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EVALUATION OF PROXIMATE, PHYTOCHEMICALS, ANTIOXIDANT CAPACITY, ENZYMATIC INHIBITION, AND ANTI-INFLAMMATORY PROPERTIES OF AVOCADO SEED MEAL AS POTENTIAL FEED ADDITIVE

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Abstract: This study examined the proximate composition, phytochemical profile, antioxidant capacity, enzymatic inhibition, and anti-inflammatory properties of avocado seed meal. The proximate analysis revealed that avocado seed meal is rich in protein (17.32%), fat (15.33%), and carbohydrates (48.73%), with moderate levels of moisture (10.26%), ash (2.39%), and crude fiber (5.97%). Phytochemical analysis indicated high contents of phenols (111.56 mg/g), saponins (96.94 mg/g) and flavonoids (66.66 mg/g), but lower levels of alkaloids (14.60 mg/g), tannins (1.17 mg/g), and steroids (0.89 mg/g). The antioxidant properties assessed showed significant DPPH (50.05%) and FRAP (63.04%) free radical scavenging activities, though lower inhibition of lipid peroxidation (34.88%) and vitamin C content (0.03 mg/g). Enzymatic inhibition assays demonstrated alpha-lipase, alpha-glucosidase, and alpha-amylase inhibition rates of 31.46%, 27.56%, and 50.88%, respectively. Anti-inflammatory properties were also notable, with 24.02% albumin denaturation inhibition and 37.99% antiprotease activity. These findings suggest that avocado seed meal holds considerable potential for nutritional and therapeutic applications as natural feed supplement for livestock and humans.

Keywords: Antioxidant, Plant-based additives, Phytochemicals, Proximate, Protein denaturation, Seed meal

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1. Introduction

In recent years, interest in the use of feed ingredients or additives endowed with bioactive properties has increased significantly, especially within the system of intensive livestock production, due to occurrence of antibiotic residues in animal products, which are injurious to consumer health (Falowo et al., 2014; Hotea et al., 2023). Part of these feed ingredients/additives could be obtained from processed fruit and vegetable wastes, which are typically discarded after processing, packing, distribution and consumption (FAO, 2013; Ikusika et al., 2024). .On yearly basis, substantial quantities of fruit and vegetable wastes are generated and disposed of in landfills or water bodies, presenting potential health and environmental hazards if not appropriately channeled into productive products (FAO, 2013; Ikusika et al., 2024). These wastes represent abundant resources on a global scale. However, fruit wastes, encompassing seeds, peels, pulp, pomace, and their extracts, can be processed and harnessed as natural feed ingredients or additives to promote growth and health in animal production, because they possessed a plethora of bioactive compounds that offers antioxidant, antimicrobial, immune-protective and other beneficial secondary metabolites (Achilonu et al., 2018). In addition, they are rich sources of dietary fiber, protein, vitamins C and E, carotenoids, minerals, and other micronutrients, offering potential as alternative to synthetic growth hormones and antibiotics within the livestock industry (Achilonu et al., 2018; Ikusika et al., 2024). Recent studies have indicated that dietary supplementation with fruit seed meals can enhance growth performance and improve nutrient digestibility and meat quality in livestock (Akure et al., 2021; Haruna et al., 2021). Also the dietary inclusion of fruit seed meals containing phytochemicals have been reported to lower blood glucose and cholesterol content and enhance liver glycogen storage in animals (Uchenna et al., 2017). Utilization of fruit seed meals as feed additives in animal diets is believed to be natural, less toxic, residue-free, and safer than synthetic ones (Manuelian, et al., 2021).

One of the fruit seeds that can be explored as natural feed additives is avocado fruit seeds. Avocado (*Persea americana*) is a dicotyledonous tropical plant belonging

to the *Lauraceae* family (Barbosa-Martín et al., 2016; Bangar et al., 2022). Renowned for its sensory attributes and nutritional benefits, avocado is widely cultivated and highly sought after internationally, often regarded as the world's healthiest fruit for human consumption (Mahawan et al., 2015; Bangar et al., 2022; Siol and Sadowska, 2023). Apart from its use as food, the avocado fruit is traditionally used for several medicinal purposes including hypotensive, hypoglycemic, anti-viral and anti-diarrheal and cardiovascular diseases (Vinha et al., 2020). The processing of avocado fruits into products such as avocado oil and paste often generate a sizeable amount of waste products, including peels and seeds which are always discarded as waste (Bangar et al., 2022). Each fruit contains a single large seed, accounting for approximately 13–18% of the fruit's weight (Barbosa-Martín et al., 2016; Siol and Sadowska, 2023). These seeds are rich in nutrients and possess various medicinal properties, making them potential candidates as valuable feed additives in animal diets. This is primarily due to their substantial reserves of inherent phytochemicals such as alkanols, terpenoid glycosides, furan ring-containing derivatives, flavonoids and coumarin (Uchenna et al., 2017; Setyawan et al., 2021). Emerging research indicates that the phytochemicals in seed meals may offer health benefits, including antioxidant and antimicrobial effects (Siol and Sadowska, 2023). Understanding these compounds could pave the way for new health supplements and functional foods, while reevaluating them could enhance the commercial use of these resources in food and feed industries. It is known that different varieties of seed meals including avocados produce different content of nutrients and bioactive compounds (Setyawan et al., 2021). Therefore, this study aims to evaluate the proximate composition, phytochemical profile, and potential anti-diabetic, anti-inflammatory, and antioxidant properties of avocado seed meal.

2. Materials and Methods

2.1. Collection and Processing of Avocado Seed Meal

Fresh avocado fruits were purchased from a local market in Ondo State, Nigeria. The seeds were extracted from the fruits, cleaned, sliced into small cubes, and air-dried in an open shade for 14 days. After drying, the seeds were ground into a meal and stored at 4 °C until analysis. All chemicals used for the analyses were of analytical reagent grade and were purchased from Sigma-Aldrich.

2.2. Determination of Proximate Composition of Avocado Seed Meal

The proximate composition (moisture, fat, protein, ash, crude fiber) of the seed meal was determined according to the procedures described by the Association of Official Analytical Chemists (AOAC, 2010). The carbohydrate content of the seed meal was calculated by the difference method (AOAC, 2010), subtracting the sum (g/100g dry matter) of crude protein, crude fat, ash, and fiber from 100g.

2.3. Determination of Phytochemical Composition of Avocado Seed Meal

2.3.1. Tannin content

Approximately 0.2g of each finely ground sample was weighed into a 50mL sample bottle. Then, 10mL of 70% aqueous acetone was added, and the bottle was properly sealed. The bottle was placed in an ice bath shaker and shaken for 2 hours at 30 °C. Each solution was then centrifuged, and the supernatant was stored on ice. From each solution, 0.2mL was pipetted into a test tube, and 0.8mL of distilled water was added. Standard tannic acid solutions were prepared from a 0.5mg/mL stock solution, and the volume was adjusted to 1mL with distilled water. Next, 0.5mL of Folin-Ciocalteu reagent was added to both the sample and the standard, followed by 2.5mL of 20% Na_2CO_3. The solution was then vortexed and allowed to incubate for 40 minutes at room temperature. Its absorbance was read at 725nm against a reagent blank. The tannin concentration was determined using a standard tannic acid curve (Makker and Goodchild, 1996).

2.3.2. Saponins content

Saponin was quantified using the vanillin and concentrated sulfuric acid colorimetric method described by He et al. (2014). A 0.1mL sample was combined with 0.5mL of 50% ethanol, 4.0mL of 77% sulfuric acid (w/w), and 0.5mL of freshly prepared 8% vanillin solution (w/v). The mixture was allowed to reach ambient temperature before being heated in a water bath at 60 °C for 15 minutes. The absorbance was measured at 545nm using a UV/Vis spectrophotometer. A tea saponin calibration curve was used to quantify the total saponin content in the seed sample, which was expressed as mg tea saponin equivalent per gram dry weight (TSE/g DW).

2.3.3. Alkaloids content

The alkaloid content of the seed sample was determined using the gravimetric technique described by Adeniyi et al. (2009). Five grams of the sample were dispersed in 50mL of 10% acetic acid in ethanol. After stirring, the mixture was left to stand for approximately 240 minutes before being filtered. The filtrate was then reduced to a smaller volume on a heated plate. Concentrated ammonium hydroxide was added dropwise to precipitate the alkaloids. The precipitate was collected on filter paper and rinsed with a 1% ammonium hydroxide solution. It was then oven-dried at 60 °C for 30 minutes, transferred to desiccators, and weighed repeatedly until a constant weight was achieved. The weight of the alkaloids was calculated as a percentage of the total sample weight.

2.3.4. Steroids content

The steroid content of the seed sample was determined using the method described by Odeyemi et al. (2023). One gram (1.0g) of the powdered sample was mixed with 100mL of distilled water in a conical flask. The mixture was filtered, and the filtrate was eluted with 0.1N ammonium hydroxide solution. Two milliliters (2mL) of the eluent were transferred to a test tube and mixed with

2mL of chloroform. Subsequently, 3mL of ice-cold acetic anhydride was added to the mixture. A standard sterol solution (200mg/dL) was prepared and treated as a blank. The absorbance of the standard and the test sample was measured using a spectrophotometer set to 420nm, zeroed with the blank.

2.3.5. Vitamin C

The vitamin C content of the seed sample was determined using the method described by Benderitter et al. (1998). A 500 µL extract mixture (300 µL extract diluted with 100 µL of 13.3% trichloroacetic acid and water) was prepared, to which 75 µL of DNPH solution (containing 2g dinitrophenyl hydrazine, 270mg copper sulfate ($CuSO_4.5H_2O$), and 230mg thiourea in 100mL of 5mL/L H_2SO_4) was added. The reaction mixture was incubated at 37 °C for 3 hours. After incubation, 0.5mL of 65% H2SO4 (v/v) was added to the mixture, and the absorbance was measured at 520nm using a UV spectrophotometer. The vitamin C content of the seed sample was then quantified using ascorbic acid as a reference standard.

2.3.6. Phenol content

The total phenol content of the seed aqueous extract was determined using the method described by Singleton et al. (1999). Briefly, 0.2mL of the extract was mixed with 2.5mL of 10% Folin-Ciocalteu reagent and 2mL of 7.5% sodium carbonate. The reaction mixture was incubated at 45 °C for 40 minutes, and the absorbance was measured at 700nm using a spectrophotometer. The phenol content was expressed as mg gallic acid equivalent.

2.3.7. Flavonoid content

The total flavonoid content of the seed aqueous extract was determined using a colorimetric assay described by Bao et al. (2005). Briefly, 0.2mL of the extract was added to 0.3mL of 5% $NaNO_3$ at zero time. After 5 minutes, 0.6mL of 10% AlCl3 was added. Six minutes later, 2mL of 1M NaOH was added to the mixture, followed by 2.1mL of distilled water. The absorbance was read at 510nm against a reagent blank. The flavonoid content was expressed as mg rutin equivalent.

2.4. Determination of Anti-oxidant Properties of Avocado Seed Meal

2.4.1. 2,2-Diphenyl-2-picrylhydrazyl radical scavenging capacity

The free radical scavenging ability of the seed aqueous extracts against DPPH (1,1-diphenyl-2-picrylhydrazyl) was estimated according to the method described by Gyamfi et al. (1999). One milliliter of the extract was mixed with 1mL of a 0.4 mM methanolic solution of DPPH, and the mixture was left in the dark for 30 minutes before measuring the absorbance at 516 nm

2.4.2. Lipid peroxidation inhibition

The lipid peroxidation inhibition of the seed aqueous extract was determined using a method previously described by Bajpai et al. (2015). In the presence or absence of seed extract (50-250 g/mL) or a control substance, a reaction mixture containing 1 mM $FeCl_3$, 50 µL of bovine brain phospholipids (5 mg/L), and 1 mM ascorbic acid in 20 mM phosphate buffer was incubated at 37 °C for 60 minutes. Malondialdehyde (MDA), a byproduct of lipid peroxidation caused by hydroxyl radicals, was measured using the 2-thiobarbituric acid (TBA) reaction. The percentage of inhibitory activity was calculated using the equation 1:

$$\% \text{ inhibition} = [(AC - AT)/(AC)] \times 100 \quad (1)$$

where, AC= absorbance of control, AT= absorbance of test

2.4.3. Ferric reducing antioxidant power (FRAP)

The reducing property of the seed aqueous extracts was assessed by their ability to reduce ferric chloride ($FeCl_3$) solution, following the method described by Oyaizu (1986). A 2.5 mL aliquot of the extract was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was then incubated at 50 °C for 20 minutes, followed by the addition of 2.5 mL of 10% trichloroacetic acid. After centrifugation at 2000 x g for 10 minutes, 5 mL of the supernatant was mixed with an equal volume of water and 1 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm using a spectrophotometer, and the ferric reducing antioxidant property was subsequently calculated, with ascorbic acid used as the standard.

2.5. Determination of Antidiabetic Properties of Avocado Seed Meal

2.5.1. Alpha-amylase inhibitory activity

The α-amylase inhibition study was conducted using the 3,5-dinitrosalicylic acid (DNSA) method, as outlined by Wickramaratne et al. (2016). Seed extract concentrations ranging from 10 to 1000 g/mL were prepared by treating the extract with at least 10% dimethylsulfoxide and diluting it in buffer solution (0.006 M NaCl, 0.02 M Na_2HPO_4/NaH_2PO_4, pH 6.9). Two hundred microliters of extract and 200 µL of α-amylase solution were mixed and incubated at 30 °C for 10 minutes. Subsequently, 200 µL of starch solution (1% in water, w/v) was added to each tube, and the mixture was further incubated for 3 minutes. The reaction was stopped by adding 200 µL of DNSA reagent (12 g sodium potassium tartrate tetrahydrate in 8.0 mL 2 M NaOH and 20 mL 96 mM 3,5-dinitrosalicylic acid solution) to a water bath at 85-90 °C, followed by boiling for 10 minutes. After cooling to room temperature, the mixture was diluted with 5 mL distilled water and analyzed using a UV-Visible spectrophotometer at 540nm. A blank with 100% enzyme activity was created by substituting 200 µL of buffer for the plant extract, while a blank reaction without the enzyme solution was generated using the plant extract at each concentration. As a positive control, acarbose (100–200 µg/mL) was used, and the reaction was conducted similarly to the plant extract reaction. The inhibitory activity of α-amylase was calculated as a percentage of inhibition using the equation provided. IC50 values were determined by plotting the percentage of α-amylase inhibition versus the extract concentration (Equation 2):

% α - amylase inhibition = 100 x (A%C – (AS/A%C) (2)

where, A%C= absorbance 100% control, AS= absorbance sample

2.5.2. Alpha-glucosidase inhibitory activity

An assay for assessing the glucosidase inhibitory activity of the seed was adapted from Dej-adisai and Pitakbut (2015). The glucosidase enzyme converts the substrate, p-nitrophenyl-D-glucopyranoside (pNPG), into the yellow product, p-nitrophenol (pNP), which is used to analyze the glucosidase reaction. Fifty microliters of a 10 mM phosphate buffer solution (pH 7), containing 0.2 mg/mL sodium azide and 2 mg/mL bovine serum albumin, were added to a well plate. One unit/mL of Saccharomyces cerevisiae α-glucosidase and 50 µL of an 8 mg/mL sample solution were added to the phosphate buffer solution (Type I, lyophilized powder, Sigma, EC 3.2.1.20). The solvent control was a 5% DMSO solution, and the positive control was 8 mg/mL of acarbose in each well. The mixtures were incubated at 37 °C for 2 minutes. Fifty microliters of 4 mM pNPG were then added to each well, and the mixture was further incubated for 5 minutes under the same conditions. The release of pNP was measured every 30 seconds for 5 minutes using a microplate reader at 405 nm. The velocity (V) was calculated using the following linear relationship equation (3) between absorbance and time:

Velocity = ΔAbsorbance at 405 nm / ΔTime (3)

The initial highest velocity of each sample's reaction was recorded, and the percentage of inhibition was calculated using the equation (4) provided:

% Inhibition = ((V control − V sample) / (V $control$)) X 100 (4)

2.5.3. Lipase inhibition activity

The lipase inhibitory activity of the seed samples was determined following the method described by Ambigaipalan et al. (2017), with modifications as mentioned by Fathi et al. (2021). In brief, lipase enzyme (5 mg) was dissolved in 1 M Tris-HCl (pH 8.5). One hundred microliters of each tested sample (concentrations ranging from 0.2 to 1 mg/mL) were added to an equal volume of lipase solution and 4 mL of Tris-HCl buffer (1 M, pH 8.5). The mixtures were then incubated at 37 °C for 25 minutes, and the enzymatic reactions were initiated by adding 100 µL of the substrate (5 mM palmitate in dimethyl sulfoxide (DMSO): ethanol (at 1:1 w/v)) to the reaction mixtures, followed by a second incubation period (37 °C, 25 minutes). The absorbance of the samples was measured in a microplate reader (BioTek, Winooski, Vermont, US) at 412 nm. Lipase inhibitory activity was determined using the following equation 5:

Lipase inhibitory (%) = ((As − Asb)/(Ac − Acb)) × 100 (5)

where As, Asb, Ac, and Acb represent the absorbance of the sample, sample blank, control, and control blank, respectively. The blank and control samples were generated by following the aforementioned experimental steps without adding the enzyme or without adding the inhibitor and enzyme, respectively.

2.6. Determination of Anti-inflammatory Properties of Avocado Seed M

2.6.1. Albumin denaturation inhibition

The assay was conducted following the protocol outlined by Osman et al. (2016). Ibuprofen and diclofenac, two positive standards, were prepared at a concentration of 0.1% each (1.0 mg/mL), alongside the seed extracts. Each reaction vessel contained 1000 µL of the test extract, 1400 µL of phosphate-buffered saline, and 200 µL of egg albumin. Distilled water was used as a negative control in place of the extracts. The mixtures were incubated at 37 °C for 15 minutes and then heated for 5 minutes at 70 °C. After cooling, their absorbances at 660 nm were measured. The protein denaturation inhibition percentage was calculated using the following formula given in Equation 6:

% DI = (1 – ARTS/ARTS (−ve control)) ∗ 100% (6)

where, DI= denaturation inhibition, ARTS= absorbance reading of the test sample

2.6.2. Antiproteinase activity

The test was conducted following the procedure outlined by Rajesh et al. (2019). The reaction mixture (2 mL) consisted of 1 mL of 20 mM Tris-HCl buffer (pH 7.4), 0.06 mg of trypsin, and 1 mL of the test sample with varying concentrations (100–500 µg/mL). The mixture was heated at 37 °C for five minutes. Subsequently, 1 mL of 0.8% (w/v) casein was added to the mixture, and it was further incubated at 37 °C for 20 minutes. To terminate the reaction, 2 mL of 70% perchloric acid was added to the mixture. After centrifugation, the absorbance of the supernatant was measured at 210 nm using buffer as a blank. The experiment was conducted in triplicate. The percentage inhibition of proteinase inhibitory activity was calculated using the following formula given in Equation 7:

% inhibition = (Abs control-Abs sample)*100/Abs control. (7)

2.7. Statistical Analysis

Data obtained on each experiment were carried out three times and the results were analyzed using descriptive statistic of SPSS version 2021 to compute the mean. The means were used to construct bar graphs in Excel.

3. Results

The result of proximate composition of avocado seed meal is presented in Figure 1. The result revealed that avocado seed meal possessed high protein (17.32%), fat (15.33%) and carbohydrate (48.73%) content and moderate moisture (10.26%), ash (2.39%) and crude fibre (5.97%) content. As presented in Figure 2, the result of the phytochemical analysis showed that avocado seed meal contained high phenol (111.56mg/g), saponins

(96.94mg/g) and cardiac glycosides (56.78mg/g) contents and low flavonoid (66.66mg/g), alkaloid (14.60mg/g), tannin (1.17mg/g) and steroid (0.89mg/g) contents. The antioxidant properties of avocado seed meal is presented in Figure 3. The result showed that avocado seed meal had strong DPPH (50.05%) and FRAP (63.04%) free radical scavenging ability and low percent inhibition of lipid peroxidation (34.88%) and vitamin C content (0.03mg/g). The percentage of lipase inhibition, alpha-glucosidase and alpha-amylase inhibition activities in the avocado seed meal were 31.46, 27.56 and 50.88%, respectively (Figure 4). The result of the anti-inflammatory properties showed that avocado seed meals had a 24.02 percent albumin denaturation inhibition and a 37.99 percent antiprotease activity, respectively (Figure 5).

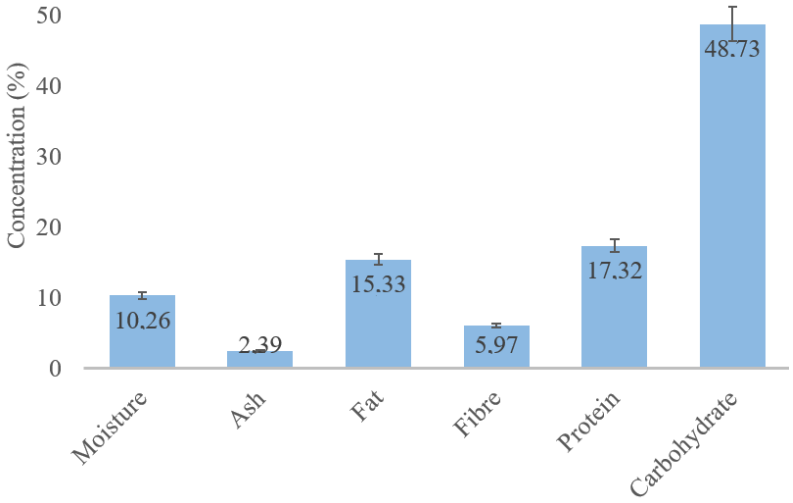

Figure 1. Proximate composition of avocado seed meal.

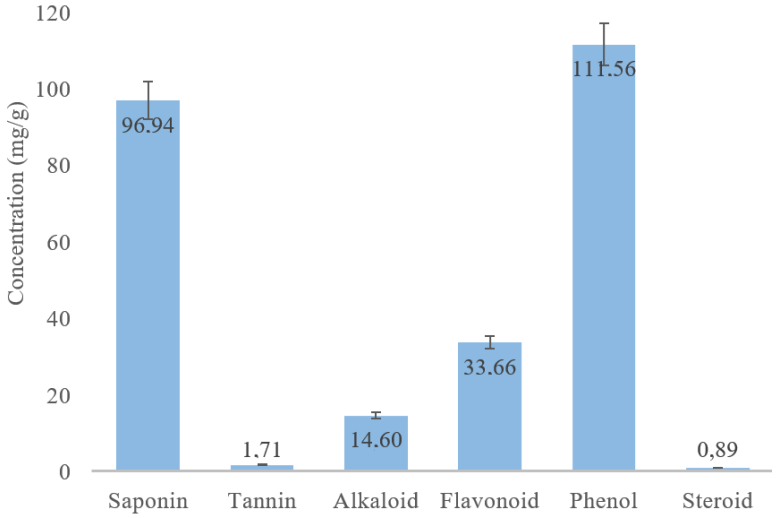

Figure 2. Phytochemical composition of avocado seed meal.

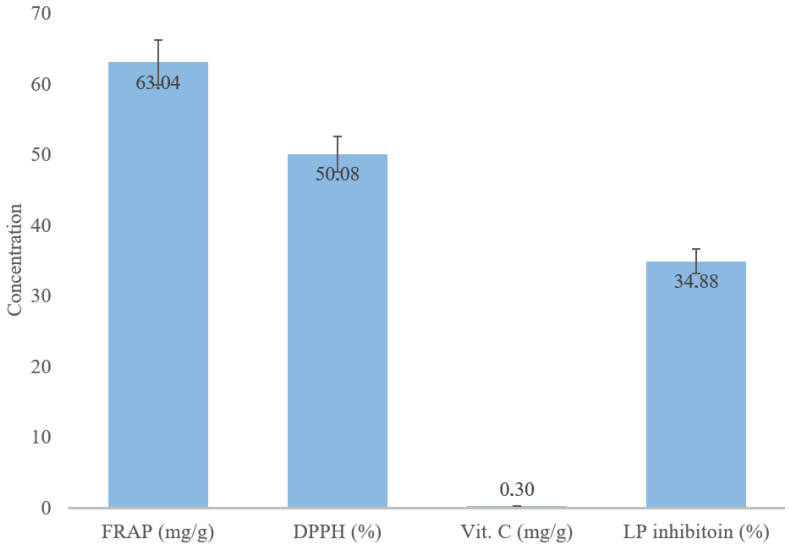

Figure 3. Anti-oxidant properties of avocado seed meal: LP= lipid peroxidation, DPPH= 2,2-diphenyl-1-picrylhydrazyl, FRAP= Ferric reducing antioxidant power, Vit. C= Vitamin C.

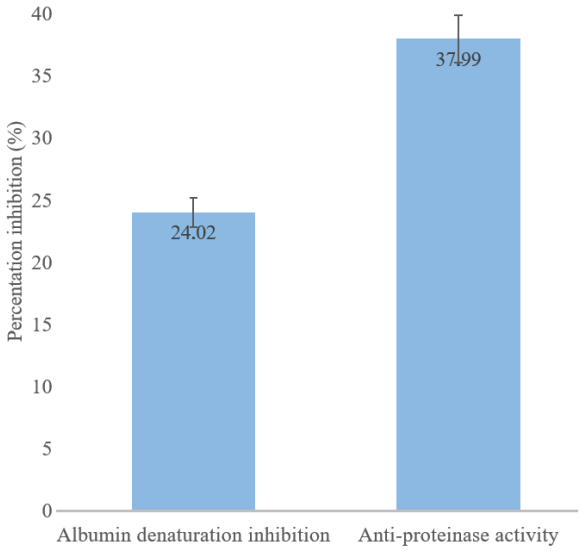

Figure 4. Anti-inflammatory properties of avocado seed meal

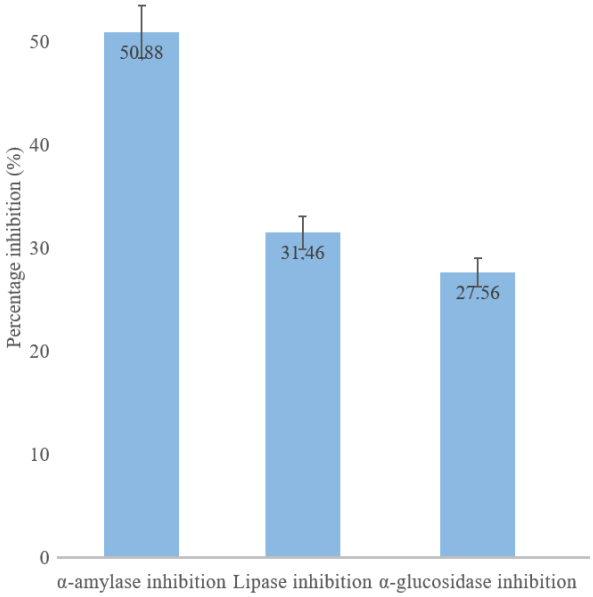

Figure 5. Antidiabetic properties of avocado seed meal.

4. Discussion

Evaluation of the proximate composition of food and feedstuffs is crucial for determining their safety, suitability, and nutritional integrity before consumption or incorporation as ingredients in animal diets (Oloruntola and Ayodele, 2022). In this study, the analysis of the proximate composition of avocado seed meal revealed significant nutritional attributes, positioning it as a promising food supplement or feed ingredient. The high protein content observed in avocado seed meal suggests its potential as an alternative protein source when utilized as feed ingredient to reduce the cost of using conventional protein in diet. Proteins are vital complex molecules that play pivotal roles in growth and development, cell signaling, enzyme regulation, and serving as biocatalysts in animals (Bangar et al., 2022).

The recorded level of fat content in avocado seed meal indicates its capacity to provide additional energy to the diet, thereby promoting efficient nutrient utilization and feed conversion efficiency. Studies have shown that the inclusion of feed ingredients rich in fat content can elevate dietary energy levels, leading to improved feed efficiency, growth rates, and enhanced quality of animal products (Hao et al., 2020; Omidi et al., 2020). Similarly, the substantial carbohydrate content found in avocado seed meal suggests it could be used as energy source in animal diets, particularly when combined with other carbohydrate or energy sources. Carbohydrates serve as supplementary energy sources for animals, supporting metabolic functions and aiding in maintaining body temperature (Navarro et al., 2019). The observed low crude fiber content in avocado seed meal indicates that the seed meal could easily be digested and absorbed in

the gastrointestinal tract when included as a feed ingredient or additive in animal diets, especially for monogastric animals. Fiber is crucial in the diet of farm animals, as it acts as a diluent to enhance proper bowel movement (Odoemelam and Ahamefule, 2006; Udo et al., 2018). However, feed with higher crude fiber content has been reported to remain longer in the stomach, widening the interior wall of the colon, facilitating waste movement, and preventing constipation (Kolu et al., 2021). The low ash content of avocado seed meal implies that the seeds may not be a rich source of minerals, necessitating supplementary diets when included in animal diets. The ash content of feed samples is typically used to determine the organic content, from which the mineral content can be derived (Bello et al., 2008; Kolu et al., 2021). The values of moisture content obtained for avocado seed meal in this study fall within the range (5-12%) previously reported for other seed meals (Ogunbode and Raji, 2024). Moisture content in feed is indicative of its water activity, with higher moisture content samples having a higher tendency for spoilage of food materials (Famuwagun and Taiwo, 2023). The moisture content levels observed in avocado seed meal in this study suggest a prolonged shelf life when included in animal diets. In overall, the proximate composition values (protein, fat, crude fiber, carbohydrate, and moisture) obtained for avocado seed meal in this study align with the range of values reported by Bangar et al. (2022) and Nyakang et al. (2023) for avocado seed meal.

Phytochemical screening of plant materials, including seeds, is crucial to determine their potential and suitability as natural feed additives before inclusion in animal diets (Oloruntola et al., 2024). This is because the presence of specific phytochemical contents can elucidate the product's action or reaction when used in biological systems. The amount of phenol, flavonoid, and alkaloid contents obtained in avocado seed meal revealed that the seed contained rich antioxidants and could be utilized as a potential natural food and feed supplement/additive. The presence of phenol and flavonoid content in avocado seed has been associated with its high antioxidant and antimicrobial bioactivity (Rodriguez-Carpena et al., 2004; Achilonu et al., 2018). Phenol assists in the prevention of several chronic diseases that arise through the activity of free radicals, while flavonoid and alkaloids help combat various resistant bacterial strains (Achilonu et al., 2018; Kurek, 2019). The application of feed additives rich in antioxidant content, such as phenol and flavonoid, has been reported to increase serum and muscle antioxidants, improve the growth, nutrient digestibility and absorption, and meat quality of livestock (Falowo, 2023; Oloruntola et al., 2024).

The presence of saponins and tannins shows that inclusion of avocado seed meal in animal diets can boost the immune system and reduce the level of cholesterol content in animals. Saponins, steroids, and tannins are important secondary metabolites that play significant roles as immunostimulants and hypocholesterolaemic properties in animals (Das et al., 2012; Oloruntola and Ayodele, 2022). Specifically, saponins are used to reduce serum cholesterol levels, enhance feed efficiency and body weight gain of animals, increase the permeability of intestinal mucosal cells in vitro, inhibit active mucosal transport, and facilitate uptake of substances that are usually not absorbed (Oloruntola et al., 2021). Tannins are also used to control bloat, pathogenic bacteria load, and intestinal parasites in animals. Steroids are used to increase feed intake, induce hypocholesterolemia, and increase muscle yield in animals (Petit et al., 1995; Skoupa et al., 2022). The results of the antioxidant content (phenol, flavonoid, saponin, tannins, steroids, and alkaloid) of avocado seed meal obtained in this study are inconsistent with values reported by Setyawan et al. (2021). This variation may be due to factors such as variety, season of harvest, geographical origin and location, processing, and methods of extraction (Kupnik et al., 2023).

The analysis of ferric reduction activity potential (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity is utilized to determine the antioxidant potentials of plant materials including seed meal. It was evident that extracts of the avocado seed meals displayed good antioxidant activity in both the DPPH and FRAP assays. This could be attributed to the high contents of inherent phenolic compounds in the seed meal. Numerous studies have shown that the antioxidant activities of plant materials are mainly due to the concentration of inherent phenolic compounds (Falowo et al., 2014; Oloruntola et al., 2021; Falowo, 2022). The level of lipid peroxidation inhibition recorded in this study is a direct confirmation that when avocado seed meal is utilized as feed additives/ingredient, it could inhibit the production of oxidation due to its high inherent phenolic content and antioxidant activities as shown earlier. Lipid peroxidation is a complex chemical process that occurs when free radicals attack lipids to alter the physicochemical properties of membrane lipid bilayers and cause severe cellular dysfunction in animals (Ayala et al., 2014). The low amount of vitamin C recorded in this study shows that the use of avocado seed meals in the diet may require additional supplementation for vitamin C. Vitamin C is a natural antioxidant that helps to scavenge free radicals and strengthen the body's immunity against infections (Robert et al., 2003).

In addition to the inherent phytochemicals and antioxidant activities, avocado seed meal can act as good inhibitors of enzymes. Inhibition of digestive enzymes such as α-amylase, α-lipase, and α-glucosidase has been reported as one therapeutic strategy for the treatment and management of chronic health conditions such as diabetes and obesity (Oluwagunwa et al., 2021; Ogundipe et al., 2022). The results of the α-amylase, α-lipase, and α-glucosidase inhibition activities recorded in this study suggest that avocado seed meal could be used to decrease the digestion of carbohydrates and postprandial

hyperglycemia, particularly in diabetic and obese patients (Zhang et al., 2015; Poovitha and Parani, 2016). The antidiabetic activity of avocado seed has been linked to its polyphenolic content, antioxidant activity, and polyunsaturated fatty acids content (Razola-Díaz et al., 2023). This result is similar to the antidiabetic activities of medicinal herbs and fruits recently reported by Oloruntola et al., 2024, and Rybak and Wojdyło, 2023, respectively. The percentage albumin denaturation and anti-proteinase activity of avocado seed meal revealed its anti-inflammatory properties, which also makes it eligible for use as natural food supplements to combat inflammation in body. This result is in line with other studies that have revealed the utilization of plants and their parts as anti-inflammatory agents, protecting the body against denaturation of tissue proteins and proteinases causing tissue damage development during inflammatory reactions (Iqbal et al., 2019; Truong et al., 2019; Mustafa et al., 2023).

5. Conclusion

This study have showed that avocado seed meal could be utilized as potential source of dietary protein, lipid and energy for livestock. Its phytochemicals, anti-diabetic, anti-inflammatory, and antioxidant properties also showed it could be utilized as supplements to improve the health of humans and livestock.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	O.I.A.	A.F.	O.D.O.	O.J.O.	M.A.
C	20	40	20	10	10
D	30	50	20		
S		50	50		
DCP	50	20	10	10	10
DAI		50	40	10	
L	10	40	20	10	20
W	20	20	20	20	20
CR	20	20	20	20	20
SR	20	20	20	20	20
PM	20	20	20	20	20
FA	20	20	20	20	20

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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