that the dietary inclusion had no discernible impact on its activity. Overall, the results show that local

laying hens' physiological health status is improved and oxidative stress is lessened by JLM supplementation.

Hence, it is recommended that local laying hens be fed jackfruit leaf meal as a natural supplement to improve their liver health and antioxidant capability. Increasing the amount of JLM in the meal up to 60 g/kg seems safe and healthy for the birds, and it may even increase their resilience to stress and production. Further studies could look into the underlying processes and long-term benefits of JLM supplementation in laying hens, as well as the ideal inclusion rate for maximizing performance and egg quality.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

Authors Contribution

Authors contributed equally to the paper.

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