PRINT ISSN 1119-8362
Electronic ISSN 2659-1499

Full-text Available Online at
https://www.ajol.info/index.php/jasem
https://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 28 (9) 2887-2903 September 2024

Assessment of Biochemical Potential of *Rhizopus oligosporus* as Starter Culture to Enhance the Nutritional Value of Palm Kernel Cake for Animal Feed Formulation

UGHE, FO; *EGBUNE, EO; ANIGBORO, AA; TONUKARI, NJ

Department of Biochemistry, Faculty of Science, Delta state University, P.M.B. 1, Abraka, Nigeria.

**Correspondence: egbuneegoamaka@gmail.com*
**ORCID ID: 0000-0002-5526-6397*
**Tel: +2348039463794*

Co-Authors Email: fridayoughe@gmail.com; akposanigboro@yahoo.co.uk; tonukari@gmail.com

ABSTRACT: The objective of this study was to assess the biochemical potential of *Rhizopus oligosporus* as a starter culture to enhance the nutritional value of palm kernel cake (PKC) for animal feed formulation using appropriate standard methods. Among the experimental groups, the batch of birds fed with solid-state fermented PKC as a 50% maize replacement showed a noteworthy weight gain ($p < 0.05$) in relation to the control group, while the other experimental groups exhibited a weight loss. Serum levels of ALT, AST, ALP, creatinine, and blood urea did not show a notable difference from the reference group and those on the experimental diets. These findings collectively suggest that the pre-treatment of PKC using *R. oligosporus* holds the potential to elevate its nutritional quality for utilization in animal feed applications.

DOI: https://dx.doi.org/10.4314/jasem.v28i9.35

Cite this Article as: UGHE, F. O; EGBUNE, E. O; ANIGBORO, A. A; TONUKARI, N. J (2024). Assessment of Biochemical Potential of *Rhizopus oligosporus* as Starter Culture to Enhance the Nutritional Value of Palm Kernel Cake for Animal Feed Formulation. *J. Appl. Sci. Environ. Manage.* 28 (9) 2887-2903

Dates: Received: Received: 07 July 2024; Revised: 15 August 2024; Accepted: 19 August 2024 Published: 05 September 2024

Keywords: Palm Kernel Cake; Solid-state fermentation; *Rhizopus oligosporus*; Soluble protein; Animal feed

The rising demand for poultry meat and eggs in developing countries is driving an increased need for primary poultry feedstuffs, especially protein and energy sources like soybean meal and yellow maize (Alshelmani *et al*., 2021; Tonukari *et al.*, 2023). This trend is expected to cause the price of poultry diets to rise. In response to this challenge, certain developing countries are investigating alternative feed sources derived from agricultural waste materials, including wheat bran, rice bran, cottonseed meal, copra meal, and palm kernel cake. Nonetheless, these residual products frequently encompass non-starch polysaccharides (NSPs) and anti-nutritional elements, which may pose risks to monogastric animals like chickens and pigs (Nguyen *et al*., 2021). One possible solution to mitigate the consequences of increasing animal food product demand is to increase livestock feed efficiency. However, there is a need to search for and investigate alternative feed sources to replace expensive traditional feedstuffs such as maize, sorghum, soybean, and peanut due to their increasing demand for human consumption and industrial production (Ansari *et al*., 2021). The seasonality of grain production and insufficient storage technology also make their supply problematic for cattle rearing. Palm kernel cake (PKC) emerges as a fibrous byproduct during the palm kernel oil extraction process. Offering a protein-rich composition (16-18%) devoid of aflatoxin, PKC proves valuable for ruminants and monogastric animals in their dietary

**Correspondence: egbuneegoamaka@gmail.com*
**ORCID ID: 0000-0002-5526-6397*
**Tel: +2348039463794*

regimen (Wong *et al*., 2011). Nevertheless, its incorporation into monogastric species' diets, especially poultry, encounters limitations due to its elevated indigestible fiber content and palm nut shell impurities (Azizi *et al*., 2021). The predominant presence of mannan within PKC's non-starch polysaccharides adversely impacts chickens, causing taste aversion and digestive issues (Alamgir, 2017; Alshelmani *et al*., 2021).

Addressing this concern, enzymatic treatment utilizing β-mannanase offers a potential solution to break down the NSP, specifically β-mannan, into easily absorbable and metabolizable carbohydrates like mannose, thereby enhancing palatability and digestion in poultry (Saeed *et al*., 2019). Another avenue involves solid-state fermentation (SSF), a biotechnological process where microorganisms thrive on solid substrates, producing enzymes that modify the substrate's chemical and physicochemical attributes (Egbune *et al*., 2023a, b, c, d). Fungal strains like Aspergillus niger, Sclerotium rolfsii, Trichoderma spp., and Aspergillus flavus have been explored for PKC SSF (Al-Snai, 2019; Akula and Golla, 2020; Guzmán-Guzmán *et al*., 2023). Interestingly, a recently isolated fungal strain from PKC demonstrated the ability to enhance reducing sugar content and reduce hemicellulose (β-mannan) through fermentation in a unique bioreactor setup (Iluyemi *et al*., 2006). Among these options, filamentous fungi such as *R. oligosporus*, though underutilized, exhibit promise due to their capability to produce nutritive elements without generating harmful compounds. This study delves into the feasibility of *R. oligosporus* as a starter culture to transform PKC into a more nutritionally enriched component suitable for animal feed.

MATERIALS AND METHODS

Materials Collection and Starter Organism Selection: The palm kernel cake used in this study was obtained from Songhai Delta Amukpe Sapele, Nigeria, and was milled before being stored at room temperature until further analysis. The *R. oligosporus* strains used in the study were provided by PT Aneka Fermentasi Industri, Bandung, Indonesia and obtained from the laboratory at Harmony Path, Ltd. situated in Amukpe, Delta State, Nigeria.

Various pretreatment for PKC: To prepare the substrates for autoclaved PKC, 5kg of PKC was blended with 15 mL of 50 mM phosphate buffer at pH 6. Similarly, the substrates for autoclaved PKC with 1N Sulfuric acid were prepared using the same method. However, in this case, the mixture was autoclaved in 4L of water and 80 ml of sulfuric acid. For solid-state fermented PKC, 1g of *R. oligosporus*, obtained from Tonukari Biotechnology Laboratory, was blended with 15 mL of 50 mM phosphate buffer at pH 6 and 5kg of PKC. The same process was followed for SSF autoclaved PKC with 1N Sulfuric acid. Control samples were also prepared using PKC with buffer alone and without cells. After fermentation for 72 hours at room temperature, 6-gram aliquots of the cell-PKC mixture were collected and blended with a mortar and pestle. The resulting mixture was spun for 10 minutes, and the supernatant was collected as crude extract. Replicate samples of crude extracts were prepared for follow-up tests.

Biochemical parameters: Total soluble protein was measured using Gornall *et al*.'s method (1949). Glucose concentration was assessed using the Randox glucose kit. Reducing sugars concentration was measured by the method (Miller, 1959). Total phenols were determined with Singleton and Rossi's procedure (1965). Total flavonoids were measured by colorimetry (Jia *et al*., 1999). Antioxidant activity was evaluated using the DPPH assay (Hatano *et al*., 1988), and reducing power was assessed as per Benzie and Strain (1996).

Enzyme extraction: On the seventh day of the fermentation process, a sterile distilled water volume of 20 mL was introduced into the PKC substrate engaged in solid-state fermentation. Thorough mixing was achieved by swirling the mixture to ensure uniformity. Subsequently, all the flasks underwent vigorous agitation on a rotary shaker operating at 200 revolutions per minute for a duration of 30 minutes. To separate the solid biomass from the suspension, filtration through Whatman filter paper No.1 was employed. The resulting extract was then employed as the source of the enzyme preparation. This sequence of steps was repeated using various treated PKCs. The determination of Mannanase activity was executed using a modified adaptation of the Araujo and Ward (1990) method. Conversely, the assessment of Phytase activity was carried out by quantifying the release of inorganic phosphorus from a sodium phytate solution, utilizing the methodology outlined by Bogar *et al*. (2003).

Evaluation of Broiler Starter Diets: Substitution of Maize with Pretreated Palm Kernel Cake and Its Impact on Growth Performance: Eight starter diet diets were designed by replacing 50% of the maize with processed palm kernel cake (PKC), while other ingredients remained constant (see Table 1 for details). Forty newly hatched chicks were shared into 8 groups of five, each fed one diet for 4 weeks with unrestricted access to feed and water. Weight gain was assessed by recording weights on the first day and at the end of the

fourth week. The study was conducted in accordance with ethical guidelines, and approval was obtained from the Research and Ethics Committee of the Faculty of Science, Delta State University, Abraka (Ref: REC/FOS/22/06).

Table 1: Gross composition of broiler chick meals with 50% replacement maize with pretreated PKC

Component	A	B	C	D	E	F	G
PKC	0	25	0	0	0	0	0
Autoclaved PKC	0	0	25	0	0	0	0
Autoclaved PKC with 1N sulphuric acid	0	0	0	25	0	0	0
SSF PKC	0	0	0	0	25	0	0
SSF Autoclaved PKC	0	0	0	0	0	25	0
SSF Autoclaved PKC with 1N sulphuric acid	0	0	0	0	0	0	25
Maize	50	25	25	25	25	25	25
Wheat offal	10	10	10	10	10	10	10
Soybean cake	35	35	35	35	35	35	35
Bone meal	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Limestone	2	2	2	2	2	2	2
Dicalcium phosphate	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Lysine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100	100
Number of birds	5	5	5	5	5	5	5

A) Control (Maize), B) Untreated PKC, C) Autoclaved PKC, D) Autoclaved PKC with 1N Sulfuric acid, E) SSF PKC, F) SSF Autoclaved PKC, G) SSF Autoclaved PKC with 1N Sulfuric acid

Metabolizable Energy: The compound meal was analyzed according to AOAC (1995) guidelines. Metabolizable energy (ME) in Kcal/kg was calculated using the following formula from AOAC (1995):

$$\mathrm{ME} = (37 \times CP) + (81.8 \times \mathrm{CF}) + (35.5 \times \mathrm{NFE}) \quad (1)$$

Where: NFE denotes nitrogen-free extract (carbohydrates), CF represents crude fat, and CP stands for crude protein, all expressed as percentages.

Proximate Analysis of Feeds: The proximate analysis of feeds, as detailed by Kirk and Sawyer (1991) and James (1995), involved determining fat, fiber, ash, carbohydrate, moisture, and dry matter percentages. The fat percentage was calculated using the formula in equation 2

$$\% fat = \frac{(\mathrm{W2} - \mathrm{W1})}{\mathrm{W} \times 100} \quad (2)$$

Fiber content was assessed via the Weende method, and ash content was determined using the furnace incineration gravimetric method. Carbohydrate percentage was computed using the formula in equation 3

$$\% Carb. = \frac{(\%\ \text{protein} + \%\ \text{fat} + \%\ \text{fiber})}{(\%\ \text{ash} + \%\ \text{moisture content})} \quad (3)$$

Where Carb = carbohydrates

-Moisture percentage was determined using the gravimetric method from AOAC (1990). Dry matter percentage was obtained by subtracting moisture content from 100, as expressed in the formula in equation 4

$$\%\ \text{Dry matter} = 100 - \%\ \mathrm{MC} \quad (4)$$

Where MC represents Moisture Content.

Assessment of the Biochemical Impact of Feed Formulations: Upon the culmination of the feeding trial, venous blood was aseptically extracted using sterile syringes and needles from prominent veins located in the wings and/or legs of the chicks. The collected blood was subsequently transferred into test tubes. Following a clotting period, the blood was dislodged and subjected to centrifugation at 2000 g for 10 minutes. This process facilitated the separation of serum as the supernatant. The obtained sera were then carefully preserved at a temperature of 4°C and earmarked for various biochemical analyses. The biochemical markers examined in the serum encompassed serum alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, urea, albumin, calcium, cholesterol and aspartate aminotransferase (AST). The procedures for conducting these analyses adhered to the protocols stipulated in RANDOX test kits.

Statistical Evaluation: The data analysis was conducted using the SPSS software. To assess the

experimental data, analysis of variance (ANOVA) was employed. Subsequently, a Fisher's test of least significant difference (LSD) was performed to facilitate a comparison of group averages.

RESULTS AND DISCUSSION

According to Figure 1A, the concentration of soluble proteins in the pre-treated PKC exhibited a noteworthy increase ($p<0.05$). The pre-treatment method involving *R. oligosporus* in solid-state fermentation yielded the highest concentration of soluble proteins among all the tested methods.

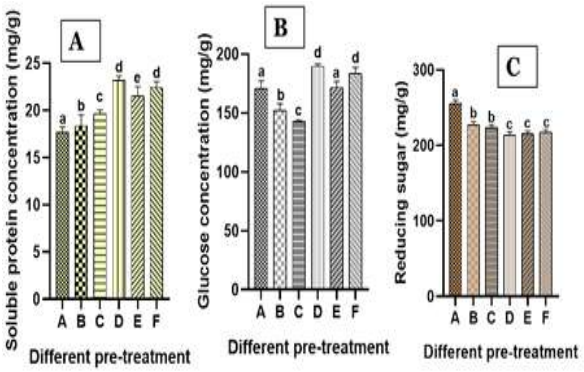

Fig. 1: (A) Levels of soluble proteins (B) Glucose concentration and (C) Reducing sugar concentration at different pretreatments of PKC. Values marked with superscript letters vary markedly from the control. A) Untreated PKC, B) Autoclaved PKC, C) Autoclaved PKC with 1N Sulfuric acid, D) SSF PKC, E) SSF Autoclaved PKC, F) SSF Autoclaved PKC with 1N Sulfuric acid

The increase in soluble protein concentration observed in the pre-treated PKC samples could be due to the proteolytic activity of *R. oligosporus* during solid-state fermentation. *R. oligosporus* is a well-known filamentous fungus with a broad range of proteolytic enzymes that can hydrolyze proteins and peptides into smaller units (Christensen *et al*., 2022; Aganbi *et al.,* 2023; Edema-Eyen *et al.,* 2023). These enzymes are secreted by the fungus to degrade the complex organic matter present in the substrate and to release nutrients for their growth and metabolism (Noman *et al*., 2019; da Silva Vilar *et al*., 2022).

The proteolytic activity of *R. oligosporus* on PKC can lead to the breakdown of proteinaceous components such as cell walls and membranes, which would increase the solubility of proteins and enhance their availability for extraction (Cabanillas-Bojórquez *et al*., 2020; Egbune *et al.,* 2024b). Additionally, the release of amino acids and peptides from protein hydrolysis can stimulate the growth and activity of the indigenous microbial communities in the PKC, which could further contribute to the increase in soluble protein concentration (Wang *et al*., 2022; Egbune *et al.,* 2024a).

Figure 1B presents the outcomes of the analysis carried out to determine the glucose concentration of pre-treated PKC. The results showed that SSF PKC, SSF Autoclaved PKC, and SSF Autoclaved PKC with 1N Sulfuric acid had a noticeable elevation ($p<0.05$) in glucose concentration compared to the PKC that was untreated. However, the glucose concentration of Autoclaved PKC and Autoclaved PKC with 1N Sulfuric acid was significantly reduced.

The increase in glucose concentration in SSF PKC, SSF Autoclaved PKC, and SSF Autoclaved PKC with 1N Sulfuric acid could be due to the breakdown of the carbohydrate polymers present in the pre-treated PKC (Zhang *et al*., 2021). These treatments likely caused the breakdown of the cell wall components, which released the carbohydrates into the solution. The SSF process, which involves simultaneous saccharification and fermentation, further breaks down the complex carbohydrates into glucose, which would explain the elevated glucose concentration in the treated PKC samples (Antia *et al*., 2021; Ezedom *et al.,* 2022, 2023). On the other hand, the reduction in glucose concentration observed in Autoclaved PKC and Autoclaved PKC with 1N Sulfuric acid could be attributed to the denaturation or degradation of enzymes responsible for the saccharification process (Zhao *et al*., 2021). Autoclaving and acid treatment are known to cause denaturation or degradation of enzymes, which would explain the reduced glucose concentration in these samples (Rivas *et al*., 2022).

Relative to the control, there was a slight increase in reducing sugar concentration in SSF PKC, SSF Autoclaved PKC, and SSF Autoclaved PKC with 1N sulfuric acid. However, Autoclaved PKC and Autoclaved PKC with 1N Sulfuric acid showed a significant reduction in the concentration of reducing sugar (Figure 1C).

The reduction of sugar concentration in autoclaved PKC and autoclaved PKC with 1N Sulfuric acid could be due to the thermal and chemical degradation of the polysaccharides in the PKC (Azizi *et al*., 2021). The high temperature during autoclaving and the acidic conditions of sulfuric acid treatment could have broken down the glycosidic bonds in the polysaccharides, resulting in a decrease in reducing sugar concentration (Constantino *et al*., 2021).

In contrast, the slight increase in reducing sugar concentration in SSF PKC, SSF Autoclaved PKC, and SSF Autoclaved PKC with 1N Sulfuric acid could be attributed to the activity of enzymes produced during solid-state fermentation (SSF) (Leite *et al*., 2019). During SSF, microorganisms secrete various enzymes

such as cellulases, hemicellulases, and amylases, which can break down the complex polysaccharides in the PKC into simpler sugars, including reducing sugars (Lee *et al*., 2019). According to a study by Gnanamani *et al*. (2010), SSF of PKC with Aspergillus niger resulted in the production of cellulases and hemicellulases, leading to an increase in reducing sugar concentration. Similarly, another study by Dasari *et al*. (2019) showed that SSF of PKC with Trichoderma reesei and Aspergillus niger increased the concentration of reducing sugars due to the activity of cellulases and xylanases.

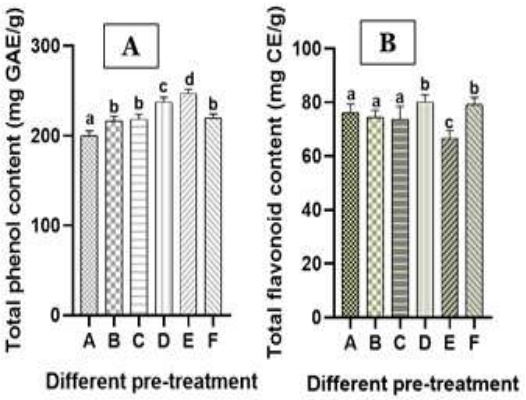

Fig. 2: (A) Total phenolic content (TPC) and (B) Total flavonoid content (TFC) at different pretreatments of PKC. Values marked with superscript letters vary markedly from the control. A) Untreated PKC, B) Autoclaved PKC, C) Autoclaved PKC with 1N Sulfuric acid, D) SSF PKC, E) SSF Autoclaved PKC, F) SSF Autoclaved PKC with 1N Sulfuric acid

Figure 2A shows a significant increase ($p<0.05$) in the total phenolic content of PKC after pretreatment compared to the untreated sample. Autoclaved PKC subjected to SSF showed the highest phenolic content among all the samples. The increase in phenolic content observed in the pretreated PKC samples could be due to the activation of plant defense mechanisms in response to the applied pretreatment methods (Suohui *et al*., 2022). Pretreatment methods such as autoclaving and SSF can cause physical and chemical changes in the structure of the plant cell wall, leading to the release of phenolic compounds (Šelo *et al*., 2022). The released phenolic compounds can also act as signal molecules to trigger the biosynthesis of additional phenolic compounds in response to the applied stress (Naikoo *et al*., 2019). Autoclaving is known to cause structural changes in plant cell walls, leading to the release of phenolic compounds that were previously inaccessible (Hu *et al*., 2022). Similarly, SSF can cause physical and chemical changes in the cell wall, leading to the release of phenolic compounds (Bodoira and Maestri 2020). The observed increase in phenolic content could also be attributed to the microbial activity during SSF, where microorganisms can produce and release various phenolic compounds as part of their metabolic processes (Torres-León *et al*., 2019).

Figure 2B displays the outcome of the evaluation of the overall flavonoid content of pre-treated PKC. The results show that there was no substantial difference ($p>0.05$) in the flavonoid content between the pre-treated PKC and the control group. Nevertheless, a significant difference ($p<0.05$) was observed in the flavonoid content between SSF PKC and SSF Autoclaved PKC when subjected to 1N Sulfuric acid treatment. Flavonoids, plant-derived compounds known for their antioxidant, anti-inflammatory, and anticancer properties (Batiha *et al*., 2020), were evaluated in pre-treated PKC to assess the impact of various treatments on their concentration. The absence of a significant difference in flavonoid content between pre-treated PKC and the control indicates that the treatments did not notably affect flavonoid levels. However, the significant difference in flavonoid content between SSF PKC and SSF Autoclaved PKC when subjected to 1N Sulfuric acid treatment indicates that the type of pre-treatment used can have a significant effect on the concentration of these compounds.

One possible explanation for this observation is that the pre-treatment methods used in this study may have differentially affected the availability of flavonoids in the samples (Song *et al*., 2022). For example, autoclaving has been shown to cause degradation of some flavonoids, whereas other methods such as solid-state fermentation (SSF) can enhance the production of certain types of flavonoids by promoting the growth of specific microorganisms (Xiao *et al*., 2022). Therefore, it is possible that the SSF pre-treatment method used in this study resulted in an increase in the production of specific flavonoids that were more susceptible to hydrolysis by the 1N sulfuric acid treatment.

The pre-treated PKC exhibited a significant increase in FRAP percentage inhibition ($p<0.05$) compared to the control (Figure 3A). On the other hand, Autoclaved PKC and Autoclaved PKC with 1N Sulfuric acid showed a significant decrease. Ferric reducing antioxidant power (FRAP) is a commonly used assay for measuring the antioxidant capacity of a sample (Munteanu and Apetrei 2021). The results of the current study suggest that the pre-treatment of PKC can have a significant effect on its antioxidant capacity, as measured by the FRAP assay.
The observed increase in FRAP percentage inhibition in the pre-treated PKC compared to the control group

suggests that the pre-treatment methods used in this study may have enhanced the antioxidant capacity of the samples. One possible explanation for this observation is that the pre-treatments may have increased the availability of antioxidant compounds in the PKC, such as phenolic compounds and flavonoids, which have been shown to have strong antioxidant activity (Posadino *et al*., 2021).

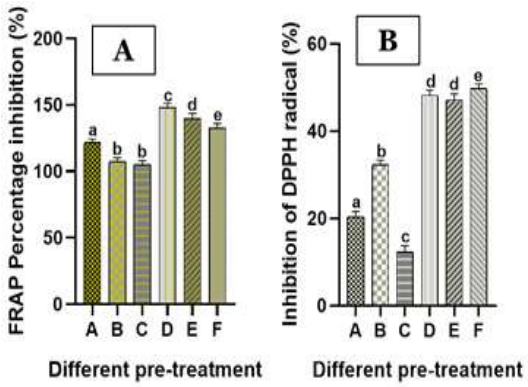

Fig. 3: (A) FRAP percentage inhibition and (B) Free radical scavenging activity of DPPH (%) at different pretreatments of PKC. Values marked with superscript letters vary markedly from the control. A) Untreated PKC, B) Autoclaved PKC, C) Autoclaved PKC with 1N Sulfuric acid, D) SSF PKC, E) SSF Autoclaved PKC, F) SSF Autoclaved PKC with 1N Sulfuric acid

In contrast, the significant decrease in FRAP percentage inhibition in Autoclaved PKC and Autoclaved PKC with 1N Sulfuric acid suggests that these pre-treatment methods may have had a negative impact on the antioxidant capacity of the samples. Autoclaving is known to cause thermal degradation of some antioxidant compounds, while sulfuric acid treatment can hydrolyze certain types of flavonoids and other phenolic compounds, resulting in a decrease in their antioxidant activity (Kataria *et al*., 2022). Figure 3B displays the outcomes of the analysis conducted to determine the free radical scavenging activity of pre-treated PKC using 1,1-diphenyl-2-picrylhydrazyl (DPPH)-2,2-diphenyl-1-picrylhydrazyl (DPPH). The results demonstrate that solid-state fermented SSF PKC had an enhanced free radical scavenging activity compared to the control. In contrast, all other pre-treatments resulted in a significant reduction in scavenging activity. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay is a widely used method to evaluate the antioxidant activity of various compounds, including natural products, due to its simplicity and reliability (Munteanu and Apetrei 2021). The free radical scavenging activity of pre-treated palm kernel cake (PKC) was determined by measuring the ability of the sample to neutralize the stable free radical DPPH. In this assay, the purple-colored DPPH radical is reduced by the addition of an antioxidant compound, resulting in a color change from purple to yellow (Jin *et al*., 2022). The results presented in Figure 3B shows that the solid-state fermented (SSF) PKC had the highest free radical scavenging activity, which indicates that SSF treatment could enhance the antioxidant potential of PKC. This could be due to the generation of bioactive compounds such as phenolic acids, flavonoids, and enzymes during the fermentation process, which have been reported to exhibit antioxidant activity (Ketnawa *et al*., 2022). On the other hand, all other pre-treatments, including autoclaving, soaking, and oven-drying, resulted in a significant reduction in scavenging activity. This could be due to the degradation or loss of bioactive compounds during these pre-treatments, resulting in a decrease in antioxidant potential (Santos *et al*., 2022).

Figure 4A demonstrates that the phytase activity of PKC subjected to pre-treatment showed a notable rise ($p<0.05$) relative to the phytase activity of PKC that was not subjected to any pre-treatment.

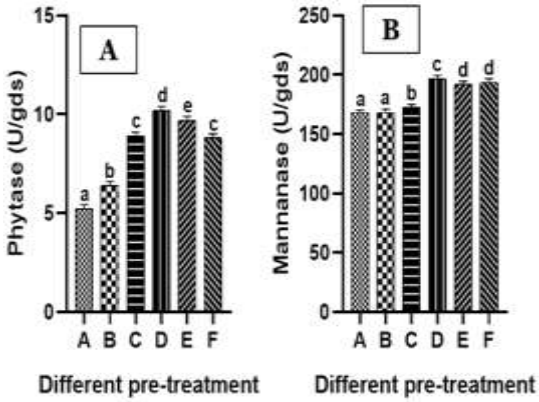

Fig. 4: (A) Phytase and (B) Mannanase activity at different pretreatments of PKC. Values marked with superscript letters vary markedly from the control. A) Untreated PKC, B) Autoclaved PKC, C) Autoclaved PKC with 1N Sulfuric acid, D) SSF PKC, E) SSF Autoclaved PKC, F) SSF Autoclaved PKC with 1N Sulfuric acid

Phytase is an enzyme that hydrolyzes phytic acid, releasing inorganic phosphate and reducing the anti-nutrient effect of phytic acid on plant-based feed ingredients (Kumar *et al*. 2021). The increase in phytase activity observed in pre-treated PKC may be due to several factors. Firstly, pre-treatment may break down the cell wall components of PKC, resulting in the release of intracellular enzymes, including phytase. This is supported by a study by Abdullah *et al*. (2020), which found that pre-treatment with protease and xylanase significantly increased the phytase activity in PKC. Secondly, pre-treatment may improve the accessibility of phytase to its substrate, leading to an increase in enzyme activity. The pre-

treatment process may disrupt the binding of phytic acid to other molecules in PKC, making it more available for phytase to act upon. This is consistent with a study by Yigit *et al*. (2018), which found that pre-treatment with a mixture of protease and cellulase significantly increased the phytase activity in soybean meal. Lastly, pre-treatment may cause changes in the protein structure of phytase, leading to an increase in enzyme activity. This is supported by a study by Kortekangas *et al*. (2020), which found that pre-treatment with acid and alkali improved the activity of phytase enzymes by changing their structure. In summary, the increase in phytase activity observed in pre-treated PKC may be due to the release of intracellular enzymes, improved accessibility of phytic acid to phytase, and changes in the protein structure of phytase. These findings highlight the potential of pre-treatment as a strategy to enhance the nutritional value of PKC and other plant-based feed ingredients. The higher phytase activity observed in SSF PKC (solid-state fermentation pre-treated palm kernel cake) may be due to the degradation of complex substrates present in the PKC by the microbial community during SSF (Lee *et al*., 2019). This process results in the release of intracellular enzymes, including phytase, which can be further improved by optimizing the fermentation conditions such as pH, temperature, and moisture content (Qasim *et al*., 2017). Several studies have shown that microbial fermentation can enhance the phytase activity in different feed ingredients. For instance, studies by Jannathulla *et al*. (2018) and Grela *et al*. (2019) reported an increase in phytase activity in fermented soybean meal and fermented rapeseed meal, respectively. The increase in phytase activity was attributed to the degradation of phytate by the microbial community during fermentation (Nkhata *et al*., 2018). Furthermore, SSF pre-treatment may have caused changes in the structure and composition of PKC, leading to an increase in phytase activity (Cebrián and Ibarruri 2023). The microorganisms involved in the SSF process may also produce organic acids, which can improve the solubility and accessibility of phytate to phytase enzymes, leading to increased enzyme activity (Cebrián and Ibarruri 2023). Figure 4B illustrates that pre-treatment of PKC resulted in a significant increase ($p<0.05$) in its mannanase activity, as compared to untreated PKC. Among the pre-treated PKC samples, SSF PKC showed the highest mannanase activity. The increase in mannanase activity observed in pre-treated PKC, as compared to untreated PKC, suggests that the pre-treatment may have caused structural changes in the protein that enhanced its enzymatic activity. Previous studies have reported similar effects of pre-treatment on enzymatic activity in various proteins. For instance, pre-treatment with denaturants, such as urea or guanidine hydrochloride, has been shown to increase the activity of several enzymes, including trypsin, subtilisin, and papain (Liu *et al*., 2020; de Jong *et al*., 2022). Pre-treatment with other chemicals, such as surfactants or detergents, has also been reported to affect the activity of enzymes (Mohd *et al*., 2017). It is worth noting that the effect of pre-treatment on protein activity can be complex and may depend on several factors, such as the nature of the protein, the specific pre-treatment conditions, and the type of enzymatic assay used to measure activity. Therefore, the observed increase in mannanase activity in pre-treated PKC should be interpreted in the context of these factors. Studies have reported the use of various pre-treatment methods, such as heat treatment or exposure to surfactants, to enhance the activity of other enzymes, including cellulases and xylanases (Yadav 2017; Reis *et al*., 2022). These studies suggest that pre-treatment may be a viable strategy to improve the activity of certain enzymes.

Table 2: Proximate composition of formulated diet.

Composition	A	B	C	D	E	F	G
Protein (%)	27.8±0.5	9.3±0.6	27.8±0.5	32.2±0.7	36.7±0.5	33.4±0.5	32.1±0.5
Fat content (%)	4.2±0.2	2.2±0.2	4.2±0.2	3.2±0.2	3.2±0.2	3.1±0.2	3.2±0.2
Fiber (%)	23.2±0.5	34.7±0.5	23.2±0.5	16.2±0.5	13.2±0.5	14.2±0.4	13.2±0.5
Ash (%)	5.9±0.1	6.3±0.2	6.1±0.1	5.9±0.1	6.3±0.1	5.4±0.1	6.3±0.1
Carbohydrate (%)	37.4±0.4	40.5±0.5	38.4±0.4	37.4±0.4	36.9±0.4	36.9±0.4	37.2±0.4
Moisture (%)	10.1±0.2	16.3±0.3	12.1±0.2	10.1±0.2	11.1±0.1	11.1±0.1	10.7±0.1
Dry matter (%)	89.6±0.7	90.4±0.7	81.5±0.5	89.6±0.7	80.3±0.5	80.3±0.5	80.3±0.5
Metabolizable energy (kcal/kg)	2696.9	1960.3	1369.2	2778.6	2927.4	2797.2	2767.8

A) Control, B) Untreated PKC, C) Autoclaved PKC, D) Autoclaved PKC with 1N Sulfuric acid, E) SSF PKC, F) SSF Autoclaved PKC, G) SSF Autoclaved PKC with 1N Sulfuric acid

Table 2 presents the outcomes of the proximate analyses conducted on the prepared diets. The broiler starter feed was composed of the following constituents: crude protein ranging from 9.3% to 36.7%, crude fat from 2.2% to 4.2%, crude fiber from 13.2% to 37.4%, ash from 5.9% to 6.3%, carbohydrate from 37.2% to 40.5%, moisture from 10.1% to 16.3%, and dry matter from 80.3% to 89.6%. Among the

different diets, SSF PKC exhibited the highest crude protein content at 36.7%, which could indicate its potential as a protein-rich ingredient. Conversely, Untreated PKC had the lowest crude protein content at 9.3%, possibly indicating its limited nutritional value. Furthermore, the calculated metabolizable energy (ME) values ranging from 2127.7 to 2882.8 Kcal/kg provide insights into the energy content of the feed samples. This parameter is crucial for assessing the diets' potential to meet the energy needs of broiler chickens (Egbune *et al*., 2023). The variations observed in the levels of proximate composition between the control and experimental meals highlight the importance of diet formulation. These differences could influence the growth, health, and overall performance of broiler chickens.

The group of birds that received SSF PKC demonstrated a significant increase in weight ($p<0.05$) compared to the control group, while all other experimental groups showed a noteworthy decrease in weight (Figure 5).

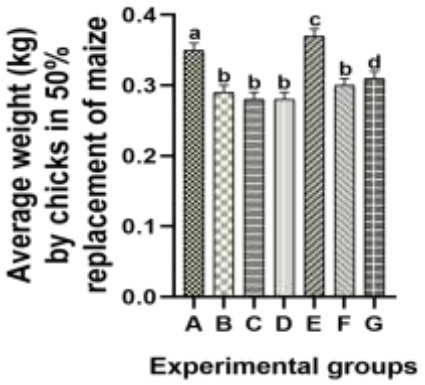

Fig. 5: Weight gained (kg). Values marked with superscript letters vary markedly from the control. A) Control, B) Untreated PKC, C) Autoclaved PKC, D) Autoclaved PKC with 1N Sulfuric acid, E) SSF PKC, F) SSF Autoclaved PKC, G) SSF Autoclaved PKC with 1N Sulfuric acid

Solid-state fermentation (SSF) of palm kernel cake (PKC) can enhance the nutritional value of the feed by breaking down the anti-nutritional factors and increasing the bioavailability of nutrients (Azizi *et al*., 2021). The improved nutrient profile of the SSF PKC may have contributed to the significant weight gain observed in the birds fed with this feed. Several studies have shown that SSF of PKC can increase the protein content, amino acid profile, and mineral content of the feed, while reducing the levels of tannins, phytates, and other anti-nutritional factors that interfere with nutrient absorption and utilization (Londoño-Hernandez *et al*., 2020; Magbanua *et al*., 2022). The fermentation process also produces enzymes and organic acids that improve digestion and absorption of nutrients, enhance gut health, and modulate the gut microbiota (Peredo-Lovillo *et al*., 2020; Melaku *et al*., 2021).

Increased nutrient absorption and utilization can lead to improved growth performance, as demonstrated by the significant weight gain observed in the birds fed with SSF PKC (Egbune and Tonukari 2023). This finding is consistent with previous studies that have reported improved growth rates, feed conversion ratios, and nutrient retention in birds fed with fermented PKC (Egbune *et al.*, 2021; Hakim *et al*., 2022). Furthermore, SSF PKC may have a beneficial effect on lipid metabolism, as PKC is known to contain high levels of medium-chain fatty acids (MCFAs), which have been shown to promote weight gain and improve lipid profiles in animals (Huang *et al*., 2021). The fermentation process can enhance the bioavailability and digestibility of MCFAs, which may have contributed to the weight gain observed in the birds fed with SSF PKC.

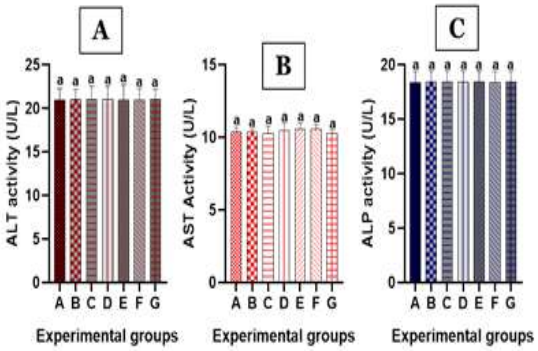

Fig. 6: (A) Activity of serum alanine aminotransferase (ALT), (B) aspartate aminotransferase (AST) and (C) alkaline phosphatase (ALP). Values marked with superscript letters vary markedly from the control. A) Control, B) Untreated PKC, C) Autoclaved PKC, D) Autoclaved PKC with 1N Sulfuric acid, E) SSF PKC, F) SSF Autoclaved PKC, G) SSF Autoclaved PKC with 1N Sulfuric acid

Figure 6A displays the blood levels of the liver enzyme alanine aminotransferase (ALT) in birds. Based on this information, it can be concluded that there were no significant variations in serum ALT activity between the group of birds that consumed the control diet and the groups that consumed the experimental diets. Alanine aminotransferase (ALT) is primarily found in the liver, and it is an enzyme that is involved in the metabolism of amino acids (Ndrepepa 2021). The normal range of ALT in the blood can vary depending on the laboratory and method of analysis, but in general, elevated levels of ALT are considered to be a marker of liver damage or disease (Kathak *et al*., 2022). The result of the study concluded that there were no significant differences in serum ALT activity between birds fed the control diet and those fed the

experimental diets. This suggests that the experimental diets did not cause any significant liver damage or dysfunction. Several studies have shown that elevated levels of ALT are associated with various liver diseases, including viral hepatitis, nonalcoholic fatty liver disease (NAFLD), alcoholic liver disease, autoimmune hepatitis, and drug-induced liver injury (DILI) (Wu *et al*., 2020). In viral hepatitis, such as hepatitis B and C, elevated ALT levels are commonly observed, and monitoring ALT levels is an important tool for assessing the severity of liver inflammation and damage (Kim *et al*., 2008). Figure 6B illustrates the blood levels of the liver enzymes aspartate aminotransferase (AST) in birds. Based on this data, it can be inferred that there were no significant differences in serum AST activity between the group of birds that consumed the control diet and the groups that consumed the experimental diets. Aspartate aminotransferase (AST) is an enzyme found in the liver, heart, skeletal muscles, and other organs (Glinghammar *et al*., 2009). Similar to ALT, elevated levels of AST in the blood can indicate liver or muscle damage (Radi *et al*., 2011). In the context of the study, the lack of significant differences in serum AST activity between the control and experimental groups suggests that the experimental diets did not have a significant effect on liver or muscle function in birds. This finding is consistent with a study conducted on broiler chickens that found no significant differences in serum AST levels between groups fed different levels of dietary crude protein (Akbarian *et al*., 2015). Alkaline phosphatase (ALP) is a hydrolase enzyme that catalyzes the hydrolysis of phosphate esters in an alkaline environment. It is found in various tissues, including the liver, bones, and intestine, and is released into the blood when there is damage or dysfunction in these tissues (Lowe *et al*. 2022). Therefore, elevated levels of ALP in the blood are often used as a marker for liver and bone disease. In the context of the study, the blood levels of ALP were measured to assess the potential impact of the experimental diets on liver function in the broiler chickens. Figure 6C shows that there were no significant differences in serum ALP activity between the group of birds that consumed the control diet and the groups that consumed the experimental diets. This suggests that the consumption of solid-state fermented PKC did not have a negative impact on liver function in the birds. The lack of significant differences in ALP levels between the groups could be due to the fact that the fermented PKC diets did not cause any liver damage or dysfunction. This is consistent with the findings of other studies that have investigated the effects of fermented feed on liver function in animals. For example, a study on weaned piglets fed with fermented wheat showed no significant differences in serum ALP levels compared to those fed with non-fermented wheat (Seo *et al*., 2021). Similarly, a study on rainbow trout fed with fermented soybean meal found no significant differences in serum ALP levels compared to those fed with non-fermented soybean meal (Barmes *et al*., 2015). Overall, the results of the study suggest that the use of solid-state fermentation in the production of PKC feed for broiler chickens does not negatively impact liver function, as evidenced by the lack of significant differences in serum ALP levels between the control and experimental groups. Figure 7A indicates that there were no significant differences in blood creatinine levels between the birds that received the experimental diets and those that were given the control diet. Creatinine is a waste product that is produced by muscle metabolism and excreted by the kidneys Pasala and Carmody 2017). Blood creatinine levels are a commonly used indicator of kidney function in both humans and animals. Elevated blood creatinine levels can indicate impaired kidney function or disease, while low creatinine levels are generally not a concern (Sargent *et al*., 2021). In this case, the finding that there were no significant differences in blood creatinine levels between the birds that received the experimental diets and those that were given the control diet suggests that the experimental diets did not have a negative impact on kidney function.

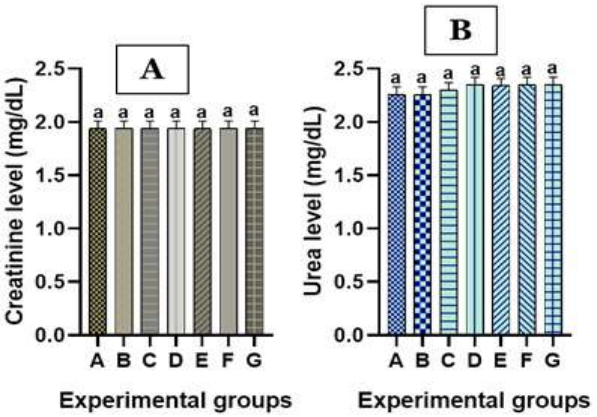

Fig. 7: (A) Level of serum creatinine and (B) Urea. Values marked with superscript letters vary markedly from the control. A) Control, B) Untreated PKC, C) Autoclaved PKC, D) Autoclaved PKC with 1N Sulfuric acid, E) SSF PKC, F) SSF Autoclaved PKC, G) SSF Autoclaved PKC with 1N Sulfuric acid

Figure 7B shows that there were no significant differences in blood urea levels between the birds that received the experimental diets and those that were given the control diet. Urea is a waste product produced by the liver as a result of protein metabolism and excreted by the kidneys (Getahun *et al*., 2019). Blood urea levels can be used as a measure of renal function, as impaired kidney function can lead to an

accumulation of urea in the blood (Gyurászová *et al*., 2019). However, in the case of the birds in the study, the blood urea levels were not significantly different between the group that received the experimental diets and the group that received the control diet, suggesting that the experimental diets did not have any adverse effects on renal function. The levels of albumin in birds that consumed the experimental diets, including solid state fermented PKC, SSF autoclaved PKC, and autoclaved PKC with 1N sulfuric acid, showed significant differences ($p<0.05$) when compared to the control diet (as seen in Figure 7A). However, the group of birds that were fed the autoclaved PKC diet displayed a notable decrease in albumin levels. Albumin is an essential protein synthesized by the liver that functions as a transport protein, aiding in the maintenance of colloidal osmotic pressure, and the transport of various molecules, including hormones, lipids, and drugs (Raghubeer *et al*., 2022). In birds, albumin levels are a critical indicator of protein metabolism, and low levels can indicate protein malnutrition or liver dysfunction (Loftus *et al*., 2019).

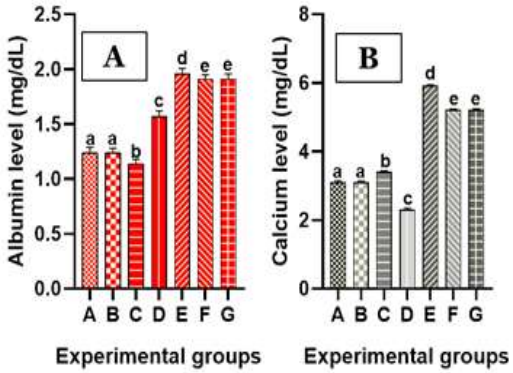

Fig. 8: (A) Levels of serum albumin and (B) calcium. Values marked with superscript letters vary markedly from the control. A) Control, B) Untreated PKC, C) Autoclaved PKC, D) Autoclaved PKC with 1N Sulfuric acid, E) SSF PKC, F) SSF Autoclaved PKC, G) SSF Autoclaved PKC with 1N Sulfuric acid.

The decrease in albumin levels in birds fed the autoclaved PKC diet could be due to the lack of essential amino acids, particularly sulfur-containing amino acids, in the diet. Sulfur-containing amino acids, such as methionine and cysteine, are essential for the synthesis of albumin (Azad *et al*., 2020). Autoclaving can also result in protein denaturation and the formation of Maillard reaction products that can reduce protein digestibility (Gu *et al*., 2022). However, the significant differences in albumin levels between the experimental diets and the control diet may suggest that the food and feed additives used in the experimental diets provided a better nutritional profile and efficient protein utilization, resulting in higher albumin levels (Disetlhe *et al*., 2018). In birds fed SSF PKC diets, the blood calcium levels were found to be significantly higher ($p<0.05$) compared to those fed the control feed, as depicted in Figure 8B. On the other hand, there were no significant differences in the blood calcium levels between birds fed Untreated PKC diets and the control diet. However, there was a notable decrease in calcium levels in birds that were fed Autoclaved PKC with 1N Sulfuric acid diet. Calcium is an essential mineral that plays a critical role in various biological processes, including bone formation, muscle contraction, and nerve function in birds (Alagawany *et al*., 2021). The calcium levels in the blood are regulated by a complex interplay between several hormones, including parathyroid hormone (PTH), calcitonin, and vitamin D (van Goor *et al*., 2017). In the case of the SSF PKC diet, the increased blood calcium levels observed in birds may be due to the presence of higher levels of calcium in the feed or the enhanced bioavailability of calcium due to the solid-state fermentation process (Olukomaiya *et al*., 2019). This is supported by a study conducted by Hsiao *et al*. (2022), which reported that solid-state fermentation of poultry feed using Aspergillus niger and Lactobacillus acidophilus resulted in increased calcium content in the feed. On the other hand, the decrease in blood calcium levels observed in birds fed Autoclaved PKC with 1N Sulfuric acid diet could be attributed to the acid treatment, which might have resulted in the solubilization and leaching of calcium from the feed (Vanderzee and Zeman 2018). Overall, the results suggest that the type of treatment applied to the feed can affect the bioavailability of calcium, which can subsequently impact the blood calcium levels in birds. The serum levels of total triglycerides were found to be significantly lower ($p < 0.05$) in birds fed experimental diets when compared to the control diet, as shown in Figure 9A. Conversely, there were no significant differences in the serum triglyceride levels of birds fed Autoclaved PKC with 1N Sulfuric acid diet when compared to the control diet.

Triglycerides are the main form of fat stored in adipose tissue and transported in the bloodstream. In birds, high levels of serum triglycerides have been associated with various metabolic disorders such as fatty liver syndrome and atherosclerosis (Song *et al*., 2021). The lower serum levels of total triglycerides observed in birds fed SSF PKC diets in this study suggest that these diets may help reduce the risk of metabolic disorders in birds.

The lower serum triglyceride levels observed in birds fed SSF PKC diets may be due to the presence of bioactive compounds, such as polyphenols and dietary fiber, which have been shown to have hypolipidemic

effects (García-García *et al*., 2020). Polyphenols have been reported to reduce lipid absorption and increase lipid excretion (Huang *et al*., 2018), while dietary fiber can reduce the absorption of dietary fats by binding to them and increasing fecal excretion (Yegin *et al*., 2020). In contrast, the lack of significant differences in serum triglyceride levels between birds fed Autoclaved PKC with 1N Sulfuric acid diet and the control diet suggests that this diet did not have a significant effect on lipid metabolism in birds.

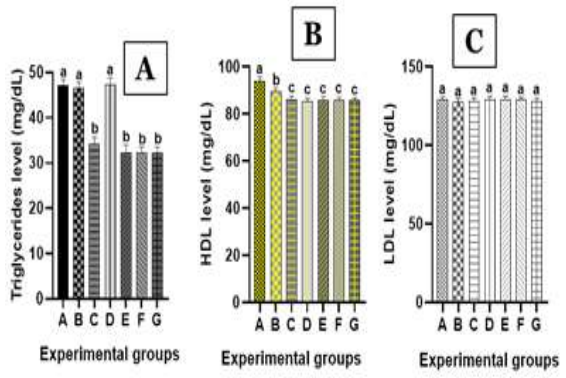

Fig. 9: (A) Levels of serum triglycerides, (B) HDL and (C) LDL. Values marked with superscript letters vary markedly from the control. A) Control, B) Untreated PKC, C) Autoclaved PKC, D) Autoclaved PKC with 1N Sulfuric acid, E) SSF PKC, F) SSF Autoclaved PKC, G) SSF Autoclaved PKC with 1N Sulfuric acid

As depicted in Figure 9B, the levels of HDL in birds fed experimental diets were significantly reduced ($p < 0.05$) when compared to the control diet. HDL, or high-density lipoprotein, is a lipoprotein that carries cholesterol from peripheral tissues back to the liver for processing and excretion, which is why it is often referred to as "good cholesterol." Several studies have shown that changes in diet can affect HDL levels in animals and humans.

One possible explanation for the observed decrease in HDL levels in birds fed the experimental diets could be the altered lipid metabolism resulting from the different nutrient compositions of the diets. For example, diets high in carbohydrates and low in fat have been associated with decreased HDL levels in both humans and animals (Yegin *et al*., 2020). Similarly, diets high in polyunsaturated fatty acids (PUFAs) have been shown to increase HDL levels (Yanai *et al*., 2018). It is possible that the trial feed used in this study may have had different ratios of carbohydrates, fats, and PUFAs compared to the control diet, leading to changes in HDL levels.

As depicted in Figure 9C, the levels of LDL in birds fed experimental diets were not significantly different ($p < 0.05$) when compared to the control diet. Low-density lipoprotein (LDL) is often referred to as the "bad cholesterol" because it can build up in the walls of arteries and lead to the formation of plaque, which can eventually cause cardiovascular disease (Anlamlert *et al*., 2017). In the study, it was found that the levels of LDL in birds fed experimental diets were not significantly different ($p < 0.05$) when compared to the control diet, as shown in Figure 4.20. This suggests that the experimental diets did not have a significant impact on the levels of LDL in the blood of the birds.

Conclusion: The study showed that solid-state fermentation of PKC using *R. oligosporus* can increase its nutritional value and antioxidant potential, while other pre-treatments may lead to a decrease in scavenging activity. The results suggest that replacing 50% of maize with SSF PKC in broiler diets can result in significant weight gain in birds. The improved nutritional quality and digestibility of fermented feed may be responsible for this weight gain. Furthermore, the use of fermented PKC in broiler diets did not have any negative impacts on the birds' liver function or overall health. The study's findings suggest that solid-state fermentation has promising applications in various biotechnological processes and the production of improved bioproducts.

Declaration of conflict of interest: The authors declare no conflict of interest.

Data Availability Statement: Data are available upon request from the corresponding author.

REFERENCES

Abdullah, J; Greetham, D; Du C; Tucker, GA (2020). Viability of municipal solid waste as a source for bioenergy products production. *Int. J. Environ. Agric. Biotechnol.* 52

Aganbi, E; Egbune, EO; Orororo, O. C; Ezedom, T; Egbune, O. U; Tonukari, NJ (2023). Effect of microbial cell-size on solid state fermentation of cowpea (*Vigna unguiculata* L. Walp) and groundnut (*Arachis hypogaea* L.) by *Rhizopus oligosporus. J. Appl. Sci. Environ. Manage.* 27(6), 1093-1103.

Akbarian, A; Golian, A; Kermanshahi, H; De Smet, S; Michiels, J (2015). Antioxidant enzyme activities; plasma hormone levels and serum metabolites of finishing broiler chickens reared under high ambient temperature and fed lemon and orange

peel extracts and Curcuma xanthorrhiza essential oil. *J. Anim. Physiol. Anim. Nutr.* 99(1); 150-162

Akula, S; Golla, N (2020). Significance of process parameters on fungal cellulase production. *Biofuel Prod. Technol.: Crit. Anal. Sustain.* 299-324

Alagawany, M; Elnesr, SS; Farag, MR; Tiwari, R; Yatoo, MI; Karthik, K; Dhama, K (2021). Nutritional significance of amino acids; vitamins and minerals as nutraceuticals in poultry production and health–a comprehensive review. *Vet. Q.* 41(1); 1-29

Alamgir, ANM (2017). Therapeutic use of medicinal plants and their extracts: volume 1. Springer Int. Publ. AG

Alshelmani, MI; Kaka, U; Abdalla, EA; Humam, AM; Zamani, HU (2021). Effect of feeding fermented and non-fermented palm kernel cake on the performance of broiler chickens: a review. *World's Poult. Sci. J.* 77(2); 377–388

Al-Snai, AE (2019). Iraqi medicinal plants with antifungal effect-A review. *IOSR J. Pharm.* 9(7); 16-56

Anlamlert, W; Lenbury, Y; Bell, J (2017). Modeling fibrous cap formation in atherosclerotic plaque development: stability and oscillatory behavior. Adv. Differ. Equations (1); 1-15

Ansari, FA; Guldhe, A; Gupta, SK; Rawat, I; Bux, F (2021). Improving the feasibility of aquaculture feed by using microalgae. *Environ. Sci. Pollut. Res.* 28(32): 43234-43257

Antia, UE; Stephen, NU; Onilude, AA; Udo, IOM; Amande, TJ (2021). Bioconvertibility of mannan-containing polysaccharides to bioethanol: a comparative study of palm kernel cake and copra meal feedstocks. *Biomass Conv. Bioref.* 1-12

Araujo, A; Ward, OP (1990). Extracellular mannanases and galactanases from selected fungi. *J. Ind. Microbiol.* 6: 171–178

Azad, MAK; Liu, G; Bin, P; Ding, S; Kong, X; Guan, G; Yin Y (2020). Sulfur-containing amino acid supplementation to gilts from late pregnancy to lactation altered offspring's intestinal microbiota and plasma metabolites. *Appl. Microbiol. Biotechnol.* 104: 1227-1242

Azizi, MN; Loh, TC; Foo, HL; Teik, CEL (2021). Is palm kernel cake a suitable alternative feed ingredient for poultry?. Animals 11(2): 338

Barnes, ME; Brown, ML; Neiger, R (2015). Comparative performance of two rainbow trout strains fed fermented soybean meal. *Aquacult. Int.* 23: 1227-1238

Batiha, GES; Beshbishy, AM; Ikram, M; Mulla, ZS; El-Hack, MEA; Taha, AE; Elewa, YHA (2020). The pharmacological activity; biochemical properties; and pharmacokinetics of the major natural polyphenolic flavonoid: Quercetin. Foods 9(3): 374

Benzie, IF; Strain, JJ (1996). The ferric reducing ability of plasma FRAP as a measure of "antioxidant power". The FRAP assay. Anal. Biochem. 239: 70–76

Bodoira, R; Maestri, D (2020). Phenolic compounds from nuts: extraction; chemical profiles; and bioactivity. *J. Agric. Food Chem.* 68(4): 927-942

Bogar, B; Szakaes, G; Linden, JC; Pandey, A; Tengerdy, RP (2003). Optimization of phytase production by solid-state fermentation. *J. Microbiol. Biotechnol.* 30(3): 183-189

Cabanillas-Bojórquez, LA; Gutiérrez-Grijalva, EP; Contreras-Angulo, LA; Aviles-Gaxiola, S; Heredia, JB (2020). Biotechnology for extraction of plant phenolics. Plant Phenolics in Sustainable Agriculture: 1; 39-67

Cebrián, M; Ibarruri, J (2023). Filamentous fungi processing by solid-state fermentation. *In Curr. Dev. Biotechnol. Bioeng.* 251-292.

Christensen, LF; García-Béjar, B; Bang-Berthelsen, CH; Hansen, EB (2022). Extracellular microbial proteases with specificity for plant proteins in food fermentation. *Int. J. Food Microbiol.* 109889

Constantino, A; Rodrigues, B; Leon, R; Barros, R; Raposo, S (2021). Alternative chemo-enzymatic hydrolysis strategy applied to different microalgae species for bioethanol production. *Algal Res.* 56: 102329

da Silva, VD; Bilal, M; Bharagava, RN; Kumar, A; Kumar, NA; Salazar- Banda, GR; Romanholo Ferreira LF (2022). Lignin- modifying enzymes: a green and environmental responsive technology for organic compound degradation. *J. Chem. Technol. Biotechnol.* 97(2): 327-342

Dasari, PR; Ramteke, PW; Kesri, S; Kongala, PR (2019). Comparative study of cellulase production using submerged and solid-state fermentation. Approaches to Enhance Industrial Production of Fungal Cellulases; 37-52

de Jong, KA; Rosing, H; Huitema, AD; Beijnen, JH (2022). Optimized sample pre-treatment procedure for the simultaneous UPLC-MS/MS quantification of ipilimumab; nivolumab; and pembrolizumab in human serum. *J. Chromatogr. B* 1196: 123215

Disetlhe, ARP; Marume, U; Mlambo, V (2018). Humic acid and enzymes inclusion in canola-based diets generate different responses in growth performance; protein utilization dynamics; and hemato-biochemical parameters in broiler chickens. *Poult. Sci.* 97(8): 2745-2753

Edema-Eyen, U; Ohwode, JO; Egbune, EO; Dennis-Eboh, U; Ezedom, T; Ebosetale, MA; Tonukari, NJ (2023). Effect of boiling on the pH, glucose, and soluble proteins contents of waterleaf (*Talinum fruticosum*), bitter leaf (*Vernonia amygdalina*), and fluted pumpkin (*Telfairia occidentalis*). *Niger. J. Sci. Environ.* 21(1)

Egbune, EO; Aganbi, E; Anigboro, AA; Ezedom, T; Onojakpor, O; Amata, AI; Tonukari, NJ (2023a). Biochemical characterization of solid-state fermented cassava roots Manihot esculenta Crantz and its application in broiler feed formulation. *World J. Microbiol. Biotechnol.* 39(2): 1-12

Egbune, EO; Avwioroko, OJ; Anigboro, AA; Aganbi, E; Amata, AI; Tonukari, NJ (2022). Characterization of a surfactant-stable α-amylase produced by solid-state fermentation of cassava Manihot esculenta Crantz tubers using Rhizopus oligosporus: Kinetics; thermal inactivation thermodynamics and potential application in laundry industries. *Biocatal. Agric. Biotechnol.* 39: 102290

Egbune, EO; Avwioroko, OJ; Orororo, OC; Egbune, OU; Aganbi, E; Anigboro, AA; Tonukari, NJ (2023b). Rhizopus oligosporus alkaline protease in cassava fermentation: Characterization and detergent potential. *Biocatal. Agric. Biotechnol.* 54, 102954

Egbune, EO; Egbune, OU; Ezedom, T; Dennis-Eboh, U; Eraga, LI; Ichipi-Ifukor, PC; Tonukari, NJ (2024a). Enhancement of biochemical parameters and enzyme activity in solid-state fermented and biofortified maize cobs utilizing yeasts and plant extracts. *Bioresour. Technol. Rep.* 101874

Egbune, EO; Ezedom, T; Odeghe, OB; Orororo, OC; Egbune, OU; Ehwarieme, AD; Tonukari, NJ (2024b). Solid-state fermentation production of L-lysine by Corynebacterium glutamicum (ATCC 13032) using agricultural by-products as substrate. *World J. Microbiol. Biotechnol.* 40(1), 20

Egbune, EO; Ezedom, T; Tonukari, NJ (2021). Inclusion of a locally produced animal performance supplement in broilers' ration. *Niger. J. Sci. Environ.* 19(2)

Egbune, EO; Orororo, OC; Ezedom, T; Egbune, OU; Ebuloku, CS; Bright, OJ; Tonukari, NJ (2023c). Production of L-Lysine by Corynebacterium glutamicum using aqueous leaf extracts of tropical grasses as substrate. *Food Hum.* 1, 1592-1597

Egbune, EO; Tonukari, NJ (2023). Fermented mixture of cassava roots and palm kernel cake can substitute for maize in poultry feed formulation. *Afr. J. Biochem. Res.* 17(1): 1-8

Egbune, OU; Egbune, EO; Orororo, OC; Ezedom, T; Onojakpor, O; Sabo, AM; Amadi, K (2023d). Chronic cassava meal modulates body weight, histology and weight of reproductive organs in male albino rats. *Toxicol. Environ. Health Sci.* 15(3), 257-266

Ezedom, T; Egbune, E. O; Atseponu, SA; Charles, MO; Benson, BA; Ebbah, D; Tonukari, N (2023). Production of L-lysine under submerged fermentation by *Corynebacterium glutamicum* using different plants' leaves. *Afr. J. Trop. Med. Biomed. Res.* 6(1), 12-24

Ezedom, T; Egbune, E; Ehikordi, M; Ezeugo, N; Eledu, F; Esiete, J; Tonukari, N (2022). Biochemical evaluation of autoclaved and solid state fermented tropical pasture grasses. *J. Agric. Biotechnol. Sustain. Dev.* 14(2), 24-32

García-García, FJ; Monistrol-Mula, A; Cardellach, F; Garrabou, G (2020). Nutrition, bioenergetics, and metabolic syndrome. Nutrients 12(9): 2785

Getahun, D; Getabalew, M; Zewdie, D; Alemneh, T; Akeberegn, D (2019). Urea metabolism and recycling in ruminants. BJSTR 20: 14790-14796

Glinghammar B; Rafter I; Lindström AK; Hedberg JJ; Andersson HB; Lindblom P; Cotgreave I (2009).

Detection of the mitochondrial and catalytically active alanine aminotransferase in human tissues and plasma. *Int. J. Mol. Med.* 23(5): 621-631

Gnanamani, A; Kavitha, V; Radhakrishnan, N; Rajakumar, GS; Sekaran, G; Mandal, AB (2010). Microbial products biosurfactant and extracellular chromate reductase of marine microorganism are the potential agents reduce the oxidative stress induced by toxic heavy metals. Colloids Surf. B: Biointerfaces 79(2): 334-339

Gornall, AG; Bardawill, CJ; David, MM (1949). Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* 177: 751-756

Grela, ER; Czech, A; Kiesz, M; Wlazło, Ł; Nowakowicz-Dębek, B (2019). A fermented rapeseed meal additive: Effects on production performance; nutrient digestibility; colostrum immunoglobulin content and microbial flora in sows. *Anim. Nutr.* 5(4): 373-379

Gu, J; Bk, A; Wu, H; Lu, P; Nawaz, MA; Barrow, CJ; Suleria, HAR (2022). Impact of processing and storage on protein digestibility and bioavailability of legumes. *Food Rev. Int.* 1-28

Guzmán-Guzmán, P; Kumar, A; de los Santos-Villalobos, S; Parra-Cota, FI; Orozco-Mosqueda, MDC; Fadiji, AE; Santoyo, G (2023). Trichoderma Species: Our Best Fungal Allies in the Biocontrol of Plant Diseases—A Review. Plants 12(3): 432

Gyurászová, M; Gurecká, R; Bábíčková, J; Tóthová, Ľ (2020). Oxidative stress in the pathophysiology of kidney disease: implications for noninvasive monitoring and identification of biomarkers. *Oxid. Med. Cell. Longev.* 2020(1), 5478708.

Hakim, AH; Zulkifli, I; Farjam, AS; Awad, EA; Ramiah, SK (2022). Impact of Feeding Fermented Palm Kernel Cake and High Dietary Fat on Nutrient Digestibility; Enzyme Activity; Intestinal Morphology and Intestinal Nutrient Transporters mRNA Expression in Broiler Chickens under Hot and Humid Conditions. Animals 12(7): 882

Hatano, T; Kagawa, H; Yasuhara, T; Okuda, T (1988). Two New Flavonoids and Other Constituents in Licorice Root: Their Relative Astringency and Radical Scavenging Affects. *Chem. Pharm. Bull.* 36: 2090- 2097

Hsiao, FSH; Artdita, CA; Lin, SY; Yu, YH; Cheng, YH (2022). Mixed Solid-State Fermentation of Okara and Copra Meal by Probiotics with Non-Starch Polysaccharide Enzymes and Its Effects on the Growth Performance and Ileal Microbiota in Broilers. Fermentation 8(10): 478

Hu, X; Zhang, G; Hamaker, BR; Miao, M (2022). The contribution of intact structure and food processing to functionality of plant cell wall-derived dietary fiber. Food Hydrocolloids 107511

Huang, J; Feng, S; Liu, A; Dai, Z; Wang, H; Reuhl, K; Yang, CS (2018). Green tea polyphenol EGCG alleviates metabolic abnormality and fatty liver by decreasing bile acid and lipid absorption in mice. *Mol. Nutr. Food Res.* 62(4): 1700696

Huang, L; Gao, L; Chen, C (2021). Role of medium-chain fatty acids in healthy metabolism: a clinical perspective. Trends Endocrinol. Metab. 32(6): 351-366

Iluyemi, FB; Hanafi, MM; Radziah, O; Kamarudin, MS (2006). Fungal solid-state culture of palm kernel cake. *Bioresour. Technol.* 97: 477-482

Jannathulla, R; Dayal, JS; Ambasankar, K; Muralidhar, M (2018). Effect of Aspergillus niger fermented soybean meal and sunflower oil cake on growth; carcass composition and haemolymph indices in Penaeus vannamei Boone; 1931. Aquaculture 486: 1-8

Jia, Z; Tang, M; Wu, J (1999). The determination of flavonoid contents of mulberry and their scavenging effects on superoxide radicals. *Food Chem.* 64: 555-559

Jin, HM; Dang, B; Zhang, WG; Zheng, WC; Yang, XJ (2022). Polyphenol and Anthocyanin Composition and Activity of Highland Barley with Different Colors. Molecules 27(11): 3411

Kataria, A; Sharma, S; Dar, BN (2022). Changes in phenolic compounds; antioxidant potential and antinutritional factors of Teff Eragrostis tef during different thermal processing methods. *Int. J. Food Sci. Technol.* 57(11): 6893-6902

Kathak, RR; Sumon, AH; Molla, NH; Hasan, M; Miah, R; Tuba, HR; Ali, N (2022). The association between elevated lipid profile and liver enzymes: a study on Bangladeshi adults. *Sci. Rep.* 12(1): 1711

Ketnawa, S; Reginio, Jr FC; Thuengtung, S; Ogawa, Y (2022). Changes in bioactive compounds and antioxidant activity of plant-based foods by

gastrointestinal digestion: A review. *Crit. Rev. Food Sci. Nutr.* 62(17): 4684-4705

Kim, WR; Flamm, SL; Di Bisceglie, AM; Bodenheimer, HC (2008). Serum activity of alanine aminotransferase ALT as an indicator of health and disease. Hepatology 47(4): 1363-1370

Kortekangas, A; Silventoinen, P; Nordlund, E; Ercili-Cura, D (2020). Phytase treatment of a protein-enriched rice bran fraction improves heat-induced gelation properties at alkaline conditions. Food Hydrocolloids 105: 105787

Kumar, A; Singh, B; Raigond, P; Sahu, C; Mishra, UN; Sharma, S; Lal, MK (2021). Phytic acid: Blessing in disguise; a prime compound required for both plant and human nutrition. *Food Res. Int.* 142: 110193

Lee, FH; Wan, SY; Foo, HL; Loh, TC; Mohamad, R; Abdul Rahim, R; Idrus, Z (2019). Comparative study of extracellular proteolytic; cellulolytic; and hemicellulolytic enzyme activities and biotransformation of palm kernel cake biomass by lactic acid bacteria isolated from Malaysian foods. *Int. J. Mol. Sci.* 20(20): 4979

Leite, P; Silva, C; Salgado, JM; Belo, I (2019). Simultaneous production of lignocellulolytic enzymes and extraction of antioxidant compounds by solid-state fermentation of agro-industrial wastes. *Ind. Crops Prod.* 137: 315-322

Liu, HM; Zou, Y; Yao, CY; Yang, Z (2020). Enzymatic synthesis of vanillin and related catalytic mechanism. *Flavour Fragr. J.* 35(1): 51-58

Loftus, TJ; Brown, MP; Slish, JH; Rosenthal, MD (2019). Serum levels of prealbumin and albumin for preoperative risk stratification. Nutr. Clin. Pract. 34(3): 340-348

Londoño-Hernandez, L; Chávez-González, ML; Ascacio-Valdés, JA; Ruiz, HA; Toro, CR; Aguilar, CN. Fermentative Bioprocesses for Detoxification of Agri-Food Wastes for Production of Bioactive Compounds. *In Food Microbiol. Biotechnol.* 287-318

Lowe, D; Sanvictores, T; Zubair, M; John, S (2022). Alkaline phosphatase. In StatPearls [internet]. StatPearls Publishing

Magbanua, TO; Ragaza, JA (2022). Selected dietary plant-based proteins for growth and health response of Nile tilapia *Oreochromis niloticus*. *Aquacult. Fish.* 9(1), 3-19

Melaku, M; Zhong, R; Han, H; Wan, F; Yi, B; Zhang, H (2021). Butyric and citric acids and their salts in poultry nutrition: Effects on gut health and intestinal microbiota. *Int. J. Mol. Sci.* 22(19): 10392

Miller, GL (1959). Use of the Dinitrosalicylic Acid Reagent for the Determination of Reducing Sugar. *Anal. Chem.* 31: 426-428

Mohd Zin, NB; Mohamad Yusof, B; Oslan, SN; Wasoh, H; Tan, JS; Ariff, AB; Halim, M (2017). Utilization of acid pre-treated coconut dregs as a substrate for production of detergent compatible lipase by *Bacillus stratosphericus*. AMB Express 7(1): 1-13

Munteanu, IG; Apetrei, C (2021). Analytical methods used in determining antioxidant activity: A review. *Int. J. Mol. Sci.* 22(7): 3380

Naikoo, MI; Dar, MI; Raghib, F; Jaleel, H; Ahmad, B; Raina, A; Naushin, F (2019). Role and regulation of plants phenolics in abiotic stress tolerance: An overview. Plant signaling molecules; 157-168

Ndrepepa, G (2021). Aspartate aminotransferase and cardiovascular disease—a narrative review. *J. Lab. Precis. Med.* 6: 6

Nguyen, HT; Bedford, MR; Morgan, NK (2021). Importance of considering non-starch polysaccharide content of poultry diets. *World's Poultry Sci. J.* 77(3): 619-637

Nkhata, SG; Ayua, E; Kamau, EH; Shingiro, JB (2018). Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. *Food Sci. Nutr.* 6(8): 2446-2458

Noman, E; Al-Gheethi, A; Mohamed, RMSR; Talip, BA (2019). Myco-remediation of xenobiotic organic compounds for a sustainable environment: a critical review. *Top. Curr. Chem.* 377(1): 1-41

Olukomaiya, O; Fernando, C; Mereddy, R; Li, X; Sultanbawa, Y (2019). Solid-state fermented plant protein sources in the diets of broiler chickens: A review. *Anim. Nutr.* 5(4): 319-330

Pasala, S; Carmody, JB (2017). How to use serum creatinine; cystatin C and GFR. *Arch. Dis. Child. Educ. Pract. Ed.* 102(1): 37-43

Peredo-Lovillo, A; Romero-Luna, HE; Jiménez-Fernández, M (2020). Health promoting microbial metabolites produced by gut microbiota after prebiotics metabolism. *Food Res. Int.* 136: 109473

Posadino, AM; Cossu, A; Giordo, R; Piscopo, A; Abdel-Rahman, WM; Piga, A; Pintus, G (2021). Antioxidant properties of olive mill wastewater polyphenolic extracts on human endothelial and vascular smooth muscle cells. Foods 10(4): 800

Qasim, SS; Shakir, KA; Al-Shaibani, AB; Walsh, MK (2017). Optimization of culture conditions to produce phytase from *Aspergillus tubingensis* SKA. *Food Nutr. Sci.* 8(07): 733

Radi, ZA; Koza-Taylor, PH; Bell, RR; Obert, LA; Runnels, HA; Beebe, JS; Sadis, S (2011). Increased serum enzyme levels associated with kupffer cell reduction with no signs of hepatic or skeletal muscle injury. *Am. J. Pathol.* 179(1): 240-247

Raghubeer, S; Matshazi, DM; Motshwari, DD; Weale, CJ; Erasmus, RT; Matsha, TE (2022). The Role of Glycated Albumin as a Biomarker of Glycemic Control in Diabetes and Chronic Kidney Disease. In Biomarkers in Diabetes; 1-25

Reis, CER; Junior, NL; Bento, HB; de Carvalho, AKF; de Souza Vandenberghe, LP; Soccol, CR; Chandel, AK (2022). Process strategies to reduce cellulase enzyme loading for renewable sugar production in biorefineries. *Chem. Eng.* J. 138690

Rivas, MÁ; Benito, MJ; Martín, A; de Guía Córdoba, M; Ruíz-Moyano, S; Casquete, R (2022). Improve the functional properties of dietary fibre isolated from broccoli by-products by using different technologies. *Innov. Food Sci. Emerg. Technol.* 80: 103075

Santos, NC; Almeida, RLJ; Monteiro, SS; de Vilela Silva, ET; de Alcântara Silva, VM; André, AMM; de Brito, ACO (2022). Influence of ethanol and ultrasound on drying; bioactive compounds; and antioxidant activity of strawberries Fragaria× ananassa. *J. Indian Chem. Soc.* 99(7): 100542

Sargent, HJ; Elliott, J; Jepson, RE (2021). The new age of renal biomarkers: does SDMA solve all of our problems? *J. Small Anim. Pract.* 62(2): 71-81

Šelo, G; Planinić, M; Tišma, M; Grgić, J; Perković, G; Koceva, KD; Bucić-Kojić, A (2022). A Comparative Study of the Influence of Various Fungal-Based Pretreatments of Grape Pomace on Phenolic Compounds Recovery. Foods 11(11): 1665

Seo, K; Cho, HW; Chun, J; Jeon, J; Kim, C; Kim, M; Kim, K (2021). Evaluation of fermented oat and black soldier fly larva as food ingredients in senior dog diets. Animals 11(12): 3509

Singleton, VL; Rossi, JA (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am. J. Enol. Vitic.* 16: 144-158

Song, J; Huang, M; Shi, X; Li, X; Chen, X; He, Z; Zheng, J (2021). T329S mutation in the FMO3 gene alleviates lipid metabolic diseases in chickens in the late laying period. Animals 12(1): 48

Song, Z; Yang, Q; Dong, B; Li, N; Wang, M; Du, T; Fu, Y (2022). Melatonin enhances plant stress tolerance by promoting flavonoid enrichment; focusing on luteolin for salt stress. *J. Exp. Bot.* 73(17), 5992-6008.

Suohui, T; Yanping, C; Shuhui, Z; Zhihua, L; Honggang, J; Jun, L; Tao, L (2022). Thiamine induces resistance in tobacco against black shank. *Australasian Plant Pathol.* 51(2): 231-243

Tonukari, NJ; Anigboro, AA; Avwioroko, OJ; Egbune, EO; Ezedom, T; Ajoh, AI; Aganbi, E (2023). Biochemical properties and biotechnological applications of cassava peels. *Biotechnol. Mol. Biol. Rev.* 14(1), 1-8

Torres-León, C; Ramírez-Guzmán, N; Ascacio-Valdes, J; Serna-Cock, L; dos Santos Correi,a MT; Contreras-Esquivel, JC; Aguilar, CN (2019). Solid-state fermentation with *Aspergillus niger* to enhance the phenolic contents and antioxidative activity of Mexican mango seed: A promising source of natural antioxidants. Lwt 112: 108236

van Goor, MK; Hoenderop, JG; van der Wijst, J (2017). TRP channels in calcium homeostasis: from hormonal control to structure-function relationship of TRPV5 and TRPV6. Biochim. Biophys. *Acta BBA-Mol. Cell Res.* 1864(6): 883-893

Vanderzee, S; Zeman, F (2018). Recovery and carbonation of 100% of calcium in waste concrete

fines: Experimental results. *J. Cleaner Prod.* 174: 718-727

Wang, Y; Han, J; Wang, D; Gao, F; Zhang, K; Tian, J; Jin, Y (2022). Research Update on the Impact of Lactic Acid Bacteria on the Substance Metabolism; Flavor; and Quality Characteristics of Fermented Meat Products. Foods 11(14): 2090

Wong, YP; Saw, HY; Janaun, J; Krishnaiah, K; Prabhakar, A (2011). Solid-state fermentation of palm kernel cake with Aspergillus flavus in laterally aerated moving bed bioreactor. *Appl. Biochem. Biotechnol.* 164: 170-182

Wu, J; Song, S; Cao, HC; Li, LJ (2020). Liver diseases in COVID-19: Etiology; treatment and prognosis. *World J. Gastroenterol.* 26(19): 2286

Xiao, Y; He, C; Chen, Y; Ho, CT; Wu, X; Huang, Y; Liu, Z (2022). UPLC–QQQ–MS/MS-based widely targeted metabolomic analysis reveals the effect of solid-state fermentation with *Eurotium cristatum* on the dynamic changes in the metabolite profile of dark tea. *Food Chem.* 378: 131999

Yadav, SK (2017). Technological advances and applications of hydrolytic enzymes for valorization of lignocellulosic biomass. Bioresour. Technol. 245: 1727-1739

Yanai, H; Masui, Y; Katsuyama, H; Adachi, H; Kawaguchi, A; Hakoshima, M; Sako, A (2018). An improvement of cardiovascular risk factors by omega-3 polyunsaturated fatty acids. *J. Clin. Med. Res.* 10(4): 281

Yegin, S; Kopec, A; Kitts, DD; Zawistowski, J (2020). Dietary fiber: A functional food ingredient with physiological benefits. *In Dietary sugar; salt and fat in human health* pp 531-555 Academic Press

Yigit, NO; Bahadir, KS; Didinen, BI; Diler, I (2018). Effect of protease and phytase supplementation on growth performance and nutrient digestibility of rainbow trout (Oncorhynchus mykiss; Walbaum) fed soybean meal-based diets. J. *Appl. Anim. Res.* 46(1): 29-32

Zhang, R; Li, XY; Cen, XL; Gao, QH; Zhang, M; Li, KY; Huang, ZX (2021). Enzymatic preparation of manno-oligosaccharides from locust bean gum and palm kernel cake; and investigations into its prebiotic activity. *Electron. J. Biotechnol.* 49: 64-71

Zhao, B; Al Rasheed, H; Ali, I; Hu, S (2021). Efficient enzymatic saccharification of alkaline and ionic liquid-pretreated bamboo by highly active extremozymes produced by the co-culture of two halophilic fungi. *Bioresour. Technol.* 319: 124115