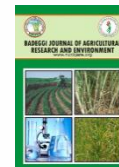




NCRI Press

Available online: www.ncribjare.org
 ISSN: 2695-2122, e-ISSN: 2695-2114
 DOI: <https://doi.org/10.35849/BJARE202303/138/011>
 Journal homepage: www.ncribjare.org



Research Article

Gamma Irradiation Doses reduce Cooking time and Improve Nutritional Profile of selected Pigeon pea [*Cajanus cajan* (L.) Millsp] Genotypes in Nigeria

Daudu, O. A. Y., Falusi, O. A., Abubakar, A., Bello, I. M., Abdulsalami, H. and Otaru, S. O.

Federal University of Technology, School of Life Sciences, Department of Plant Biology, PMB 64, Minna, Nigeria;

Corresponding e-mail: dauduoladipupoyusuf@yahoo.com

Abstract

Pigeon Pea (*Cajanus cajan* L.) is a self-pollinating perennial legume shrub with over 30,000 accessions deposited world-wide. Despite the nutritional importance of pigeon peas, its detrimental traits such as its long cooking time, has led to low cultivation of the crop and inhibit its consumers' request. Concerted efforts to improve both the quality and quantity of this crop through mutation breeding informed this research. It was based on this premise that the study was aimed to evaluate gamma irradiation influences on the nutrient quality and cooking duration of selected pigeon pea varieties in Nigeria. Four accessions of pigeon peas ('NG/SA/11/08/108', 'NG/AO/MAY/00/021/01', 'TCC-2' and 'NG/SA/07/191') were obtained from National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan. They were irradiated at the Oncological Department ABU Teaching Hospital, Shika, Zaria, using Cobalt-60 (⁶⁰CO) irradiation source. Standard procedures were followed to determine the effects of the irradiation on cookability and nutritional profile of the M1 plants and their respective controls. It was established that all the parameters tested were irradiation dose dependent. In NG/AO/MAY/00/021/01, 100 Gy produced the highest protein (20.40 %) and highest carbohydrate (68.93 %) contents. Anti-nutritional factors such as cyanide were lower (48.83, 46.22, 43.65 and 44.75 mg/kg in NG/AO/MAY/00/021/01, NG/SA/11/08/108, NG/SA/07/191 and TCC-2) at 400 Gy than those of their respective controls (54.83, 56.83, 53. and 53.55 mg/kg). The amount of Magnesium increased with higher doses of gamma irradiation (1.37 g/100 g for 200 Gy NG/AO/MAY/00/021/01; 1.03 g/100g for 400 Gy NG/SA/11/08/108 and 1.13 g/100 g for TCC-2). There were significant reductions in cooking time in 400 Gy treatments for all the genotypes. The differences between the control and the least cooking time in 400 Gy were 11, 16, 22 and 15 minutes for NG/AO/MAY/00/021/01, NG/SA/11/08/108, NG/SA/07/191 and TCC-2, respectively. It was therefore concluded that these variations could be exploited for further improvement of the crop for food security.

Keywords: *Cooking time, Pigeon pea, Legume, Mutation breeding, Gamma irradiation, Cookability, Dose dependent*

© 2021 National Cereals Research Institute (NCRI), Nigeria, all rights reserved.

Introduction

Pigeon pea [*Cajanus cajan* (L.) Millsp] is a group of pulses that belongs to the genus *Cajanus* and family Fabaceae (Makelo, 2011). In agricultural systems, the pigeon pea is amongst the most significant legume crops; the crop was first domesticated in India (Makelo, 2011). Pigeon pea is the world's sixth most prominent legume crop, per the FAOSTAT (2015); the West African sub-region of the word generates only a small percent

of global pigeon pea volume. Nepal, India, Myanmar, Nepal, Kenya, Malawi, Uganda and Tanzania were amongst the countries that grow the plant (Singh *et al.*, 2005; Lin-Qi *et al.*, 2014). In the Philippines, it is known by many names, including Kadio, Tropical green pea and Red gram (Lin-Qi *et al.*, 2014). It is known as "Waken Kurawa" in Hausa, "Fio-Fio" in Igbo and "Otili" in Yoruba languages (Esan and Ojemola, 2018).

Whole seeds of pigeon pea are cooked and then barbecued with spices and eaten with cereals in most African countries (Nwosu *et al.*, 2013). The cation distribution that marks the transition from raw to cooked pulses is known as starch unification, and it can be used to define the term "cooked" (Avola and Patane, 2010). De-podding, sanitizing, cooking, drying, and milling are all steps in the pigeon pea machining operation (Nwosu *et al.*, 2013). The seeds of the pigeon pea are protected by a semi-permeable outer layer that is tough, hard and fairly thick. Since this adhesive force that unites the mesocarp to the seed is rather solid, current can flow through the mesocarp (Wood *et al.*, 2014). As a result, very long cooking is necessary to loosen the tough seed skin and make de-hulling simpler. Such lengthy cooking time consumes a lot of energy and wastes a lot of time; thus leading to the abandonment of the crop for more easily cooked and less heat energy demanding crops. Furthermore, the high anti-nutritional content of the crop, such as oxalate, phytates and tannins, has been documented to limit protein digestion, reduce the bioavailability of some critical minerals and cause intestinal discomfort (flatulence) when taken (Adebowale and Maliki, 2011; Thompson, 2019). As a result, it has a low level of demand and utilisation.

Energy efficiency, environmental protection, and food processing waste management have attracted increasing attention in the food industry. Effective energy utilization and energy source management in food processing facilities are desirable for reducing processing costs, conserving non-renewable energy resources, and reducing environmental impact (Akinoso and Oladeji, 2017). Reduction in cooking times is therefore an important trait that could be sought in this crop because of its valid economic importance. Therefore, mutation breeding has been selected to achieve this important feat due to its ability in 'creating' new species in crop plants population.

Mutagenesis is a phenomenon in which biological, chemical or physical stimuli elicit abrupt heritable changes in plant contents that are not mediated by genetic dispersion or integration (Roychowdhury and Tah, 2013). The act of initiating a mutation in a DNA sequence at

a definite locus is termed mutagenesis and could result in point as a result of DNA insertion, either via T-DNA insertion or transposon activation (Surekha *et al.*, 2005; Kharkwal and Shu, 2009). Mutation breeding is very useful in pulses as variability in germplasm is low (Pazhamala *et al.*, 2015).

Improving pigeon pea cooking time (i.e. reduction in cooking time) has an immense benefit to the consumer in term of environmental impact of burning fuel, fuel and saving labour of burning fuel. Reduction in the cooking time is likely to increase demand, size of the market, range of value-added products and incomes of pigeon pea farmers (Ayenan *et al.*, 2017). In addition, it has been consistently established that the short cooking time is the end-user's top priority (Ayenan *et al.*, 2017; Śmiglak-Krajewska *et al.*, 2020).

Ionizing radiation has been observed to be useful in improving the overall traits in pigeon pea, including some preferred changes in useful properties of the seed (Bamidele and Akanbi, 2013). Furthermore, irradiation was shown to be efficient in lowering or abolishing anti-nutritional agents in seed crops such as soybean, cowpea, and other kindred crops (Hamdani *et al.*, 2018). These prospects through ionizing radiation informed this present research.

Materials and Methods

Source of Research Materials: Pigeon pea (*Cajanus cajan* (L.) Millsp) genotypes were obtained from breeder lines at the Federal University of Technology, Minna's Department of Plant Biology. The seed treatment was carried out at the Radiology and Oncology Department, Ahmadu Bello University Teaching Hospital Shika, Zaria, using a gamma irradiation source (Cirrus Cobalt- 60 Teletherapy). The Atomtex is self-calibrated and manufactured with range 50nSv – 10Sv/h. The leakage free Cobalt-60 machine was a 229.061 TBq (6190.84Ci) model.

Treatment of Pigeon pea Seeds with Gamma Radiation: The dried seeds of the pigeon pea [*Cajanus cajan* (L.) Millsp] were subjected to various doses of gamma rays [0 (control), 100, 200, 300, and 400 Gy] obtained from a Cobalt-60

(⁶⁰CO) source with a measured dose rate of 124.5 Gy/min for 8 hours 52 minutes.

Proximate, Anti-nutritional Factors and Minerals Compositions; Proximate composition evaluation: Proximate analyses (percent crude protein, fat, crude fibre, ash, moisture content, and carbohydrate) for each treatment were calculated using the AOAC (2010), technique.

Protein content estimation: The protein level was calculated utilizing micro-Kjedhal method, which requires wet digestion, distillation, and titration [Association of Official Analytical Chemists (AOAC), 2010]. To quantify the protein content, 3 g of sample was poured in a boiling tube with 25 ml concentrated sulfuric acid and one catalyst tablet containing 0.15 g CuSO₄, 5 g K₂SO₄ and 0.1 g TiO₂. The tubes were heated to a low temperature to allow digestion to begin. The digest was diluted with 100 mL distilled water, 5 mL Na₂S₂O₃ and 10 mL 40 percent NaOH, as well as an anti-bumping agent, before being diluted with 10 mL boric acid. The NH₄ concentration of the distillate was assessed by titrating 0.1 N standards HCl in a 25 ml burette. Without the sample, a blank was formed. The resulting protein value was then multiplied by a conversion factor, delivering the crude protein amount.

$$\text{Percentage protein} = \frac{\text{actual titre value} - \text{titre value of blank} \times 0.1 \times 0.014 \times \text{conversion factor} \times 100}{\text{Weight of food sample}} \times 100$$

Fat content estimation: Fat concentration was estimated using the AOAC (2010), method. To weigh exactly 10 g of material wrapped in filter paper, a chemical balance was used. After being packed in an extraction thimble, dried in an oven, and chilled in desiccators, it was weighed. In the jar, 25 mL of petroleum ether solvent was used to extract the fatty acid content. After extraction, the solvent was evaporated by drying in the oven. After cooling in desiccators, the flask and its contents were weighed. The following formula was used to get the percentage fat content:

$$\text{Percentage (\%)} \text{ of total fat content} = \frac{\text{Weight of fat extracted}}{\text{Weight of food sample}} \times 100$$

Estimation of crude fibre content: The AOAC (2010), method was used to calculate crude fibre. In a 500 mL Erlenmeyer flask, 5 g of each sample

was mixed with 100 mL of TCA digestion reagent. Then, starting from the beginning of the boiling procedure, it was brought to a boil and refrigerated for 40 minutes. The supernatant was removed from the heater and left to settle somewhat before being filtered through a 15.0 cm Whatman paper. After being stirred once with a spatula, the residue was washed in hot water and transferred to a porcelain dish. The sample was dried at 105⁰C overnight. It was put to desiccators after drying and weighed as G₁. It was reweighed as G₂ after being burned for 6 hours at 500°C in a muffle furnace.

$$\text{Percentage crude fibre} = \frac{G_1 - G_2}{G_0} \times 100$$

G₀=Dry weight of food sample

G₁=Weight of crucible+fibre+ash

G₂=Weight of crucible+ash

Estimation of the ash content: The AOAC (2010), technique was used to determine the ash content. Sum of 5 g of each sample was weighed into five crucibles and then burned at 550 °C in an electric furnace until a light grey ash was evident and a constant weight was established. To avoid moisture absorption, the sample was chilled in desiccators and weighed to determine the ash content.

$$\text{Percentage ash content} = \frac{\text{change in weight (\%)}}{\text{Initial weight of food before drying}} \times 100$$

Determination of Moisture content: The AOAC (2010), technique was used to determine the moisture content. 5 g sample was weighed into a petri plate with a known weight. It was dried for 4 hours at 105¹ °C in the oven. After cooling in desiccators, the samples were weighed.

The formula below was used to determine the moisture content:

$$\text{Moisture content as a percentage (\%)} = \frac{G_1 - G_2}{G_1} \times 100$$

G₁=Sample weight before drying

G₂=Sample weight after drying

Estimation of total carbohydrate content: Deducting the whole sum of fat, moisture, ash, crude fibre and protein percentages from a hundred calories yielded the carbohydrate content (AOAC, 2010).

Percentage (%) of Carbohydrate = 100 – (Crude fibre + % Ash + % Fat + % Moisture + % Protein).

Anti-nutritional Composition

Determination of oxalate content: The oxalate profile was estimated using the method of Nwosu *et al.* (2013). One (1) g of pigeon pea seeds was pounded into powder and placed into a 100 ml container. A magnetic hot plate was used to heat 20 ml of 0.30 N HCl to a temperature of 40-50 °C and agitate the liquid for an hour. A 20 ml flask was used to extract the mixture three times. The blended residue was diluted in the volumetric flask's 100 ml mark. 5 ml of extract was pipette into a conical flask and alkaline with 1.0 ml of 5 N Ammonium hydroxide. Placing an indicator paper in the conical flask displayed the alkaline sections. 1 mL of 5% CaCl₂ was added to the mixture and let to remain for 3 hours. The mixture was centrifuged for 15 minutes at 300 rpm. The precipitate was discarded, and 2 ml of 3 N H₂SO₄ was added to the test tubes, where the suspension was dissolved by heating in a water bath to a temperature of 60⁰C (70-80 ⁰C).The components of the pipette were delicately transferred into a conical flask and titrated at room temperature against newly made 0.01 N KMnO₄ until the solution turned pink. The solution was held until it became colourless, and then heated to 70-80 degrees Celsius. The solution was titrated until it yielded a continuous pink tint that persisted for 30 minutes.

Determination of Phytate content: This was done in accordance with AOAC (2010), guidelines. An electronic weighing scale was used to weigh a precise measure of 2.0 g of the sample. 0.2N HCl_(aq) was used to extract the material. Using a pipette, 0.5 mL of the extract was placed into a test tube with a glass vial. After that, 1 mL of the solution was added to the tube, which was then closed with a cork and secured with a clip. For the first 15 minutes, the cylinder was heated in a boiling water bath before being capped with the stopper. After 15 minutes of chilling in cold water, the solution was allowed to come to room temperature. The elements of the test tube were then thoroughly mixed before being centrifuged for 30 minutes. In another test tube, 1 ml of the supernatant and 1.5 ml of the solution were combined. The absorbance at 519 nm was measured in comparison to distilled water.

$$\% \text{ phytate} = \frac{Au}{As} \times \frac{C}{W} \times \frac{Vf}{Va} \times 100$$

- Au = test sample absorbance
- As = standard solution absorbent
- C = standard solution concentration
- W = weight of sample used
- Vf = extract total volume
- Va = extract volume

Determination of tannin content: The AOAC (2010), technique was used to determine the tannin concentration. After being combined with 50 mL of distilled water, the sample (5 g) was agitated. The mixture was filtered using Whatman no.4 filter paper after 30 minutes of waiting at 8 °C. In a 50 mL volumetric flask, 2 mL of the extract was added. 2 mL standard tannic solution (0.1 mg/mL tannic acid) and 2 mL distilled water were used as benchmarks in a separate volumetric flask. 2.5 mL saturated sodium carbonate (Na₂CO₃) solution and 1 mL Folin-Creagent were added to the flask and thoroughly mixed to make the total volume of 50 mL. The sample was filtered with Whatman no.4 grade filter paper after standing for 112 hours, and the mixture was measured at 760 nm against a reagent blank:

$$\text{Tannin (mg/100g)} = \frac{\text{Standard concentration} \times \text{sample absorbance}}{\text{Standard absorbance} \times \text{weight of sample}}$$

Determination of phenol content: Each treatment's seeds were ground into flour and sieved to a depth of 250 m. Five grams (5 g) of each flour were placed in Erlenmeyer flasks (250 ml capacity) containing 50 ml of 70% ethanol (v/v) and agitated for 12 hours at 130 rpm on an orbital shaker. Whatman No. 1 filter paper was used to filter the contents of the flask, and the residue was rinsed with 25 mL of 70 percent ethanol. At 40 degrees Celsius, the mixed filtrates were evaporated in a rotary vacuum evaporator. The residue left after vacuum evaporation was dissolved in 10 mL distilled water to make concentrated sugar syrup. On chromatographic plates (1919 cm) covered with cellulose powder-G, ten microlitres of the aforementioned syrup were quantified in triplicate (Acme chemicals and Bombay). The sheets were stored in a chromatographic chamber with a solvent system of n-propanol, ethyl acetate and water (6:1:3). The manufactured plates were sprayed with 1 percent - naphthol in ethyl alcohol containing 10% orthophosphoric acid to identify the sugar spots.

For quantitative estimation, a 23 cm area corresponding to each oligosaccharide was scraped out and soaked in 3 ml distilled water for 12 hours. After 12 hours, the mixture was filtered through Whatman No. 1 filter paper and the oligosaccharide content in 1 ml of filtrate was determined.

Determination of cyanide content: The alkaline titration model was used to evaluate the cyanide proportion. To detect HCN, a stable turbid KI indicator end point was reached by titrating an alkaline sample solution with 0.02 N AgNO₃ (1 ml of 0.02 N AgNO₃ = 1.08 mg HCN).

Determination of alkaloid content: Adeniyi *et al.* (2009), method was used to determine alkaloid content. An aliquot of 2 g of each sample was weighed and dispersed in 50 ml of acetic acid solution in ethanol at a concentration of 10%. The mixtures were agitated with a glass rod and set aside for about 4 hours before being filtered. The filtrates were then evaporated to one quarter of their volume with the use of a hot plate. A few drops of strong ammonium hydroxide were used to extract the alkaloids. The precipitate was removed using filter sheets with pre-weighed weights, and the residue was dried in an oven at 60 °C for 30 minutes, transferred to a desiccator to cool, and reweighed until constant weights were recorded. The weight of the alkaloid was determined using weight differencing and reported as a percentage of the sample weight assessed.

Estimation of minerals composition: One (1) g of powdered material was measured in a 100 ml digestion flask. The flask was filled with exactly 10 mL of nitric acid (HNO₃) and left overnight in the dark. Perchloric acid (HClO₄) in the amount of 5 mL was added the next day. After 15 minutes on a hot plate at 50 degrees Celsius, the combination was significantly increased to 200 degrees Celsius. After the perchloric acid fumes had dissipated, heat was applied. The contents were then chilled and filtered via Whatman filter paper after digestion. It was then transferred to a 50 mL volumetric flask and diluted with de-ionized water to the desired concentrations. With a hollow cathode lamp and an Atomic Absorption 200 Perkin Elmer Analyt, calcium, magnesium, iron,

copper, zinc, manganese and lead were all determined spectrophotometrically. A spectrophotometer has been used to evaluate the proportion of phosphorus in the seed samples, while a flame photometer was used to quantify the number of sodium and potassium (AOAC, 2010).

Tactile (Forefinger and Thumb) method: The method described by Akinoso and Lasisi (2013), was used to calculate the cooking time. Five grams (5 g) of each treatment were measured into 100 ml of boiling water in a pressure-cooking pot (pressure-cooking pots (Model: 15 psi Marlex appliance, India).) with Liquefied Petroleum Gas (LPG) as the source of heat. A specific number of seeds (5 seeds) were scooped out of the boiling water on a regular basis to measure their softness. These were achieved by squeezing the seeds between the index and thumb fingers. When approaching subjective cooking time, this is done at a regular interval of 5 minutes until a particular percentage of the seeds are deemed tender enough. The time between assessments was lowered to 3 minutes as the cooking time came to an end. This process was repeated until 80 percent of the seeds were regarded sufficiently "cooked" (Kinyanjui *et al.*, 2015). Normal and control cooking were conducted. For all types of cooking, the pots were covered. The normal cooking method is the general practice of continuous heating in closed pot until the food is satisfactorily cooked. A stopwatch was used to keep track of how long it took to cook. Cooking time was defined as the time it took for the grains to soften without any hard centres or white core.

Determination of Energy Requirements: The difference in weight of the LPG cylinder was recorded before the commencement of the cooking and after the cooking was completed. The difference in weight was assumed to be the quantity of fuel consumed. Cooking time was also recorded. Caloric values of the LPG was considered to be 47 700 kJ/kg (Akinoso and Oladeji, 2017) and the power rating of the pressure cooker was estimated at 0.6 kW/Hour (Power consumption of a 750 watt electric pressure cooker in a 30 min = 750 watt X 0.5 hours = 375 watt hours (Wh) = 0.375 kWh). Power consumption was estimated using the equation below:

$$E = p \times t$$

Where E = energy consumed (kJ)

p = power rating of the cooker (W)

t = duration of cooking (s)

Data Analysis

Data obtained were pooled for analysis, Analysis of variance (ANOVA) was used to compare the means for all the quantitative parameters; Duncan Multiple Range (DMRT) Post-hoc Test was used to separate the means. All values were considered significant at $P < 0.05$

Results and Discussion

Proximate composition of M₁ generation of the genotypes: The proximate compositions from this study were observed to have decreased with an increase in irradiation on protein, fat, carbohydrate, dry matter and energy value. However, the reverse was the trend in moisture, ash and fibre as they increase with increase on the irradiation doses. The reductions on the protein content were not variant enough from pigeon pea CODEX standard (17-24 %) except on the higher dose treatment (400 Gy) where low contents of protein (16.65, 16.64 and 13.57 %) was observed in TCC-2 (Table 1). As one might expect, there are contrasting data on the impact of gamma irradiation on nutritional contents of seed content; while Rajeev and Sridhar (2008), found a considerable increase in proteins in irradiated cowpea and *Mucuna pruriens*, El-Niely (2007), found that irradiation had no effect on crude protein, crude fat, crude fiber or ash in the legumes studied. According to Saxena *et al.* (2010), dry pigeon pea seeds contain 20 to 22 percent protein, 62.78 percent carbohydrate and 1.5 percent fat. There are minor differences when this is compared with our own results. Gamma ray is a potent mutagen in causing variation in population of crop plants; such effect could be stimulatory or inhibitory.

Crude fat showed a dose-dependent decline that was substantial at higher doses, which could be attributable to the increased irradiation dosage. These findings are consistent with those of Abu-Darwish *et al.* (2011), who found that the oil absorption capacity of cowpea flour was unaffected by low dose irradiation. Bhat *et al.* (2009), on the other hand, claimed that depolymerisation and delignification of the plant

matrix caused a rise in oil absorption capacity fibre after irradiation. It goes without saying that the consequences of gamma irradiation vary by species, period, and dose. This, however, will explain the inconsistencies between the current study's findings and those from earlier studies. El-Niely (2007), found that irradiation had no effect on moisture. Irradiation also increases the overall quality and shelf life of legume seeds, according to Rajeev and Sridhar (2008).

Ash content is the inorganic residue after the organic matter and water are removed by heating and is important in determining the total mineral composition in foods (Zubair *et al.*, 2020). According to Saxena *et al.* (2010), dry pigeon pea seeds contain 8.1 percent ash. The stimulating effects of gamma irradiation may have contributed to the rise in fibre content among the treatments. Fibre aids in food digestion, allowing for healthy growth. It also cleanses the digestive tract by eliminating impurities from the body and prevents extra cholesterol from being absorbed (de Lourdes García-Magaña *et al.*, 2013).

Anti-nutritional composition of M₁ generation of the genotypes: Generally speaking, gamma irradiation tends to reduce the anti-nutritional compositions in all the pigeon pea genotypes; this could be seen in the reduction in most of the treatment doses when compare with the control (Table 2). Reduction in anti-nutrient is an important trait for selection in any breeding programmes. According to Siddhuraju *et al.* (2002), the low nutritional value of food beans is attributable to the presence of strong anti-nutritional components. Anti-nutrients have a tendency to reduce protein digestibility and palatability by forming insoluble complexes with one another (Mbata *et al.*, 2009). Phytates are less accessible in food samples, especially for neonates, since they form complexes with zinc, iron, magnesium and calcium. Tannins are polyphenols found in plants that bind to protein and precipitate it, making it difficult to digest and absorb. Their negative effects, such as reducing the bioavailability of some essential minerals and inhibiting protein digestion, they must be reduced or eliminated entirely (Mbata *et al.*, 2009). The findings further revealed that increasing the irradiation dose resulted in a significant reduction in tannins, oxalate, phenol, alkaloid, cyanides and

phytate among the treatments (Table 2). Wakil and Kazeem (2012), discovered a decrease in cereal-soyabean polyphenol content. El-Niely (2007), found a similar pattern in legumes, claiming that radiation processing reduced phytic acid and tannin levels. ElShazali *et al.* (2011), also discovered that gamma irradiation in pearl millet genotypes have lower tannin and phytate levels than non-irradiated pearl millet genotypes. This implies that the genotypes are radio-sensitive to the mutagen in respect to anti-nutrition present in them. The reduction in anti-nutrients could be related to the gamma irradiation's inhibitory influence on the formation of free ions that enhance the activities of the phytochemicals. The drop in phytic acid, according to Bhat *et al.* (2009), is due to the formation of free radicals during irradiation, which causes the chemical connection between phytic acid and other compounds to break down. Singh *et al.* (2014) investigated the effect of irradiation on the reduction of anti-nutrient components in legumes; when compared to Harris *et al.* (2015), report, the minimum alkaloid content (2.01 g/100g) was closer to the one recorded (2.15 g/100g), while the maximum concentration (4.44 g/100g) was higher than the one recorded (3.86 g/100g). The variation in the response of the genotypes to different doses of gamma rays could be attributed to difference in their genetic make-up. The content and composition of phenolic compounds in different form in cereal and legume seeds vary widely, mainly controlled by genotype, and differently influenced by growing environment (Singh *et al.*, 2017)

Mineral composition of M_1 generation of the genotypes: The mineral contents in this study were observed to be irradiation dose dependent as it increase with an increase on the irradiation dose in Calcium, Magnesium and Phosphorus while insignificant reduction was obtained in Sodium, Potassium, Zinc and Iron. According to previous research, green seeds are a higher source of iron, copper, and zinc than mature seeds, so the increased concentration could be attributed to an increase in ash content, a decrease in anti-nutritional elements and most likely, the ripe seeds used in this study, because green seeds are a higher source of iron, copper and zinc than mature

seeds (Singh, 2016). Pigeon pea also includes tiny levels of copper, manganese and zinc, as well as a high concentration of magnesium, according to Adamu and Oyetunde (2013). Copper, along with iron, is essential for the creation of red blood cells. The trace mineral zinc aids immune function, wound healing, protein synthesis, DNA synthesis and cell division. In plant cells, magnesium is the most abundant ion. It is required by a number of enzyme systems in the body. By controlling nerve electrical potential, it also aids in the synthesis of adenosine triphosphate (ATP), the storage of carbohydrates, lipids and proteins, as well as nerve and muscle function. There was a small amount of phosphorous within and among the mutant lines, but no major changes were seen. Anderson (1997), study discovered that pigeon pea seeds contained tiny amounts of phosphorus, which is required in very minute amounts in the body for blood sugar regulation and glucose transport into cells. The findings of Okafor *et al.* (2018), were challenged by the drop-in iron levels seen in this study. Red blood cell formation, breathing and growth all require iron. It's a part of hemoglobin, which carries oxygen to the body's tissues.

Cooking time of M_1 generation of the genotypes:

In general, cooking quality is thought to be a consequence of cooking time, which is the amount of time it takes to boil the seeds in excess water until they reach the appropriate soft texture and are ready to eat (Singh *et al.*, 2005). In this investigation, the effects of irradiations on the cooking time of the treatment were dose dependent; with the cooking time tend to decrease as the irradiation dose increased. This decrease could be related to the weakening of bonds in the hemicelluloses content of the seed coats, which made seed cotyledon imbibitions easier. Water transport through the mesocarp is restricted, according to Wood *et al.* (2014), since the adhesive force that connects the mesocarp to the seed is quite significant.

References

- Adamu, A. S., & Oyetunde, J. G. (2013). Comparison of dietary proximate and mineral values of two varieties of bean. *Asian Journal of Natural and Applied Sciences*, 2(2), 103-106.

- [http://www.ajsc.leena-luna.co.jp/AJSCPdFs/Vol.2\(2\)/AJSC2013\(2.2-13\).pdf](http://www.ajsc.leena-luna.co.jp/AJSCPdFs/Vol.2(2)/AJSC2013(2.2-13).pdf).
- Adebowale, O. J., & Maliki, K. (2011). Effect of fermentation period on the chemical composition and functional properties of Pigeon pea (*Cajanus cajan*) seed flour. *International Food Research Journal*, 18(4), 1329-1333. [http://ifrj.upm.edu.my/18%20\(04\)%202011/\(18\)IFRJ-2011-244.pdf](http://ifrj.upm.edu.my/18%20(04)%202011/(18)IFRJ-2011-244.pdf).
- Adeniyi, S. A., Orjiekwe, C. L., & Ehiagbonare, J. E. (2009). Determination of alkaloids and oxalates in some selected food samples in Nigeria. *African Journal of Biotechnology*, 8(1).
- Akinoso, R., & Lasisi, I.M. (2013). Effect of Cooking Time on Select Physical and Mechanical Properties of Dried Pigeon pea (*Cajanus cajan*). *West Indian Journal of Engineering*, 35(2).
- Akinoso, R., and Oladeji, O. D. (2017). Determination of Energy and Time Requirement for Cooking Pigeon Pea (*Cajanus cajan*). *Journal of Biosystems Engineering*, 42, 56-67.
- Anderson, N. (1997). Historical changes in epilimnetic phosphorus concentrations in six rural lakes in Northern Ireland. *Freshwater Biology*, 38(2), 427-440.
- Association of Analytical Chemists (AOAC) (2010). Official Methods of Analysis. 18th Edition, Washington D.C. [https://www.scirp.org/\(S\(351jmbntvnsjt1adkposzje\)\)/reference/ReferencesPapers.aspx?](https://www.scirp.org/(S(351jmbntvnsjt1adkposzje))/reference/ReferencesPapers.aspx?)
- Avola, G., & Patanè, C. (2010). Variation among physical, chemical and technological properties in three Sicilian cultivars of Chickpea (*Cicer arietinum* L.). *International Journal of Food Science & Technology*, 45(12), 2565-2572.
- Ayenan, M. A. T., Danquah, A., Ahoton, L. E., & Ofori, K. (2017). Utilization and farmers' knowledge on pigeon pea diversity in Benin, West Africa. *Journal of Ethnobiology and Ethno-medicine*, 13(1), 1-14.
- Bamidele, O. P. & Akanbi, C. T. (2013). Influence of gamma irradiation on the nutritional and functional properties of pigeon pea (*Cajanus cajan*) flour. *African Journal of Food Science*, 7(9), 285-290.
- Bhat, R., Sridhar, K. R., Karim, A. A., Young, C. C., & Arun, A. B. (2009). Influence of γ -radiation on the nutritional and functional qualities of lotus seed flour. *Journal of Agricultural and Food Chemistry*, 57(20), 9524-9531.
- de Lourdes García-Magaña, M., García, H. S., Bello-Pérez, L. A., Sáyago-Ayerdi, S. G., & de Oca, M. M. M. (2013). Functional properties and dietary fiber characterization of mango processing by-products (*Mangifera indica* L., cv Ataulfo and Tommy Atkins). *Plant Foods for Human Nutrition*, 68(3), 254-258.
- El-Niely, H. F. (2007). Effect of radiation processing on anti-nutrients, in-vitro protein digestibility and protein efficiency ratio bioassay of legume seeds. *Radiation Physics and Chemistry*, 76(6), 1050-1057.
- ElShazali, A. M., Nahid, A. A., Salma, H. A., & Elfadil, E. B. (2011). Effect of radiation process on anti-nutrients, protein digestibility and sensory quality of pearl millet flour during processing and storage. *International Food Research Journal*, 18(4), 1401.
- Esan, V. I., & Ojemola, O. I. (2018). Evaluation of production systems, traditional knowledge of pigeon pea (*Cajanus cajan*) and risks of extinction of pigeon pea, jack bean (*Canavalia ensiformis*) and Lubia Bean (*Lablab purpureus*) in some parts of south west Nigeria. *Journal of Experimental Agriculture International*, 2 (7), 1-11.
- Food and Agriculture Organization of the United Nations Statistics Division (2015). Agricultural production: pigeon pea. Retrieved on June 6, 2016 from FAOSTAT website: <http://faostat.fao.org/site/339/default.aspx>
- Hamdani, A. M., Wani, I. A., Bhat, N. A. & Masoodi, F. A. (2018). Chemical composition, total phenolic content, antioxidant and anti-nutritional characterization of exudate gums. *Food Bioscience*, 23, 67-74

- Harris, A. C., Tally, L., Muelken, P., Banal, A., Schmidt, C. E., Cao, Q., & LeSage, M. G. (2015). Effects of nicotine and minor tobacco alkaloids on intracranial-self-stimulation in rats. *Drug and Alcohol Dependence*, 153, 330-334.
- Kharkwal, M. C., & Shu, Q. Y. (2009). The role of induced mutations in world food security. *Induced plant mutations in the genomics era. Food and Agriculture Organization of the United Nations, Rome*, 33-38.
- Kinyanjui, P. K., Njoroge, D. M., Makokha, A. O., Christiaens, S., Ndaka, D. S., & Hendrickx, M. (2015). Hydration properties and texture fingerprints of easy-and hard-to-cook bean varieties. *Food Science & Nutrition*, 3(1), 39-47.
- Lin-Qi, X., Li, T.T., Wei Z., Guo, N., Luo, M., Wang, W., Zu, Y., Fu, Y., & Peng, X. (2014). Solvent-free microwave extraction of essential oil from pigeon pea leaves and evaluation of its antimicrobial activity. *Industrial Crops and Products Journal* 58: 322-328.
- Makelo, M. N. (2011). Development of pigeon pea [*Cajanus cajan* (L.)] hybrids for the semi-arid Kenya. Doctoral dissertation, University of KwaZulu-Natal, Pietermaritzburg, South Africa.
- Mbata, T. I., Ikenebomeh, M. J., & Alaneme, J. C. (2009). Studies on the microbiological, nutrient composition and anti-nutritional contents of fermented maize flour fortified with bambara groundnut (*Vigna subterranean* L). *African Journal of Food Science*, 3(6), 165-171.
- Nwosu, J. N., Ojukwu, M., Ogueke, C. C., Ahaotu, I., & Owuamanam, C. I. (2013). The Anti-nutritional Properties and Ease of De-hulling on the Proximate Composition of Pigeon pea (*Cajanus cajan*) as Affected by Malting.
- Okafor, U. I., Omemu, A. M., Obadina, A. O., Bankole, M. O., & Adeyeye, S. A. (2018). Nutritional composition and anti-nutritional properties of maize ogi co fermented with pigeon pea. *Food Science & Nutrition*, 6(2), 424-439.
- Pazhamala, L., Saxena, R. K., Singh, V. K., Sameerkumar, C. V., Kumar, V., Sinha, P., & Varshney, R. K. (2015). Genomics-assisted breeding for boosting crop improvement in pigeon pea (*Cajanus cajan*). *Frontiers in plant science*, 6, 50.
- Rajeev, B., & Sridhar, K. R. (2008). Effect of electron beam irradiation on the quality characteristics of an economically valued tropical legumes *Mucuna pruriens*. *LDC Electronic Journal of Environmental, Agricultural Food Chemistry* 7(12), 2578-2581.
- Roychowdhury, R., & Tah, J. (2013). Mutagenesis—A potential approach for crop improvement. In *Crop Improvement*. Springer, Boston, MA. Pp 149-187.
- Saxena, K. B., Kumar, R. V., & Sultana, R. (2010). Quality nutrition through pigeon pea-a review. *Health*, 2(11), 1335-1344.
- Siddhuraju, P., Makkar, H.P.S., and Becker, K. (2002). The effect of ionising radiation on anti-nutritional factors and the nutritional value of plant materials with reference to human and animal food. *Food Chemistry*, 78 (2), 187-205. [https://doi.org/10.1016/S0308-8146\(01\)00398-3](https://doi.org/10.1016/S0308-8146(01)00398-3).
- Singh, P. K., Sohani, S., Panwar, N. and Bhagyawant, S. S. (2014), Effect of radiation processing on nutritional quality of some legume seeds. *International Journal of Biological and Pharmaceutical Research*, 5(11), 876-881.
- Singh, S., Upadhyaya, H. D., Gowda, C. L. L., & Reddy, L. J. (2005). Development of core collection in pigeon pea [*Cajanus cajan* (L.) Millspaugh] using geographic and qualitative morphological descriptors. *Genetic Resources and Crop Evolution*, 52(8), 1049-1056.
- Singh, I. P. (2016). Nutritional benefits of pigeon pea. In *Bio-fortification of Food Crops*. Springer, New Delhi. (pp. 73-81).
- Singh, B., Singh, J. P., Shevkani, K., Singh, N., and Kaur, A. (2017). Bioactive constituents in pulses and their health benefits. *Journal of Food Science and Technology*, 54, 858–870. doi: 10.1007/s13197-016-2391-9

- Śmiglak-Krajewska, M., & Wojciechowska-Solis, J. (2021). Consumption Preferences of Pulses in the Diet of Polish People: Motives and Barriers to Replace Animal Protein with Vegetable Protein. *Nutrients*, 13(2), 454. <https://doi.org/10.3390/nu13020454>.
- Surekha, C., Beena, M. R., Arundhati, A., Singh, P. K., Tuli, R., Dutta-Gupta, A., & Kirti, P. B. (2005). Agrobacterium-mediated genetic transformation of pigeon pea (*Cajanus cajan* (L.) Millsp.) using embryonal segments and development of transgenic plants for resistance against Spodoptera. *Plant Science*, 169(6), 1074-1080.
- Thompson, H. J. (2019). Improving human dietary choices through understanding of the tolerance and toxicity of pulse crop constituents. *Current Opinion in Food Science*, 30, 93-97.
- Wakil, S. M., & Kazeem, M. O. (2012). Quality assessment of weaning food produced from fermented cereal-legume blends using starters. *International Food Research Journal*, 19(4). 1679-1685 [http://www.ifrj.upm.edu.my/19%20\(04\)%202012/53%20IFRJ%2019%20\(04\)%202012%20Nigeria%20\(104\).pdf](http://www.ifrj.upm.edu.my/19%20(04)%202012/53%20IFRJ%2019%20(04)%202012%20Nigeria%20(104).pdf).
- Wood, J. A., Knights, E. J., Campbell, G. M., & Choct, M. (2014). Differences between easy-and difficult-to-mill chickpea (*Cicer arietinum* L.) genotypes. Part I: Broad chemical composition. *Journal of the Science of Food and Agriculture*, 94(7), 1437-1445.
- Zubair, M., Wang, S., Zhang, P., Ye, J., Liang, J., Nabi, M., ... & Cai, Y. (2020). Biological nutrient removal and recovery from solid and liquid livestock manure: Recent advance and perspective. *Bioresource Technology*, 301, 122-823.

Table 1: Effects of Gamma Irradiation on M₁ Generations-Proximate Composition

Dose (Gy)	Moisture (%)	Ash (%)	Crude Protein (%)	Crude Fibre (%)	Crude Fat (%)	Carbohy drate (g/100g)	Dry matter (%)	Energy value (kcal/g)
NG/AO/MAY/00/021/01								
Control	5.89±0.62 ^b	3.49±0.02 ^a	19.68±0.11 ^b	3.04±0.02 ^b	5.06±0.01 ^d	68.82±0.37 ^c	94.12±0.62 ^{bc}	193.30±1.55 ^a
100	6.15±0.35 ^{bc}	4.00±0.02 ^b	20.40±0.09 ^c	3.04±0.02 ^b	4.46±0.04 ^c	68.93±0.44 ^c	93.85±0.35 ^{bc}	192.86±0.27 ^a
200	4.90±0.10 ^a	3.49±0.01 ^a	18.14±0.19 ^a	2.65±0.01 ^a	4.17±0.05 ^b	66.62±0.19 ^c	92.10±0.10 ^b	189.83±0.87 ^a
300	5.10±0.10 ^{ab}	3.51±0.47 ^a	19.53±0.36 ^b	2.97±0.06 ^b	3.48±0.04 ^a	61.15±0.83 ^b	90.90±0.10 ^b	185.50±0.03 ^a
400	7.05±0.03 ^c	4.49±0.01 ^b	18.52±0.04 ^a	3.41±0.01 ^c	3.04±0.06 ^a	57.43±0.15 ^a	82.96±0.01 ^a	181.19±0.70 ^a
NG/SA/11/08/108								
Control	5.24±0.26 ^b	4.49±0.01 ^b	20.05±0.09 ^c	3.41±0.00 ^b	3.00±0.00 ^c	64.99±0.29 ^b	94.76±0.26 ^c	177.44±0.12 ^c
100	4.44±0.06 ^a	3.11±0.11 ^a	19.70±0.44 ^c	2.36±0.08 ^a	3.03±0.04 ^c	65.54±0.02 ^b	95.56±0.06 ^c	171.44±1.41 ^b
200	5.48±0.02 ^b	4.99±0.02 ^c	18.87±0.04 ^b	3.80±0.01 ^c	3.06±0.06 ^c	62.09±0.51 ^b	94.52±0.02 ^c	170.94±8.22 ^b
300	6.36±0.36 ^c	4.95±0.00 ^c	18.30±0.09 ^b	3.76±0.00 ^c	2.59±0.04 ^b	58.08±0.63 ^{ab}	83.64±0.36 ^b	165.29±0.78 ^a
400	5.06±0.06 ^{ab}	4.49±0.04 ^b	17.29±0.22 ^a	3.79±0.03 ^c	2.03±0.05 ^a	52.83±0.16 ^a	75.94±0.06 ^a	161.31±0.26 ^a
NG/SA/07/191								
Control	3.50±1.50 ^d	3.98±0.00 ^b	24.78±0.26 ^c	3.03±0.00 ^b	3.51±0.03 ^a	66.29±1.57 ^c	96.00±1.50 ^b	192.67±1.09 ^b
100	4.53±0.03 ^{cd}	4.47±0.01 ^c	23.69±0.04 ^b	3.40±0.01 ^c	3.94±0.06 ^b	65.61±0.10 ^c	95.48±0.03 ^b	186.27±0.65 ^a

200	4.20±0.20 ^c	3.01±0.01 ^a	21.06±0.04 ^a	2.29±0.01 ^a	3.95±0.03 ^b	61.87±0.32 ^c	95.80±0.20 ^b	185.11±0.41 ^a
300	5.05±0.10 ^b	4.04±0.04 ^b	21.00±0.05 ^a	3.07±0.03 ^b	5.71±0.24 ^c	58.86±0.66 ^b	81.95±0.05 ^a	198.29±2.68 ^a
400	5.10±0.10 ^a	4.49±0.03 ^c	20.84±0.26 ^a	3.41±0.02 ^c	3.40 ±0.04 ^a	53.54±0.16 ^a	76.53±0.30 ^a	181.37±0.34 ^a
TCC-2								
Control	3.10±0.10 ^d	5.45±0.02 ^d	19.31±0.04 ^c	4.14±0.01 ^d	2.45±0.05 ^c	72.62±0.18 ^c	96.90±0.10 ^b	198.86±0.57 ^b
100	4.41±0.11 ^d	5.46±0.01 ^d	17.34±0.26 ^b	4.15±0.01 ^d	2.45±0.05 ^c	70.97±1.16 ^c	96.59±0.11 ^b	198.53±2.17 ^b
200	3.66±0.62 ^d	3.99±0.03 ^b	16.65±0.00 ^b	3.03±0.02 ^b	3.06±0.08 ^d	67.69±0.99 ^c	95.89±0.44 ^b	192.49±1.24 ^b
300	4.20±0.70 ^d	3.46±0.02 ^a	16.64±1.24 ^b	2.63±0.01 ^a	2.07±0.06 ^a	63.53±0.24 ^b	95.80±0.70 ^b	190.22±2.03 ^b
400	3.60±0.10 ^d	4.94±0.04 ^c	13.57±0.44 ^a	3.75±0.03 ^c	2.24±0.02 ^b	57.35±0.60 ^a	80.40±0.10 ^a	188.20±0.52 ^a

Values are mean ± standard error of mean; Values followed by different superscript along the same column are significantly different at P<0.05

Table 2: Impacts of Gamma Irradiation on Anti-nutritional Compositions in M₁ Generation of Pigeon Pea

Dose(Gy)	Ox (mg)	Cy (mg/kg)	Phy (%)	T (%)	Ph (%)	Alk (mg/100g)
NG/AO/MAY/00/021/01						
Control	3.19±0.11 ^c	54.83±0.00 ^b	3.67±0.00 ^d	0.83±0.06 ^c	58.82±0.37 ^c	4.44±0.03 ^c
100	2.62±0.02 ^a	54.80±0.01 ^b	2.32±0.00 ^b	0.64±0.00 ^b	55.93±0.44 ^b	4.31±0.01 ^c
200	3.52±0.00 ^d	53.60±0.00 ^b	3.00±0.04 ^c	0.55±0.03 ^b	52.62±0.19 ^{ab}	3.89±0.01 ^b
300	2.99±0.04 ^{bc}	52.83±0.02 ^b	2.46±0.15 ^b	0.19±0.01 ^a	51.23±2.91 ^a	3.73±0.16 ^b
400	2.89±0.03 ^b	48.83±0.03 ^a	2.04±0.07 ^a	0.29±0.03 ^a	50.43±0.15 ^a	3.30±0.01 ^a
NG/SA/11/08/108						
Control	2.87±0.12 ^d	56.31±0.00 ^b	2.10±0.03 ^a	0.74±0.03 ^d	67.35±0.60 ^b	4.39±0.01 ^c
100	2.51±0.11 ^{cd}	55.10±0.01 ^b	2.30±0.02 ^b	0.15±0.00 ^a	65.83±0.56 ^b	4.30±0.10 ^c
200	2.32±0.02 ^c	53.11±0.00 ^{ab}	3.49±0.06 ^c	0.39±0.03 ^c	62.09±0.51 ^{ab}	3.89±0.00 ^{ab}
300	2.27±0.02 ^b	50.63±0.02 ^{ab}	3.35±0.01 ^c	0.35±0.05 ^{bc}	60.11±0.63 ^a	3.88±0.01 ^{ab}
400	1.54±0.22 ^a	46.22±0.03 ^a	4.00±0.06 ^d	0.29±0.03 ^b	58.06±0.59 ^a	3.16±0.14 ^a
NG/SA/07/191						
Control	3.63±0.02 ^d	53.75±0.06 ^b	2.07±0.00 ^c	1.03±0.00 ^a	62.61±1.50 ^b	4.27±0.25 ^b
100	3.01±0.02 ^{cd}	52.60±0.01 ^b	2.01±0.00 ^d	1.22±0.06 ^b	62.29±0.10 ^b	4.21±0.01 ^b
200	2.93±0.02 ^c	50.12±0.04 ^b	1.70±0.01 ^c	1.28±0.00 ^b	61.86±0.66 ^b	4.11±0.01 ^b
300	1.91±0.02 ^b	47.82±0.01 ^{ab}	1.63±0.01 ^b	1.22±0.06 ^b	57.76±0.15 ^a	4.05±0.08 ^b
400	1.34±0.06 ^a	43.65±0.03 ^a	1.45±0.02 ^a	1.06±0.03 ^a	54.54±0.50 ^a	3.85±0.13 ^a
TCC-2						
Control	2.90±0.02 ^c	53.55±0.03 ^b	3.00±0.00 ^c	1.00±0.03 ^c	68.62±0.18 ^c	4.11±0.10 ^c
100	0.99±0.02 ^b	53.00±0.01 ^b	3.05±0.03 ^c	0.42±0.03 ^a	67.11±0.99 ^c	3.65±0.18 ^{bc}
200	1.85±0.04 ^d	50.73±0.02 ^b	2.21±0.11 ^b	0.71±0.06 ^b	65.26±0.91 ^{ab}	3.22±0.35 ^b
300	0.84±0.04 ^a	48.22±0.00 ^{ab}	2.28±0.00 ^b	0.35±0.03 ^a	62.16±0.24 ^b	3.06±0.05 ^b

400 1.62±0.03^c 44.75±0.02^a 1.94±0.00^a 0.38±0.00^a 59.34±0.52^a 2.01±0.12^a

Values are mean ± standard error of mean; Values followed by different superscript along the same column are significantly different at P<0.05.

Ox = Oxalate (mg), Cy = Cyanide (mg/kg), Phy = Phytate (%), T = Tannin (%), Ph = Phenol (%), Alk = Alkaloid (mg/100g)

Table 3: Effects of Gamma Irradiation on Cooking Time (minutes) of M₁ Generations

Dose (Gy)	Energy		Energy		Energy		Energy	
	NG/AO/MAY /00/021/01	Consumed (kJ)	NG/SA/11/08 /108	Consumed (kJ)	NG/SA/07/191	Consumed (kJ)	TCC-2	Consumed (kJ)
0	155.00±0.00 ^c	58125±612 ^c	148.00±0.00 ^d	55500±450 ^c	132.50±2.50 ^c	49688±170 ^c	121.50±1.50 ^c	45563±200 ^c
100	154.50±0.50 ^c	57938±287 ^c 56625±390 ^b	147.50±0.50 ^d	55313±470 ^c	131.50±0.50 ^c	49313±150 ^c	120.00±0.00 ^{bc}	44995±996 ^{bc}
200	151.00±0.00 ^{bc}	^c	143.50±1.50 ^c	53813±850 ^b	126.00±1.00 ^b	47250±435 ^b	115.00±0.00 ^b	43125±657 ^b
300	148.50±1.50 ^b	55688±302 ^b	138.00±0.00 ^b	51750±970 ^b	121.00±1.00 ^b	45375±977 ^b	112.00±1.00 ^b	42100±610 ^b
400	144.00±0.50 ^a	54000±201 ^a	132.00±0.50 ^a	49500±100 ^a	111.00±1.00 ^a	41625±225 ^a	106.50±1.28 ^a	38750±97 ^a

Values are mean ± standard error of mean; Values followed by different superscript along the same column are significantly different at P<0.05.