TOXICITY, ANTI-NUTRITIONAL FACTORS, AND PERFORMANCE CHARACTERISTICS OF WEANER'S RABBIT-FED SPROUTED MORINGA SEED MEAL

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ABSTRACT

A ten-week feeding trial was conducted to determine the performance of rabbits fed diets containing graded levels of sprouted Moringa seed meal (SMSM). Twenty-four male growing rabbits of mixed breeds were randomly allotted to four dietary treatments. Each treatment group was further subdivided into three (3) replicates of two (2) rabbits each in a completely randomized design (CRD). Four diets were formulated to include SMSM at varying inclusion levels of 0, 5, 10, and 15% for treatments T1, T2, T3, and T4 respectively, which were pelletized. Feed and water were provided ad libitum. Data were collected on the growth performance of rabbits fed the experimental diets. Results revealed that the average daily feed intake (71.57 ± 3.73 – 85.07 ± 3.83 g) decreased across the dietary treatments as the SMSM inclusion levels increased but a rise was noticed at the highest inclusion level of SMSM (T4). The weight gains and feed conversion ratio (FCR) were not significantly influenced (p>0.05) by the dietary treatments. It can be concluded that SMSM can be supplemented in growing rabbit's diet without any deleterious effects on the performance. The utilization of SMSM can be used up to 15% on diets and increased income from diets of weaner rabbits.

Keywords: Sprouted Moringa seed meal, Growth, Weight, Feed intake, Rabbits

INTRODUCTION

Rabbit is the most prolific animal among all domesticated animals. They have several beneficial characteristics for the sustenance of the farming system, such as the small size of the body and the short generational interval with a short relative period of gestation of around 30 – 31 days (Safwat *et al*., 2014). Rabbit meat is a very good source of animal protein in Nigeria, particularly in rural and urban areas (Kalio *et al*., 2008; Mikstas, 2022). They possess some unique qualities that make them suitable as meat-producing animals, as well as smallholder subsistence integrated farming and business when compared with other herbivorous animals. Some of these qualities include prolific breeders, rapid growth rate and high fecundity, short generational interval, genetic diversity, high nutritional meat quality (including exceptional protein value, low cholesterol content, and sodium levels), high efficiency in converting forage to meat, low-cost management requirements, ability to utilize wide arrays of feedstuffs and forages, ability to adapt to different ecological environment and consumption bereft of cultural and religious biases (Blasco *et al.,* 2017; Jiwuba and Ogbuewu, 2019).

However, the feeding of rabbits just like other monogastric animals accounts for about 70 to 80% of its production cost (Arijeniwa *et al.,* 2000). These high costs of feed coupled with the ignorance of possible alternative and cheap feed ingredients are among important factors militating against increased commercial rabbit production in Nigeria. According to Ahaotu *et al.* (2010), the high cost of feed ingredients in most tropical countries indicates that the production of feeds for livestock business is grossly inadequate. This major constraint has brought about the search for alternative feed resources that can serve as non-conventional replacements or supplements of conventional feedstuffs in rabbits and other livestock diets.

Although rabbits can survive on a foliage diet, there is a need to explore varying feedstuff and feeding regimes that will enhance optimum performance. One priority area of research now is the production of rich nutrient-based forage-based diets alongside concentrates (Akinmoladun *et al.*, 2018). The inclusion of tree foliage in animal nutrition tends to serve as a rich source of protein, minerals, vitamins (Jiwuba *et al.,* 2017), and important phytochemicals with enhanced anti-microbial, anti-oxidant, and palatability (Valenzuela-Grijalva *et al.*, 2017). Thus, the provision of sustainable, locally available, and perennial feeding systems for rabbits and other livestock is paramount for sustainable livestock production (Dida *et al.,* 2019). Most farmers in less developed countries use available forages to feed their rabbits without having the proper knowledge about the anti-nutritional factors that may be in them (Kimsé *et al.,* 2017).

Presently, researchers' interest has been focused on proteinous dietary ingredients mostly from legumes and forages, which when incorporated into livestock diets will reduce proteinous food acute shortage in tropical and developing countries, caused by population explosion and the high cost of animal protein (Abdelnour *et al.*, 2018). A lot of forages could be used in animal rations (Odetola *et al.,* 2012). Green plant leaf ingredients with a high percentage crude protein content are presently used in the ration of livestock to reduce the cost of production, enhance productive performance, enhance health status, and promote the growth of farm animals, thus increasing overall livestock production when used as a feed component or additive. One of such plants is *Moringa oleifera* Lam. (Brassicales: Moringaceae) (Abiodun and Olubisi*,* 2017). *Moringa* is thus one of the world's most useful plants for a variety of food and medicinal purposes in many countries in Africa, Southeast Asia, the Pacific and Caribbean Islands, and South America (Henuk, 2018). *M. oleifera* is considered to be one of the best antioxidant plants worldwide (Wadhwa *et al.*, 2013). It has been reported that all the parts of *M. oleifera* are rich in phytochemicals (Ayirezang *et al.*, 2020).

Moringa oleifera possesses a good quality of dietary protein for the rabbits' optimal growth. In addition, it can also be used to improve the daily weight gain. Moreover, it can replace the soya bean meal in the diet of rabbits with up to a 15% level of inclusion without any detrimental effect on the rabbit's performance (Alemede *et al.*, 2014). Given the aforementioned, the quest to search for an alternative feed source that can produce economically viable results in a relatively short period becomes expedient. This study aimed to determine the growth performance of weaner rabbits that were fed sprouted *Moringa* seed meal (SMSM).

MATERIALS AND METHODS

A total of 24 (6 weeks old) New Zealand white rabbits with an average weight of 750 ± 12 g were used for a ten-week feeding trial. The rabbits were randomly allocated to four dietary treatments, and replicated three times, having two rabbits in each replicate (pen) in a completely randomized design (CRD). Fresh feed and clean water were offered *ad libitum* twice daily, in the morning and evening throughout the experimental period. Feeders and drinkers were cleaned daily and disinfected every week. At the onset of the study, the rabbits were weighed before placement into experimental pens.

Preparation of *Moringa* Seed: *Moringa* seeds were separately sorted, cleaned, and washed with cold tap water. The seeds were soaked in cold tap water for 12 hours (the water for soaking the seeds was changed every two hours to prevent fermentation) at room temperature (32^{O}C). After soaking, the seeds were drained and spread on a clean jute bag and also covered with a damp cotton cloth and left for 72 hours to germinate. The germination period was chosen based on the results of earlier studies (Chinma *et al*., 2014). Water was sprinkled at 12-hour intervals to facilitate the germination process. At the end of germination, root hairs, and seed coats were manually removed from the germinated seeds. Sprouted *Moringa* seeds were dried at 60^{O}C in an air-dry oven followed by grinding into fine powder that was sieved using a 0.45 mm mesh size sieve. The SMSM was packed in a vacuum bag and stored in a plastic container with a lid for further use in rabbit feed formulation.

Acute Toxicity of Sprouted Moringa Seed Meal: The extracts of SMSM were evaluated for possible toxic effects in the rabbits. The rabbits were kept under strict observation for physical and behavioral changes for 24 hours with special attention during the first 4 hours. The rabbits were observed and monitored for a period of 14 days for any signs of toxicity or mortality (Asare *et al.,* 2012).

Phytochemical Assay in Sprouted *Moringa* Seed Meal

Phytate: The phytate content of the SMSM was determined using the colorimetric procedure described by Vaintraub and Lapteva (1988). Phytate was extracted using 3.5% HCl, the aliquot was diluted with distilled water to make 3ml and treated with 1ml Wade reagent (0.03% Fecl3.6H2O+0.3% sulfosalicylic acid) and determined spectrophotometrically at 500 nm suing phytic acid as a standard. The phytate was expressed as mg phytic acid equivalent (PAE)/100 g as described by León-López *et al.* (2013).

Saponin: Total saponin content was determined following the method described by Hiai *et al.* (1976). The extract was obtained with 80% aqueous methanol. The quantitative analysis was done using the method described by León-López *et al.* (2013) where a standard curve of diosgenin was adopted and expressed as (DE)/100 g.

Tannin: was determined using a method described by Xu and Chang (2007) where 80% methanol was extracted and assayed colorimetrically according to the modified Vanillin method of Price *et al.* (1978) and expressed as (CE)/100 g (dry weight).

Flavonoid: The aluminum tri-chloride assay was used to determine flavonoids in the methanolic extracts. To 1.0 ml of each sample, 1.0 ml of 2.0% (w/v) aluminum tri-chloride in ethanol was added. Absorbance was measured at 420 nm, after 1 hour, on a UV/Vis spectrophotometer (Jenway 6405). Absolute methanol was used as a blank. The extracts' flavonoid concentrations were determined from the standard curve of catechin (Roberts and Vaughan, 1971).

Alkaloids: Quantitative determination of alkaloids was done according to the method described by AOAC (2000). 200 ml of 20% acetic acid in ethanol was added to 5 g of the sample in a 250 ml beaker and allowed to stand for 4 hours at 25^{0}C. The extract was concentrated in a water bath to one-quarter of the original volume and some drops of concentrated ammonium hydroxide were until the precipitation was complete immediately after filtration. After some 3 hours of mixture of the sedimentation, the supernatant was discarded and the precipitates were washed with dilute NH_4OH solution and then filtered using Gem filter paper. An electronic weighing balance was used to dry the residue in an oven at 80^{0}C and the percentage of the alkaloid is estimated as: % Alkaloids = Weight of Alkaloids / Weight of Sample x 100.

Experimental Diets: Four experimental diets were formulated based on the nutrient requirements of growing rabbits. The dietary treatments consisted of basal diets supplemented with SMSM at levels of 0 (control), 5, 10, and 15%. The diets were: Treatment 1 (control) without SMSM, Treatment 2 (contained 5% of SMSM), Treatment 3 (contained 10% of SMSM), and Treatment 4 (contained 15% of SMSM). The ingredients for each diet were ground, mixed, and pelletized. The dietary ingredients and the resultant diet were analyzed (AOAC, 2000) for their proximate compositions (Table 1).

Table 1: Composition of diets containing graded levels of sprouted *Moringa* seed meal

Ingredients	Dietary Treatments			
	Diet 1 (0%)	Diet 2 (5%)	Diet 3 (10%)	Diet 4 (15%)
Maize	34.5	33	31	28
Soya beans meal	23	20	18	16.5
SMSM	0	5	10	15
Palm kernel cake	9	9	9	9
Rice husk	11	11	11	11
BDG	12	12	12	12
Groundnut cake	6.5	6	5	4.5
Bone meal	2.5	2.5	2.5	2.5
Methionine	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Vitamin-mineral premix*	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5
Total	100	100	100	100
Calculated Analysis				
ME (Kcal/kg)	2399.925	2431.4	2471.449	2491.944
Crude protein (%)	19.78	19.93	20.27	20.96
Crude fibre (%)	10.13	9.9935	9.867	9.783

**Each 1 kg premix contains: vitamin A 22,000 IU; vitamin D3 5000 IU; vitamin E 300 mg; vitamin K3 10.00 mg; B1 20.00 mg; B2 25.00 mg; vitamin C 300.00 mg; niacin 120.00 mg; calcium pantothenate 60.00 mg; B6 10.00 mg; B12 0.05 mg; folic acid 5.00 mg; biotin 1.00 mg; choline chloride 500.00 mg; inositol 50.00 mg; manganese 30.00 mg; iron 35.00 mg; zinc 45.00 mg; copper 3.00 mg; iodine 5.00 mg; cobalt 2.00 mg; selenium 0.15 mg; lysine 85.00 mg; methionine 100.00 mg; and antioxidant 80.00 mg*

Data Collection: The rabbits were weighed individually at the beginning of the experiment and subsequently every week using a sensitive hanging weighing scale before feeding in the morning. The initial live weight was subtracted from the final live weight to determine the weight gained by each animal. Feeds were offered and remnants were weighed daily to determine the actual feed intake per animal. Feed conversion ratio (FCR) was calculated from feed intake and weight gain values; FCR = Feed Intake (g)/Weight gain (g). Weight gain (g) = Final weight – Initial weight (g), and Feed Intake = Feed offered – Feed refused (g) (Saka *et al*., 2019).

Statistical Analyses: Data were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS, 2007). Data collected were subjected to a one-way analysis of variance (ANOVA) as prescribed for a completely randomized design (CRD). Mean separation was done using Duncan's New multiple range tests, and differences were declared significant at $p<0.05$.

RESULTS AND DISCUSSION

In the oral toxicity assay, all treated groups of the rabbits at different doses of SMSM (5, 10, and 15%) did not show any alteration in behavioural signals, with no mortality during the period of the study. Several literature reviews on the toxicological effects of different *Moringa* parts like leaves, stems and seeds in animals have proven the safety of *Moringa* plants (Ijarotimi *et al*., 2013; Oloyede *et al.,* 2015; El-Hak *et al*., 2017). Silva *et al*. (2017) investigated the eco-toxicity of lectins isolated from *M. oleifera* seeds on zebrafish embryos and found that the lectins showed LC_{50} values ranging from 0.05 to 0.19 mg/mL after treatment of embryos for 48 – 96 hours. Teshome *et al.* (2021) agreed with this study, they reported that the acute toxicity study of *Moringa stenopetala* seed extract did not show mortality in the animals at a dose of 5000 mg/kg during the observation period. Thus, the median lethal dose (LD_{50}) of the seed extract was greater than 5000 mg/kg and there were not any signs of toxicity such as behavioural, neurological, autonomic, or physical changes.

The anti-nutritional components in SMSM were saponins, tannins, flavonoids, alkaloids, and phytate (Table 2). According to León-López *et al.* (2020), the anti-nutrient profile detected in *Moringa* seeds were phytate, total phenolic, and saponins. SMSM contained 80.21 mg/g of saponin.

Table 2: Anti-nutrient in the sprouted *Moringa oleifera* seeds

Anti-nutrients	Composition
Saponins (mg/g)	80.21
Tannin (mg/g)	1.24
Flavonoids (mg/g)	0.72
Alkaloids (%)	3.70
Phytate mg/100g^{-}	40.00

The saponin content of SMSM in this study differed from its content in a previous study by Ijarotimi *et al.* (2013) which reported a substantially lower saponin amount (9.83 mg/100 g). Furthermore, the saponin content recorded for SMSM (80.21 mg/g) was lower when compared with values reported for quinoa seeds (1260 mg/100 g) (Liu *et al.*, 2020) and other edible legumes such as soybean (800 mg/100g) (Mohan *et al.*, 2016). SMSM contained 1.24 ± 0.24 mg/g of tannin. Tannins bind dietary proteins making them nutritionally unavailable, decreasing animal consumption of feed, and may result in digestive enzymes forming indigestible complexes. Tannins may also slow the rate of growth and decrease palatability (Emire *et al.*, 2013). The tannins content of SMSM (1.24 ± 0.24 mg/g) in this study was lower than 2.22 ± 0.32 mg/100g in *Adansopnia digitata* (Baobab) and 7.40 ± 0.14 mg/100g in *Balanite aegyptiaca* (Desert date) (Umaru *et al.*, 2007). These indicated that low levels of tannins in the sample will not exert a negative effect on the bioavailability of Cu and Zn. Anti-nutrients like tannins, oxalate, and phytates may be harmful when consumed in unrefined food.

Flavonoid content of SMSM in this study was 0.72 ± 0.15 mg/g. Purified flavonoids given in high doses as dietary supplements may affect trace elements, folate, and vitamin C status (Egert and Rimbach, 2011). Additionally, SMSM has 3.70% alkaloids. Neurological and gastrointestinal disorders have been attributed to alkaloids (Hussain *et al.*, 2018). Glycoalkaloids (α-solanine and α-chaconine) found in potatoes (*Solanum* spp.) have been reported to be haemolytically active and harmful to human beings (Aziz *et al.,* 2012). Gastrointestinal and neurological disorders are the toxicological consequences of potato glycoalkaloids especially when the intake level is above 20 mg/100g (Emire *et al.*, 2013).

Phytate are potent inhibitor of iron and zinc absorption. In legumes and most oil seeds, phytate is uniformly distributed within the protein bodies of the endosperm (Gibson *et al.*, 2018). The report on phytate content in this study was 40.00 mg/100g^{-1} which was similar to those of *Moringa* sprouts reported for rice sprouts (0.096 g/100g^{-1}), corn (0.072 g/100g^{-1}) and wheat (0.079 g/100g^{-1}) (Azeke *et al.*, 2011). León-López *et al.* (2020) also reported that whole *Moringa* seed contained 1380 mg PAE/100 g which fell within the range reported for other oil seeds such as soybeans, sesame seeds, sunflower kernels, linseeds, and rape seeds (1000 – 5400 mg/100 g) (Gupta *et al.,* 2015). It was observed that the concentration of phytates in *Moringa* sprouts was not so different from those found in conventional cereal sprouts. Though most reports proved that phytate is an antinutritional agent. Abdulwaliyu *et al.* (2019) reported that dietary phytate has been associated with specific therapeutic effects like anti-diabetic, anti-cancer, and anti-inflammatory properties.

Performance characteristics of rabbits fed diets containing graded levels of SMSM indicated that there were no significant differences (p>0.05) among the parameters observed (Table 3). The average daily feed intake (ADFI) values obtained decreased across the treatment with an increase in the levels of SMSM except T4. This significant decline in feed intake may be attributed to the presence of some anti-nutritional factors, which are prevalent in *Moringa* (Awodele *et al.*, 2012). Previous studies have indicated the presence of some anti-nutritional factors like tannins and phytate in diets resulted in poor palatability, and a decrease in feed intake due to its astringent property as a result of its ability to bind with protein of saliva and mucous. León-López *et al.* (2020) reported phytate was the most abundant antinutritional factor in *Moringa* seeds. The phytate contents were 1380.6 mg PAE/100g, saponins were 154.95 mg DE/100g, and tannins amounted to 62.6 mg CE/100g. Moreso, phytate reduces the bioavailability of critical nutrients in non-ruminant animals (Weaver and Kannan, 2001).

Table 3: Weaner's rabbits fed varying levels of sprouted *Moringa* seed meal diet

Parameters	Dietary Treatment			
	SMSM (T1 - 0%)	SMSM (T2 - 5%)	SMSM (T3 - 10%)	SMSM (T4 - 15%)
Initial live weight (g/rabbit)	649.13 ± 11.93[b]	670.93 ± 11.98[d]	668.58 ± 11.94[c]	645.87 ± 11.91[a]
Final live weight (g/rabbit)	1585.88 ± 72.27[b]	1690.92 ± 72.32[d]	1500.57 ± 72.24[a]	1654.27 ± 72.30[c]
Daily weight gain (g/rabbit)	16.73 ± 1.22[a]	17.99 ± 1.27[b]	17.99 ± 1.28[b]	18.01 ± 1.30[c]
Daily feed intake (g/rabbit/day)	85.07 ± 3.83[d]	75.89 ± 3.78[b]	71.57 ± 3.73[a]	83.46 ± 3.80[c]
Feed conversion ratio	5.13 ± 0.20[c]	4.24 ± 0.15[a]	4.87 ± 0.18[b]	4.86 ± 0.16[b]

SMSM: sprouted Moringa seed meal, [a,b,c,d] Means with different letter superscripts on a row are significantly different (p<0.05)

This result did not agree with the findings of (Nuhu, 2010) who reported an increase in feed intake of rabbits fed graded levels of *M. oleifera* leaf meal simply as a result of their acceptability of diets supplemented with *M. oleifera* leaf meal. The variation observed may be attributed to different parts used and SMSM had been confirmed to show greater concentration of anti-nutrients than *Moringa* leaf meal (Stevens *et al.,* 2015).

The comparatively higher amounts of anti-nutrients in the seeds than in the leaves may explain why *Moringa* seeds are used more in ethno-medicine than the leaves (Saka *et al.*, 2019). The highest ADFI values were observed in rabbits on T1 (85.07 ± 3.83 g/dL) followed by those on T4 (83.46 ± 3.80 g/dL) and higher than rabbits on T3 (71.57 ± 3.73 g/dL).

Total weight gain was not significantly affected (p>0.05) by the dietary treatments but values obtained in animals on T4 were however numerically higher than those on T1, T3, and T4 indicating that the inclusion levels of 5% supported weight gain. This could be attributed to an increase in the anti-nutrient content of the diet as the SMSM inclusion level increased thereby resulting in reduced nutrient utilization (Shimelis and Rakshit, 2005). The result from this study was in agreement with the findings of Odeyinka *et al.* (2008) who reported that the replacement for *Centrocema pubescens* with *M. oleifera* meal had no significant effect (p>0.05) on the average body weight of rabbits. FCR values observed were not significantly influenced (p>0.05) by the dietary treatment. FCR is an important index of performance, which is a direct indication of how best feed offered to animals was utilized for meat production. The values of FCR ranged from 4.24 – 5.13 with rabbits on T2 having the lowest FCR numerical values. The FCR values obtained were comparable with the range of values (4.22 – 5.13) reported by (Nuhu, 2010).

Conclusion: The findings of this study suggested that the inclusion of up to 15% SMSM in the experimental diets favoured the growth performance of the rabbits. SMSM supplementation in the diets of rabbits enhanced the growth and development of the rabbits as well as reduce the cost of production in terms of rabbit farming enterprise. SMSM also has a broad safety potential for therapeutic use, since no mortality or any sign of toxicity is observed. There is a need to encourage massive afforestation of the *Moringa* plant to enable farmers to produce rabbit diets at a lower cost for economic use in animal feed formulation.

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REFERENCES

ABDELNOUR, S. A., ABD EL-HACK, M. E. and RAGNI, M. (2018). The efficacy of high-protein tropical forages as alternative protein sources for chickens: a review. *Agriculture*, 8(6): 86. https://doi.org/10.3390/agriculture8060086

ABDULWALIYU, I., AREKEMASE, S. O., ADUDU, J. A., BATARI, M. L., EGBULE, M. N. and OKODUWA, S. I. R. (2019). Investigation of the medicinal significance of phytic acid as an indispensable anti-nutrient in diseases. *Clinical Nutrition Experimental*, 28: 42 – 61.

ABIODUN, A. A. and OLUBISI, E. E. (2017). Growth performance and organ indices of rabbit bucks fed *Moringa oleifera* leaf meal. *World Applied Science Journal,* 35(8): 1229 – 1234.

AHAOTU, E. O., EKENYEM, B. U., AGIANG, E. A., BALAKRISHNAN, V. and MADUBUIKE, F. N. (2010). Effects of dietary substitution of rubber seed cake for groundnut cake on the body conformations of finisher broilers. *Animal Production Research Advances*, 6(1): 49 – 52.

AKINMOLADUN, O. F., ADEJORO, V. and JIMOH, A. (2018). Performance and carcass characteristics of rabbits fed diets with total or partial replacement of *Tridax procumbens* by bamboo (*Bambusa arundinancea*) leaves. *Journal of Experimental Agriculture International*, 27(1): 1 – 10.

ALEMEDE, I. C., ONYEJI, E. A., TSADO, D. N. and SHIAWOYA, E. L. (2014). Reproductive response of rabbits is to diets containing varying levels of horseradish (*Moringa oleifera*) leaf meal. *Journal of Biology, Agriculture and Healthcare*, 4(19): 62 – 68.

AOAC (2000). *Official Methods of Analysis.* 17th Edition, The Association of Official Analytical Chemists, Gaithersburg, MD, USA.

ARIJENIWA, S., OTAIKHIAN, O. and IMASEUM, J. A. (2000)**.** Performance of weaner rabbits fed: Poultry grower mash supplemented with different grass legume rations. Pages 103 – 105. *In: Proceedings of 5th Annual Conference of Animal Science Association of Nigeria (ASAN).* September 19 – 22, 2000.

ASARE, G. A., GYAN, B., BUGYEI, K., ADJEI, S., MAHAM, R., OTU-NAYARKO, L., ADDO, P., WIREDU, E. K. and NYARKO, A. (2012). Toxicity potentials of the nutraceutical *Moringa oleifera* at supra supplementation levels. *Journal of Ethnopharmacology*, 139: 265 – 272.

AWODELE, O., OREAGBA, I. A., ODOMA, S., DA SILVA, J. A. and OSUNKALU, V. O. (2012). Toxicological evaluation of the aqueous leaf extract of *Moringa oleifera* Lam. (Moringaceae). *Journal of Ethnopharmacology*, 139(2): 330 – 336.

AYIREZANG, F. A., AZUMAH, B. K. and ACHIO, S. (2020). Effects of *Moringa oleifera* leaves and seeds extracts against food spoilage fungi. *Advances in Microbiology,* 10: 27 – 38.

AZEKE, M. A., EGIELEWA, S. J., EIGBOGBO, M. U. and IHIMIRE, I. G. (2011). Effect of germination on the phytase activity, phytate and total phosphorus contents of rice (*Oryza sativa*), maize (*Zea mays*), millet (*Panicum miliaceum*), sorghum (*Sorghum bicolor*) and wheat (*Triticum aestivum*). *Journal of Food Science and Technology,* 48(6): 724 – 729.

AZIZ, A., RANDHAWA, M. A., BUTT, M. S., ASGHAR, A., YASIN, M. and SHIBAMOTO, T. (2012). Glycoalkaloids (α-chaconine and α-solanine) contents of selected Pakistani potato cultivars and their dietary intake assessment. *Journal of Food Science*, 77(3): T58 – T61.

BLASCO, A., MARTÍNEZ-ÁLVARO, M., GARCÍA, M. L., IBÁÑEZ-ESCRICHE, N. and ARGENTE, M. J. (2017). Selection for environmental variance of litter size in rabbits. *Genetics Selection Evolution*, 49: 48. https://doi.org/10.1186/s12711-017-0323-4

CHINMA, C. E., GBADAMOSI, K. B., OGUNSINA, B. S., OLOYEDE, O. O. and SALAMI, S. O. (2014). Effect of addition of germinated *Moringa* seed flour on the quality attributes of wheat-based cake. *Journal of Food Processing and Preservation*, 38(4): 1737 – 1742.

DIDA, M. F., CHALLI, D. G. and GANGASAHAY, K. Y. (2019). Effect of feeding different proportions of pigeon pea (*Cajanus cajan*) and neem (*Azadirachta indica*) leaves on feed intake, digestibility, body weight gain and carcass characteristics of goats. *Veterinary and Animal Science*, 8: 100079. https://doi.org/10.1016/j.vas.2019.100079

EGERT, S. and RIMBACH, G. (2011). Which sources of flavonoids: complex diets or dietary supplements? *Advances in Nutrition*, 2(1): 8 – 14.

EL-HAK, H. N. G., MOUSTAFA, A. R. A. and MANSOUR, S. R. (2017). Toxic effect of *Moringa peregrina* seeds on histological and biochemical analyses of adult male albino rats. *Toxicology Report,* 12: 38 – 45.

EMIRE, S. A., JHA, Y. K. and MEKAM, F. (2013). Role of anti-nutritional factors in food industry. *Beverage and Food World*, 2: 23 – 28.

GIBSON, R. S., RABOY, V. and KING, J. C. (2018). Implications of phytate in plant-based foods for iron and zinc bioavailability, setting dietary requirements and formulating programs and policies. *Nutrition Reviews*, 76(11): 793 – 804.

GUPTA, R. K., GANGOLIYA, S. S. and SINGH, N. K. (2015). Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. *Journal of Food Science and Technology*, 52(2): 676 – 684.

HENUK, Y. L. (2018). Many uses of Moringa (*Moringa oleifera*) in human and animal nutrition. *Research and Reviews: Journal of Agriculture and Allied Sciences*, 7(1): 41 – 45.

HIAI, S., OURA, H. and NAKAJIMA, T. (1976). Color reaction of some sapogenins and saponins with vanillin and sulfur1c acid. *Planta Medica*, 29(02); 116 – 122.

HUSSAIN, G., RASUL, A., ANWAR, H., AZIZ, N., RAZZAQ, A., WEI, W., ALI, M., LI, J. and LI, X. (2018). Role of plant derived alkaloids and their mechanism in neurodegenerative disorders. *International Journal of Biological Sciences*, 14(3): 341 – 357.

IJAROTIMI, O. S., ADEOTI, O. A. and ARIYO, O. (2013). Comparative study on nutrient composition, phytochemical, and functional characteristics of raw, germinated, and fermented *Moringa oleifera* seed flour. *Food Science and Nutrition*, 1(6): 452 – 463.

JIWUBA, P. C., AHAMEFULE, F. O., OGBUEWU, I. P. and IKWUNZE, K. (2017). Blood chemistry and haematology of West African Dwarf goats fed *Moringa oleifera* leaf meal (MOLM) in their diet. *Comparative Clinical Pathology*, 26: 621 – 624.

JIWUBA, P. D. C. and OGBUEWU, I. P. (2019). Potential of *Moringa oleifera* leaf meal to replace soybean meal in rabbit diets and its influence on production parameters. *Asian Journal of Biological Sciences*, 12(4): 656 – 663.

KALIO, G., ETELA, I. and CINIKIA, V. (2008). Rabbit meat as a preferred animal protein source in Ekpeye Kingdom of Rivers State, Nigeria. *Livestock Research for Rural Development*, 20: 9. https://www.lrrd.cipav.org.co/lrrd20/1/kalicit.htm

KIMSÉ, M., YAPI, M. Y., KARAMOKO, M., GIDENNE, T., ZONGO, M., GNANDA, B. I., AKOUTEY, A., BODJI, N. C., FANTODJI, A. and OTCHOUMOU, A. (2017). Effect of tropical green forage *Pueraria phaseoloides* addition to a pelleted complete feed on rabbit growth performance and digestion. *World Rabbit Science*, 25(3): 225 – 231.

LEÓN-LÓPEZ, L., DÁVILA-ORTIZ, G., JIMÉNEZ-MARTÍNEZ, C. and HERNÁNDEZ-SÁNCHEZ, H. (2013). Sequentially integrated optimization of the conditions to obtain a high-protein and low-antinutritional factors protein isolate from edible *Jatropha curcas* seed cake. *International Scholarly Research Notices*, 2013: 197201. https://doi.org/10.5402/2013/197201

LEÓN-LÓPEZ, L., ESCOBAR-ZÚÑIGA, Y., MILÁN-CARRILLO, J., DOMÍNGUEZ-ARISPURO, D. M., GUTIÉRREZ-DORADO, R. and CUEVAS-RODRÍGUEZ, E. O. (2020). Chemical proximate composition, antinutritional factors content, and antioxidant capacity of anatomical seed fractions of *Moringa oleifera*. *Acta Universitaria*, 30: e-2892. http://doi.org/10.15174/au.2020.2892

LIU, Y., JIN, X., HONG, H. G., XIANG, L., JIANG, Q., MA, Y., WANG, K., CHEN, Z., CHENG, L., JIAN, Z., WEI, Z. and AI, J. (2020). The relationship between gut microbiota and short chain fatty acids in the renal calcium oxalate stones disease. *The FASEB Journal*, 34(8): 11200 – 11214.

MIKSTAS, C. (2022). *What Are the Health Benefits of Rabbit Meat?* Health and Diet Guide, WebMD. https://www.webmd.com/diet/health-benefits-rabbit-meat

MOHAN, V. R., TRESINA, P. S. and DAFFODIl, E. D. (2016). Antinutritional factors in legume seeds: characteristics and determination. Pages 211 – 220. *In:* CABALLERO, B., FINGLAS, P. M. and TOLDRÁ, F. (Eds.). *Encyclopedia of Food and Health.* Elsevier, Amsterdam, The Netherlands.

NUHU, F. (2010). *Effect of Moringa Leaf Meal (MOLM) on Nutrient Digestibility, Growth, Carcass and Blood Indices of Weaner Rabbits.* M.Sc. Thesis, Department of Animal Science, Faculty of Agriculture and Natural Resources, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

ODETOLA, O. M., ADETOLA, O. O., IJADUNOLA, T. I., ADEDEJI, O. Y. and ADU, O. A. (2012). Utilization of moringa (*Moringa oleifera*) leaves meal as a replacement for soya bean meal in rabbit's diets. *Scholarly Journal of Agricultural Science*, 2(12): 309 – 313.

ODEYINKA, S. M., OYEDELE, O. J., ADELEKE, T. O. and ODEDIRE, J. A. (2008). Reproductive performance of rabbits fed *Moringa oleifera* as a replacement for *Centrosema pubescens*. Pages 411 – 416. *In: Proceedings of the 9th World Rabbit Congress,* World Rabbit Science Association. Verona, Italy, June 10 – 13, 2008.

OLOYEDE, D. O., AVIARA, N. A. and SHITTU, S. K. (2015). Measurement of engineering properties necessary to the design of drumstick (*Moringa oleifera* L.) Pod Sheller. *Journal of Biosystems Engineering*, 40(3): 201 – 211.

PRICE, M. L., VAN SCOYOC, S. and BUTLER, L. G. (1978). A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *Journal of Agricultural and Food Chemistry*, 26(5): 1214 – 1218.

ROBERTS, R. J. and VAUGHAN, P. F. T. (1971). Hydroxylation of kaempferol, dihydrokaempferol and naringenin by a phenolase preparation from spinach beet. *Phytochemistry*, 10(11): 2649 – 2652.

SAFWAT, A. M., SARMIENTO-FRANCO, L. and SANTOS-RICALDE, R. H. (2014). Rabbit production using local resources as feedstuffs in the tropics. *Tropical and subtropical agroecosystems*, 17(2): 161 – 171.

SAKA, R. O., ANURUDU, N. F., ADEBIYI, A. O., ADETORO, B. O., ADETOLA, O. O., BOLARINWA, M. O., ADEWUMI, M. O., BALOGUN, I. O. and SAKA, A. A. (2019). Performance characteristics, nutrient digestibility and blood profile of rabbits fed diets containing graded levels of *Moringa oleifera* seed powder. *Nigerian Journal of Animal Science*, 21(2): 202 – 213.

SHIMELIS, E. A. and RAKSHIT, S. K. (2005). Proximate composition and physico-chemical properties of improved dry bean (*Phaseolus vulgaris* L.) varieties grown in Ethiopia. *LWT-Food Science and Technology*, 38(4): 331 – 338.

SILVA, L. L. S., ALVES, R. N., DE PAULO, D. V., DA SILVA, J. D. F., DE OLIVEIRA, A. P. S., COELHO, L. C. B. B., NAVARRO, D. M. A. F., NAPOLEÃO, T. H., DO AMARAL, I. P. G., DE CARVALHO, P. S. M. and PAIVA, P. M. G. (2017). Ecotoxicity of water-soluble lectin from *Moringa oleifera* seeds to zebrafish (*Danio rerio*) embryos and larvae. *Chemosphere*, 185: 178-182

SPSS (2007). *SPSS Statistics 17.0 Brief Guide.* SPSS Incorporated, South Wacker Drive, Chicago, Illinois, USA.

STEVENS, C. G., UGESE, F. D., OTITOJU, G. T. and BAIYERI, K. P. (2015). Proximate and anti-nutritional composition of leaves and seeds of *Moringa oleifera* in Nigeria: a comparative study. *Agro-Science*, 14(2): 9 – 17.

TESHOME, D., TIRUNEH, C. and BERIHUN, G. (2021). Toxicity of methanolic extracts of seeds of *Moringa stenopetala*, Moringaceae in rat embryos and fetuses. *BioMed Research International*, 2021: 5291083. https://doi.org/10.1155/2021/5291083

UMARU, H. A., ADAMU, R., DAHIRU, D. and NADRO, M. S. (2007). Levels of antinutritional factors in some wild edible fruits of Northern Nigeria. *African Journal of Biotechnology*, 6(16): 1935 – 1938.

VAINTRAUB, I. A. and LAPTEVA, N. A. (1988). Colorimetric determination of phytate in unpurified extracts of seeds and the products of their processing. *Analytical Biochemistry*, 175(1): 227 – 230.

VALENZUELA-GRIJALVA, N. V., PINELLI-SAAVEDRA, A., MUHLIA-ALMAZAN, A., DOMÍNGUEZ-DÍAZ, D. and GONZÁLEZ-RÍOS, H. (2017). Dietary inclusion effects of phytochemicals as growth promoters in animal production. *Journal of Animal Science and Technology*, 59: 8. https://doi.org/10.1186/s40781-017-0133-9

WADHWA, S., PANWAR, M. S., SAINI, N., RAWAT, S. and SINGHAL, S. (2013). A review on commercial, traditional uses, phytoconstituents and pharmacological activity of *Moringa oleifera*. *Global Journal of Traditional Medicinal Systems*, 2(1): 1 – 13.

WEAVER, C. M. and KANNAN, S. (2001). Phytate and mineral bioavailability. Pages 227 – 240. *In:* REDDY, N. R. and SATHE, S. K. (Eds.). *Food Phytates.* CRC Press, Boca Raton, Florida, United States.

XU, B. J. and CHANG, S. K. C. (2007). A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. *Journal of Food Science*, 72(2): S159 – S166.