

Bioavailability of Iron and Zinc in Biofortified Common Beans (*Phaseolus vulgaris* L.) from Burundi: An In Vivo *Gallus gallus* Feeding Trial

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Abstract

Background

Micronutrient malnutrition, particularly deficiencies in iron and zinc, remains a significant global public health issue, impacting over half of the world's population. To address this problem, common bean breeders have developed biofortified bean varieties rich in iron and zinc. These common beans are widely consumed in low-income areas like Burundi, where they serve as a primary food source. However, evidence from recent studies indicate that a high concentration of these minerals does not necessarily ensure high bioavailability, as antinutritional factors such as phytic acid interfere with mineral absorption.

Methods

This study determined the bioavailability of iron and zinc in dry cooked biofortified common bean varieties (MAC44 and RWR2245), a non-biofortified variety (*Kinure*) and commercial broiler mash as the control, using an in vivo *Gallus gallus*. Thirty six broiler chickens were randomly allocated to the four treatments over a seven-week feeding trial. Standard methods were used to determine dietary proximate analysis, phytic acid, and mineral concentration and experiments replicated thrice. Data analysis was conducted at a 95% confidence level and included Analysis of Variance and Pearson's correlation. *Post-hoc* analysis was performed using Tukey's Honestly Significant Difference.

Results

Results indicated that while common bean-based diets had higher iron concentrations compared to the commercial broilers mash, their higher phytic acid content limited iron bioavailability, resulting in significantly similar Hemoglobin Maintenance Efficiency (HME) across the common bean-based diets. While the commercial broiler mash yielded the highest HME and fractional zinc absorption, RWR2245 demonstrated the most favorable fractional zinc absorption among the common bean-based formulations, though it remained slightly below the non-biofortified common bean variety, *Kinure*.

Conclusions

Should the results from this *Gallus gallus* model be extrapolated to human nutrition, the high phytate-to mineral ration in these biofortified beans would similarly limit fractional absorption ad of zinc and utilization of iron for HME. Therefore, there is a need to conduct human clinical trials to determine other factors necessary to overcome these bioavailability barriers in target populations.

Clinical trial number Not applicable.

1.0 Introduction

Hidden hunger, defined as a lack of key micronutrients, particularly iron and zinc, continues to be a serious global public health concern, affecting more than half of the world's population [1] . This type of malnutrition is especially common among preschool children, adolescents, and women of reproductive age in low-income countries like Burundi [2,3]. Iron deficiency anemia (IDA) is the most common micronutrient-related illness, affecting an estimated 4.9 billion individuals, or almost 65% of the global population, owing to insufficient dietary iron consumption [4]. Although, nutritional approaches such as supplementation and food fortification have been traditionally been used to curb the problem, they face considerable challenges in terms of accessibility, scalability, and long-term viability [3].

Biofortification is defined as the process of breeding staple crops such rice, maize, wheat, and legumes containing higher concentrations of important micronutrients such as iron and zinc [5]. In recent years, this intervention has emerged as a major agriculture-based, nutrition-sensitive technique for combating hidden hunger, particularly among vulnerable and low-income communities in developing countries. Furthermore, micronutrient biofortification, which complements traditional methods such as supplementation and food fortification [6,7], offers a scalable and long-term approach to enhance nutritional outcomes [8,9].

In Africa, biofortification of common beans (*Phaseolus vulgaris*) is a flagship initiative aimed at addressing food and nutrition insecurity [2,10]. Common beans act as a nutritionally significant pulse crop that provide both macronutrients, particularly protein and micronutrients, for millions across Africa, and other low-income countries. These beans naturally accumulate trace minerals such as iron and zinc through a system of ion transporters and chelating molecules, including phytate, nicotianamine, and polyphenols [11]. Biofortification breeding techniques have successfully produced common bean varieties with enhanced iron and zinc concentration [12]. These crops are then utilized as food and raw materials for common bean-based products to alleviate trace mineral deficiencies in resource-limited regions such as Burundi, where beans are widely accepted as a dietary staple [13].

However, beyond mineral concentration, agronomic traits, environmental factors, cooking properties and consumer acceptance, nutrient bioavailability is a major concern for interventions dealing with micronutrients. [14] defines bioavailability as the amount of the nutrient that is biologically accessible for utilization in normal physiological functions, metabolism, and storage. However, the presence of other food components and food-processing techniques can either inhibit or enhance this accessible amount of micronutrients [15]. For instance, phytic acid (PA) present in common beans negatively impacts the bioavailability of iron and other minerals by reducing

solubility at the lumen level due through formation of mineral complexes with phytate [16,17]. Therefore, to properly estimate the minimum micronutrient concentrations that breeders must reach, and predict the success of these interventions, the amount of the micronutrients present in the ready-to-eat portion of the plant and available for absorption must be assessed to ensure nutritional efficacy. The modern broiler chicken is a fast-growing animal that is sensitive to dietary deficiencies of trace minerals such iron [9,16]. As such, the animal holds a greater potential as a relevant model for in vivo feeding trials, commonly referred to as *Gallus gallus* model.

The model has been used widely as an efficient and biologically realistic technique to estimate the availability of iron and other minerals [14]. This poultry-based approach allows for the measurement of food consumption and blood haemoglobin levels, making it easier to calculate total body haemoglobin iron and haemoglobin maintenance efficiency (HME), which are important criteria in determining iron status and bioavailability [9]. In parallel, Miller's equation is commonly used to predict zinc bioavailability and efficiently accounts for the inhibitory effects of phytic acid, which is commonly found in legume and cereal-based diets, enhancing the accuracy of bioavailability assessments in these food matrices [18,19]. The *Gallus gallus* model and Miller's equation work together to give a strong foundation for assessing mineral bioavailability in animal and human nutrition research.

While previous research [20] identified biofortified common bean varieties in Burundi with superior iron and zinc content than local non-biofortified varieties, the mineral bioavailability remains unknown. This study was a continuation of that work and the objective was to investigate the bioavailability of iron and zinc in selected biofortified common bean varieties using the *Gallus gallus* model. Iron bioavailability was evaluated for each of the diets as the ability to maintain total body haemoglobin iron (Hb-Fe) during a seven week *in vivo* (*Gallus gallus*) feeding trial. Zinc bioavailability was assessed as the fractional absorption determined using miller equation. By extension, effect of incorporating biofortified common beans on growth characteristics of the broiler chicken utilized in the feeding trials was also evaluated.

2.0 Materials and Methods

2.1 Study location, selecting, justification and handling of common bean variety samples

The feeding experiment was set up at the Kenya Agricultural and Livestock Research Organization (KALRO) within the Naivasha Poultry Research Unit located in Naivasha sub-county, Nakuru County, Kenya. The Research Centre is located at an elevation of roughly 1,700 meters above sea level, receives an average annual rainfall of 1,100 millimeters and temperature ranges of 8°C to 25°C. All feed preparation ingredients were purchased from reputable agrovet in Nairobi, Kenya. Stainless steel appliances were used for all the processing steps. Three dry common bean varieties namely *Kinure* (non-biofortified), RWR2245 (*Kaneza*) and MAC44 (*Magogori*) (biofortified) were sourced from farmers working with the Pan African Bean Research Alliance (PABRA) through the Institut des Sciences Agronomiques du Burundi (ISABU) in Bujumbura, Burundi. These common bean varieties were selected due to their high concentration of iron and zinc compared to other new varieties in the region, are commonly grown, widely consumed, and were also available at the time of the study. The common bean samples were packed in Purdue Improved Crop Storage (PICS) bags and transported to the Food Chemistry laboratory at Egerton University for analysis. All preparation procedures and laboratory analyses were conducted in the Food Chemistry laboratory plant. Zinc analyses were conducted at the Chemistry Laboratory at Egerton University, Faculty of Sciences while iron bioavailability assays were conducted at the Kenya Medical Research Institute in Nairobi, Kenya.

2.2 Ethical clearance

This study was conducted in strict accordance with the recommendation in the guide for the care and use of Laboratory Animals of the National Institute of Health [21] and the International Livestock Research Institute (ILRI) [22]. This research was reviewed and approved prior to commencement by the Egerton University Research and Ethics Committee (EU/RE/DVC/009 Approval No. EUREC/APP/149/2021) and the National Commission for Science, Technology and Innovation, Kenya under License No: NACOSTI/P/21/14502. All animal procedures including housing, handling, sampling and euthanasia adhered to these guidelines. Surgical procedures were performed under sodium pentobarbital anesthesia and all efforts were made to minimize animal suffering. Birds were monitored at least twice a day for health, welfare. At the end of the trial, all birds were humanely euthanized by carbon dioxide exposure in accordance to the American Veterinary Medical Association (AMVA) and the governance of animal care use for scientific purposes in Africa and Middle East guidelines for the Euthanasia of Animals [23, 24].

2.3 Feed preparation and formulation

Experimental diets were prepared according to [14] to meet the nutrition requirement for the broilers. All feed ingredients were purchased from local suppliers in Nairobi. All dried common bean grains were rinsed in ultra-pure (18Ω) water and then prepared according to [21]. The cooked common beans were then oven-dried and ground before mixing with other feed ingredients. Studies show that pretreatment of beans such as boiling is important before feeding to poultry [21]. The nutrient requirement for broilers is 3000 Kcal/kg, CP 20–22%. Therefore, corn oil that contained 884 kcal and fat 100g was incorporated to enhance the energy content [22, 23].

Table 1
Formulation of bean-based experimental diets for broilers

Ingredient g/kg	<i>Kinure</i>	MAC44	RWR2245
Dry common beans	400.00	400.00	400.00
Corn meal	350.00	350.00	350.00
Corn oil	30.00	30.00	30.00
Dry skim milk	100.00	100.00	100.00
Corn starch	46.75	46.75	46.75
Broiler premix (no Fe)	70.00	70.00	70.00
DL-Methionine	2.50	2.50	2.50
Choline Chloride	0.75	0.75	0.75
Total	1000.00	1000.00	1000.00

Broiler premix per 2 kg: vit A 15,000,000IU, vit D3 5,000,000IU, vit E 1000m, vit B12 10,000mg, vit B2 4500mg, vit B6 2500mg, vit C 1.500mg, niacin 16,000mg, methionine 10,000mg, lysine 15,250 mg, zinc sulphate 12,500mg, copper sulphate 12,500mg, sodium chloride 50,000mg, sodium sulphate 200,000mg, magnesium sulphate 200,000mg, manganese sulphate 12,500mg, potassium chloride, 85000 mg.

2.4 Poultry assays for iron bioavailability tests

This experiment was set up according to the method described by [24] with some modifications in that broilers were used in place of layers. All animal protocols were approved by the Committee on Ethics in the Use of Animals in Kenya. Fifty one-day-old broiler chicks were sourced from Kenchic, Nakuru Kenya and transported to the Kenya Agricultural and Livestock Research Organization, Poultry Unit in Naivasha, Kenya. From day 0 to day 7, all birds were feed on a standard starter broilers mash for adaptability purposes. At the age of 7 days, the chicks were randomly allocated into 4 treatment groups (n = 9), which included chicks fed on two feeds constituting dry biofortified common bean varieties (RWR2245 and MAC44), a standard broiler's feed and a traditional non-biofortified bean variety (*Kinure*). Diet composition is shown on Table 1. Chicks were housed in a total confinement building (3 chicks per 1 m² metal cage). Before the trial, the house was properly cleaned and disinfected using kupacide® disinfectant. Wood shavings were used as a deep litter system, which was continuously aerated and changed whenever it became damp. The birds were put under indoor controlled temperatures, and provided with 16 hours of light. Each cage was equipped with an automatic nipple drinker and a manual self-feeder. All birds were given *ad libitum* access to water. Feed intakes were measured daily (as from day 1) and body weight recorded each week.

2.5 Management of experimental broiler chicken (housing, feeding and disease control)

The house hosting the broiler chicken was managed according to [24]. The unit was properly cleaned and disinfected using kupacide® disinfectant prior to setting up the feeding trial. Wood shavings were used as litter in a deep litter system for the broilers and changed after every two weeks. The birds were fed daily at 8:00 a.m., and leftovers collected and weighed using a digital electronic weighing balance (KERNEW, d = ± 0.01g) to aid in computing feed consumption. Body weight for each chicken was also weighed on weekly basis and used to compute body weight gains. Body weight gain and daily feed intake were used to compute the feed conversion ratio. The birds were vaccinated against fowl typhoid at 8 weeks, 3rd dose Newcastle disease vaccine at 18 weeks, and deworming done using piperazine® at 19 weeks. Feeding and growth characteristics were computed as shown below:

2.5.1 Feed intake (FI)

Feed intake was measured every after 24 hours and determined using the difference between the amount of feed supplied and the amount left over (refusal).

$$\text{Feed Intake (FI) per broiler (g)} = \frac{\text{Feed Given (g)} - \text{Feed Leftover (g)}}{\text{Number of broilers}}$$

2.5.2 Average daily gain (ADG)

Every week, the broiler chicken in a cage were weighed together before being fed. The average daily increase was calculated by dividing the discrepancy between the weight of the broiler chicken after 7 days and the weight of the broiler chicken at the start of the 7 days.

$$\text{Average Daily Gain per broiler (g)} = \frac{\text{Weight after 7 days (g)} - \text{Weight at the start of 7 days (g)}}{7 \text{ days}}$$

2.5.3 Feed conversion ratio (FCR)

The feed conversion ratio was determined by the average feed intake (g) consumed by the bird divided by the average weight gain per grower (g) during each week.

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Average feed intake per broiler (g)}}{\text{Average weight gain per grower (g)}}$$

2.6 Proximate analyses

Proximate composition of the feed samples was analyzed using standard methods [25, 26]. Moisture content was determined using the oven-drying method according to AACC International (2007), Method 44 – 15 A. Ash content was measured using the AOAC (2000), method 942.05. Crude protein was assessed using AOAC (2000), method 984.13 with crude protein content calculated by multiplying the nitrogen content by a factor of 6.25. Crude fat extraction was based on AOAC (2000), method 920.39, while crude fibre was determined according to AACC (2000), method 6865. The analyses were conducted in replicates.

2.7 Iron and zinc analyses

Iron and zinc analyses was determined using the AOAC (2000), method 985.35 [26]. Exactly 5mls of Conc. HNO_3 and 1 ml Conc. HClO_4 was used to digest 1g of each feed sample. Allowed to stand closed overnight at room temperature to predigest the sample and thereafter placed in an oven at 100°C for 8hrs. and cooled to room temperature in a fume hood. UV/visible spectrophotometer (JENWAY 7315) was used to analyse zinc, and iron, and measured at wavelengths of 510 and 880 nm respectively.

2.8 Determination of phytic acid content

Phytic acid analysis was based on phytate precipitation, as described by [27]. Each feed sample (about 500 mg) was accurately weighed and phytate was extracted using 50 ml of 3% trichloroacetic acid (TCA) by shaking on Ratek Orbital Incubator (Boronia, Victoria, Australia) for 40 min. The suspension was then centrifuged at ($3000 \times g$, 10 min) using an Eppendorf centrifuge (Model 5804, Eppendorf, and Hamburg, Germany), a 10 ml aliquot of the supernatant was transferred to a 50 ml centrifuge tube and 4 ml of FeCl_3 solution added rapidly. The contents in the tubes were then heated in boiling water for 45 min, then centrifuged at $3000 \times g$ for 10 min using an Eppendorf centrifuge (Model 5804, Eppendorf, Hamburg, Germany) and the clear supernatant was decanted. The precipitate was washed twice by dispersing in 25 ml 3% TCA, and then heated in boiling water for 10 min, then centrifuged at ($3000 \times g$, 10 min) using Eppendorf centrifuge (Model 5804, Eppendorf, Hamburg, Germany), and there after washed with 20-ml distilled water. The precipitate was washed twice by dispersing in 5 ml of 51 distilled water and 3 ml of 1.5N NaOH added, then topped up 30 ml with distilled water and S for 30 min in boiling water. The contents were then filtered using Whatman No. 2 filter paper with a pore size of $8\mu\text{m}$ and then washed with 70 ml hot distilled water. The precipitate was transferred and dissolved into the 100 ml volumetric flask containing 40 ml hot 3.2N HNO_3 . The filter paper was washed using distilled water, and the washings were collected in the same flask. The flask was cooled to room temperature and the volume made to 100 ml with distilled water. An aliquot (5 ml) was transferred to another 100-ml volumetric flask and mixed with 65 ml distilled water, 20-ml 1.5M potassium thiocyanate (KSCN) then added. The volume was added to 100 ml with distilled water, and the color read at 480 nm using a spectrophotometer (model pharماسpec UV1700 Shimadzu, Japan) within 1 minute. Reagent blank in which distilled water would replace the sample was included. A calibration curve was made from iron (III) nitrate solution stock solution. Iron (in micrograms), present in the test solution was determined from the calibration curve and phytate P calculated as follows:

$$\text{Phytate P mg/g} = \frac{\text{Fe } (\mu\text{g}) \times 15}{\text{Weight of sample (g)}}$$

2.9 Blood analysis and hemoglobin (Hb) measurements

Blood samples collection and hemoglobin analyses were conducted following the method describes by [28] with modification in that samples were collected on day 21 and 42, rather than on weekly basis to avoid hemorrhage in the birds. Blood samples were collected from the wing vein ($n = 9$, $\sim 100 \mu\text{L}$) using micro-hematocrit heparinized capillary tubes, and stored on ice in BD Vacutainer® vials (lithium heparin; 95 USP Units) before analysis. All samples collected in the morning following an 8h overnight fast. The wing vein was not clearly visible between day 7 and day 14 hence analysis on blood samples collected on day 21 and day 42 were used as the initial and final hemoglobin concentration, respectively. The samples were then analyzed for hemoglobin (Hb) concentration determined spectro-photometrically using the cyanmethemoglobin method (H7506-STD, Pointe Scientific Inc., Canton, MI) following the manufacturer's instructions. Fe bioavailability was calculated as hemoglobin maintenance efficiency (HME) (28) as shown in the equation below:

$$\text{HME} = \frac{\text{Hb} - \text{Fe, mg Initial} - \text{Hb} - \text{Fe, mg Final}}{\text{Total Fe Intake mg}} \times 100$$

Where Hb-Fe (index of Fe absorption) = total body hemoglobin Fe. Hb-Fe was calculated from hemoglobin and estimates of blood volume based on body weight (a blood volume of 85 mL per kg body weight is assumed).

$$Hb - Fe \text{ (mg)} = Bwt \text{ (kg)} \times 0.085L \frac{\text{blood}}{\text{kg}} \times Hb \left(\frac{g}{l} \right) \times 3.35 \text{mgFe/gHb}$$

Dietary Fe intakes determined by multiplying the cumulative amount of diet consumed during the experiment with the iron concentrations measured for each diet. At the end of the experiment (day 42), birds were euthanized by carbon dioxide exposure.

2.11 Zinc bioavailability assessment

Zinc bioavailability from the experimental diets was estimated using the Miller Equation, which predicts fractional zinc absorption based on the phytate-to-zinc molar ratio in the diet [18]. For each dietary treatment, the phytate and zinc concentrations were used to calculate the total daily intake of each component per bird, considering the average daily feed intake and the proportion of beans in the formulated diet. The phytate intake (mg) was converted to millimoles using a molecular weight of 660 g/mol, while zinc intake (mg) was converted to millimoles using an atomic weight of 65.4 g/mol as shown:

$$\text{Phytate : Zinc molar ratio} = \frac{\text{Phytate intake in (mmol)}}{\text{Zinc intake (mg)} / 65.4}$$

The resulting molar ratio was then applied in the Miller predictive model as follows:

$$\text{Fractional zinc absorption} = \frac{0.5}{1 + (0.015 * \text{Pytate : Zinc molar ratio})}$$

The value obtained was used to estimate the proportion of dietary zinc that was bioavailable in each treatment group as follows:

$$\text{Estimated absorbed zinc (mg)} = \text{Cumulative zinc intake (mg)} * \text{Fractional zinc absorption}$$

3.0 Data analysis

The data were tested for normality and homogeneity using Levene's test prior to analyses in SAS® Software version 9.4. Analysis of variance (ANOVA) was conducted to test the experimental hypothesis, followed by post-hoc analysis using Tukey's HSD (Honestly Significant Difference) method. Pearson's correlation was also performed. Data analysis was carried out at a 95% confidence level, and results are presented in tables and graphs.

4.0 Results

4.1 Nutritional composition of feed formulated with biofortified common beans

Kinure (90.37%), MAC44 (90.16%), and RWR2245 (90.39%) feed formulas had significantly ($p < 0.05$) high dry matter content compared to the Broilers' mash (89.34%) (Table 2). The MAC44 (6.44%) and RWR2245 (6.42%) formulas exhibited the significantly highest ash content as compared to *Kinure* (6.22%) and Broilers' mash (5.43%) had the lowest ash content. The Broilers' mash (17.75%) recorded the significantly highest crude protein content compared to the common bean-based formulas while RWR2245 (12.27%) had the least. On the other hand, the RWR2245 formula (6.48%) had the remarkably high crude fibre content, followed by MAC44 (6.14%) and *Kinure* (5.93%) while the Broilers' mash (5.41%) recorded the lowest fibre content. Similarly, the RWR2245 formula (13.47%) had the significantly highest crude fat content, followed by MAC44 (12.65%) and *Kinure* (11.79%) but the Broilers' mash (4.64%) exhibited the lowest crude fat content. The Broilers' mash (56.12%) had the highest carbohydrate content, followed by *Kinure* (52.88%) while MAC44 (51.84%) and RWR2245 (51.74%) had considerably low carbohydrate content.

Table 2
Proximate composition (%DM) of broiler chicken feed formulated from different bean varieties

Feed formula	Dry Matter	Ash	Crude Protein	Crude Fibre	Crude Fat	Carbohydrates
Broilers' mash	89.34 ± 0.06 ^b	5.43 ± 0.01 ^c	17.75 ± 0.00 ^a	5.41 ± 0.03 ^d	4.64 ± 0.02 ^d	56.12 ± 0.03 ^a
<i>Kinure</i>	90.37 ± 0.09 ^a	6.22 ± 0.05 ^b	13.54 ± 0.01 ^b	5.93 ± 0.03 ^c	11.79 ± 0.00 ^c	52.88 ± 0.04 ^b
MAC44	90.16 ± 0.03 ^a	6.44 ± 0.03 ^a	13.09 ± 0.06 ^c	6.14 ± 0.02 ^b	12.65 ± 0.02 ^b	51.84 ± 0.08 ^c
RWR2245	90.39 ± 0.20 ^a	6.42 ± 0.01 ^a	12.27 ± 0.01 ^d	6.48 ± 0.01 ^a	13.47 ± 0.02 ^a	51.74 ± 0.24 ^c

Within rows, means followed by different letters differ significantly ($p < 0.05$).

The RWR2245 feed formula (58.16 mg/kg) exhibited significantly ($p < 0.05$) high iron concentration, followed by *Kinure* (50.17 mg/kg) and MAC44 (43.99 mg/kg) while the Broilers' mash (24.00 mg/kg) had the lowest concentration (Table 3). On the contrary, the broilers'

mash (50.00 mg/kg) recorded the highest zinc concentration, followed by RWR2245 (42.31 mg/kg) and *Kinure* (38.81 mg/kg) while MAC44 (36.93 mg/kg) had the lowest concentration.

Table 3
Iron and Zinc concentrations (Mg/Kg) in broiler chicken feed formulated different bean varieties

Feed formula	Fe	Zn
Broilers' mash	24.00 ± 0.00 ^d	50.00 ± 0.00 ^a
<i>Kinure</i>	50.17 ± 0.00 ^b	38.81 ± 0.01 ^c
MAC44	43.99 ± 0.05 ^c	36.93 ± 0.08 ^d
RWR2245	58.16 ± 0.00 ^a	42.31 ± 0.03 ^b

Within rows, means followed by different letters differ significantly ($p < 0.05$).

It was noted that the feed formula with *Kinure* (20.32 mg/g) recorded significantly ($p < 0.05$) highest concentration of phytic acid, followed by MAC44 (19.00 mg/g) and RWR2245 (14.75 mg/g) while the broilers mash (1.06 mg/g) had the least concentration (Fig. 1).

4.2 Growth characteristics, iron and zinc intake of broilers

Broiler chicken fed on the control diet (boilers' mash) recorded the significantly ($p < 0.05$) highest weight gain (89.32%), average daily gain (41.56 g) and feed intake (24.98 g) but also significantly lowest feed conversion ratio (0.60) in comparison to the feed formulations that had common beans (Table 4).

Table 4
Growth characteristics of broiler chicken fed on feed formulated with different bean varieties

Feed formula	Weight Gain (%)	Avg. Daily Gain (g)	Feed Intake (g)	FCR
Broilers' mash	89.32 ± 0.54 ^a	41.56 ± 0.09 ^a	24.98 ± 0.02 ^a	0.60 ± 0.00 ^d
<i>Kinure</i>	74.95 ± 1.16 ^b	10.19 ± 0.05 ^c	17.11 ± 0.05 ^b	1.68 ± 0.01 ^a
MAC44	67.14 ± 5.44 ^b	8.84 ± 0.07 ^d	13.56 ± 0.03 ^d	1.54 ± 0.01 ^b
RWR2245	78.49 ± 0.44 ^{ab}	11.79 ± 0.03 ^b	16.15 ± 0.06 ^c	1.37 ± 0.01 ^c

Within rows, means followed by different letters differ significantly ($p < 0.05$).

The Broilers' mash resulted in the significantly ($p < 0.05$) highest zinc absorption into the blood (0.50 mmol/g), followed by the RWR2245 formula (0.49 mmol/g), while the *Kinure* (0.47 mmol/g) and MAC44 (0.47 mmol/g) formulas showed the lowest and similar levels (Table 5). Similarly, the Broilers' mash recorded the significantly highest Hemoglobin Maintenance Efficiency (HME) at 85.98%, followed by the *Kinure* (18.48%) and RWR2245 (16.06%) formulas, with the MAC44 formula exhibiting the lowest HME (14.16%).

Table 5
Zinc absorbed into blood and Hemoglobin Maintenance Efficiency of broiler chicken fed on feed formulated with different bean varieties

Feed formula	Fractional Zn Absorbed (mmol/g)	HME (%)
Broilers	0.50 ± 0.01 ^a	85.98 ± 2.97 ^a
<i>Kinure</i>	0.47 ± 0.00 ^c	18.48 ± 0.45 ^b
MAC44	0.47 ± 0.01 ^c	14.16 ± 2.41 ^b
RWR2245	0.49 ± 0.01 ^b	16.06 ± 0.63 ^b

Within rows, means followed by different letters differ significantly ($p < 0.05$)

4.3 Association between nutritional composition and broiler chicken growth characteristics

Strong positive significant ($p < 0.05$) correlations were observed between Hemoglobin Maintenance Efficiency (HME) and Average Daily Gain (ADG) ($r = 0.994$), HME and Daily Feed Intake (DFI) ($r = 0.961$), as well as HME and weight gain ($r = 0.805$) (Table 6). Similarly, very strong positive significant correlations were found between Dry Matter (DM) and ash ($r = 0.875$), ash and crude fat ($r = 0.988$), Crude Protein (CP) and ADG ($r = 0.963$), CP and DFI ($r = 0.937$), crude fat and Phytic Acid (PA) ($r = 0.905$), PA and Feed Conversion Ratio (FCR) ($r = 0.996$), weight gain and ADG ($r = 0.801$), and ADG and DFI ($r = 0.965$). Conversely, very strong negative

significant correlations were observed between DFI and FCR ($r = -0.901$), HME and ash ($r = -0.976$), HME and crude fat (CF) ($r = -0.985$), HME and PA ($r = -0.952$), HME and FCR ($r = -0.953$), DM and CP ($r = -0.902$), CP and ash ($r = -0.981$), CP and crude fat ($r = -0.998$), CP and PA ($r = -0.882$), crude fat and ADG ($r = -0.976$), crude fat and DFI ($r = -0.953$), PA and ADG ($r = -0.975$), PA and DFI ($r = -0.919$), as well as ADG and FCR ($r = -0.973$). The correlation between iron concentration in the feed and overall body weight gain was not significant ($r = -0.529$).

Table 6
Coefficients of correlation of nutritional composition and broiler chicken growth characteristics

	HME	DM	Ash	CP	Fibre	CF	PA	Fe	Bwt. gain	ADG	DFI	FCR
HME	1.000	-0.894***	-0.976***	0.976***	-0.866***	-0.985***	-0.952***	-0.905***	0.805***	0.994***	0.961***	-0.9
DM		1.000	0.875****	-0.902***	0.808***	0.901***	0.857***	0.905***	-0.617*	-0.898***	-0.812***	0.8
Ash			1.000	-0.981***	0.912***	0.988***	0.900***	0.893***	-0.761***	-0.970***	-0.974***	0.8
CP				1.000	-0.949***	-0.998***	-0.882***	-0.955***	0.711***	0.963***	0.937***	-0.8
Fibre					1.000	0.933***	0.696*	0.924***	-0.563*	-0.835***	-0.841***	0.7
CF						1.000	0.905***	0.941***	-0.735***	-0.976***	-0.953***	0.9
PA							1.000	0.799***	-0.804***	-0.975***	-0.919***	0.9
Fe								1.000	-0.529 ^{ns}	-0.884***	-0.795***	0.8
Wt. gain									1.000	0.801***	0.847***	-0.7
ADG										1.000	0.965***	-0.9
DFI											1.000	-0.9
FCR												1.0

Key: HME= Hemoglobin Maintenance Efficiency; DM = Dry Matter; CP= Crude Protein; CF= Crude Fat; PA= Phytic Acid; ADG= Average Daily Gain; DFI= Daily Feed Intake; FCR= Feed Conversion Ratio; ns = Not Significant at $p < 0.05$; ***= Significant at $p < 0.001$; *= Significant at $p < 0.05$

5.0 Discussion

5.1 Nutritional composition of experimental diets

The nutritional composition findings revealed significant variations in nutrient composition between commercial broiler mash and common bean-based diets. The bean-based diets had approximately 1% increase in dry matter, ash and crude fibre and approximately 7% increase in crude fat compared to the commercial broilers' mash. In contrast, the commercial broiler's mash had a higher protein and carbohydrate content by approximately 4% and 3% respectively. The high ash content in the common bean feed formulas could be as a result of the biofortification process that increased mineral content [34]. The higher protein and carbohydrate content in the broilers mash could be attributed to broilers' mash being formulated to meet specific protein and carbohydrates needs of broilers. A higher crude protein content is generally desirable for broiler growth [24, 29].

Regarding mineral concentration, the broilers mash had a higher Zn content unlike the common bean-based diets, which had more Fe concentration. Among the three common bean formulated feeds, the feed containing RWR2245 biofortified variety had better nutritional composition whereas the indigenous variety *Kinure*, exhibited the highest phytic acid content. Phytic acid is anti-nutrient compound that impairs bioavailability of proteins, minerals and other compounds in the feed [16]. This observation implies that common-bean based diet utilized in the study may not be ideal to support optimal nutritional and growth needs of broilers due to their higher phytic acid concentration, which may have compromised the overall growth of birds utilized in the study.

5.2 Growth characteristics of broiler chicken

Results from the study indicate that commercial broilers' mash (control diet) outperformed the common bean-based diets in terms of weight gain, average daily weight gain, and feed intake. Additionally, these birds had a much lower feed conversion ratio (FCR) compared to the common bean-based diets, indicating that the feed was used more efficiently for growth. The lower feed intake of common bean-based formulas compared to commercial broilers' mash may suggest these feeds have lower palatability due to factors like taste, texture, or odor [22]. Additionally, weight gain differences can be attributed to the commercial broiler mash being formulated to meet the precise nutritional requirements of broilers for optimal growth, with a balanced ratio of macronutrients, vitamins, and minerals [22, 29].

This observation could indicate that common bean-based diets might have imbalances in the nutrients of commercial broiler mash. Furthermore, the lower feed conversion ratio in the control diet could be due to the high digestibility of commercial broiler mash, allowing efficient nutrient absorption and utilization [29]. Evidence from an intervention study by [30] suggests that pulse consumption leads to reduction in body weight with or without energy restriction. Results from a study by [14] that assessed iron bioavailability using a similar animal model indicated that broilers that received the white and red kidney bean diets had low feed intakes and slower growth rates. Consequently, the birds did not accumulate the Hb-Fe values that were achieved by the animals that received yellow bean diets. Common bean-based diets particularly those with darker seed coat contain anti-nutritional factors, such as high phytic acid and condensed tannins levels, which reduce nutrient digestibility [19]. Biofortified common beans utilized in this study are characterized by a darker seed coat and this attribute could explain the low feed intake, low weight gain and low average daily gain in birds fed on the common bean-based diets unlike those fed on the commercial broilers mash.

5.3 Iron and zinc bioavailability

Results in the present study demonstrated that while bean-based diets had higher iron concentrations than the control variety *Kinure* and the commercial broiler chicken mash, iron and zinc bioavailability findings was significantly lower in common bean-based diets as compared to the control. This observation could be due to the influence of the high phytate content in the common bean-based diets. In plant-based diets, phytate is a known inhibitor of iron absorption via chelating mechanisms that reduces their solubility and absorption in the intestines [31–33]. This study indicated that Hemoglobin Maintenance Efficiency (HME) which is a sensitive indicator of functional iron status [28], was highest in birds that fed the control diet (commercial broilers mash), which also contained supplemental iron and zinc. However, among the common bean-based diets, birds fed on the non-biofortified diet (*Kinure*) had a slightly higher HME than the RWR2245 and the MAC44, though the differences were not statistically significant. This observation suggests that even though dietary zinc and iron were in higher concentration in the common bean-based diets, the high phytate levels or probably poor retention mechanisms could have compromised their bioefficacy [16, 34].

Results from a previous similar study that used white beans indicated that white beans contained more bioavailable iron than red beans due to the lower polyphenolic content of the white beans [35]. The lower polyphenolic content translated to lower concentration antinutritional factors. Results from another study by [36] differed from those of the present study. Iron deficient birds receiving the high Fe common bean diet gained significantly more Hb-Fe than the birds on the diet containing standard beans. These discrepancies could be attributed to the differences in the varieties of biofortified common bean used and the feeding periods. The present study used RWR2245 and MAC44 with iron concentrations of 58.16 and 43.99 ug/g respectively over a six-week feeding period while the later study utilized Fe biofortified red-mottled common beans with an iron concentration of 71 µg/g over a four-week feeding period. Results from the present study are similar to those from a study in Rwanda that utilized biofortified cream seeded carioca beans and standard beans with Fe concentrations of 33.7 and 48.7µg, respectively, [9] concentrations that are quite closer to those of the present study. Moreover, the Rwanda study results indicated that hemoglobin concentrations were not significantly different at any time point when compared to the standard (control) group.

The strong negative significant correlation between phytic acid concentration and weight gain, average daily gain, crude protein, daily feed intake as well as hemoglobin maintenance efficiency confirms the role of anti-nutritional factors in compromising the bioavailability of iron and zinc and other nutrients [15, 16, 37]. It was also noted in the present study that there was a strong negative significant correlation between daily feed intake and iron content which could be as result of low consumption of common bean-based diets. The nutritional efficacy of biofortified common beans depends not only on the concentration of minerals in the raw state and the bioavailable fraction, which crosses the intestinal barriers and is available to the body, but also on the balance of the fraction of promotive and inhibitory compounds present in the final cooked product [38]. Although, [36] and [9] suggest that counteracting the Fe absorption inhibitory effect of polyphenols could be possible by increasing Fe concentration in beans, a vital aspect particularly for developing plant breeding strategies to alleviate dietary iron-deficiency anemia is crucial. To mitigate the effects of antinutrients, the most effective approach involves using food processing techniques proven to reduce their concentration or eliminate them entirely [37, 39].

5.4 Relevance of bioavailability assays to human nutrition

The results of this study indicate that while the biofortified common bean variety RWR2245 provided a higher absorbable zinc (Zn) fraction compared to MAC44, both varieties exhibited significantly lower iron bioavailability compared to non-biofortified variety, *Kinure*. However, it is important to note that the presence of phytic acid negatively impacted mineral bioavailability in the broiler chicken model. As discussed by [31, 43], various antinutritional factors in biofortified crops, including common beans, are known to interfere with Fe and Zn bioavailability. If the findings from this *Gallus gallus* model were extrapolated to human nutrition, a higher fractional Zn absorption would be expected from RWR2245 and a lower percentage of iron bioavailability from the two biofortified common beans varieties. This observation highlights the need to further evaluate and potentially modify the phytic acid profile in biofortified common beans. Furthermore, common household practices, such as soaking and cooking, which significantly reduce antinutrient levels in common beans before consumption could be explored to overcome these bioavailability barriers in target populations [17, 40–42].

6.0 Conclusions and recommendations

The findings from this *in vivo* feeding trial using the *Gallus gallus* model provide foundational insights into the potential of selected biofortified common beans varieties, such as RWR2245 variety in alleviating hidden hunger, particularly zinc deficiency among vulnerable populations in Burundi and similar contexts. Notably, this is the first study to conduct bioavailability assays on these new biofortified common bean varieties grown and consumed in Burundi. The results confirm that high grain mineral does not automatically translate to high blood-mineral concentration and absorption in the broiler chicken nor in human blood since there other dietary and physiological factors to be considered. However, by identifying RWR2245 as the most favorable variety, these results establish a practical and feasible baseline for advancing to pilot human studies and for designing targeted nutritional interventions within Burundi and similar regions. It is recommended that future human clinical trials to evaluating the bioavailability of iron and zinc utilize validated biomarkers, including serum ferritin and fractional absorption, to accurately assess mineral uptake and bioavailability and fully ascertain the impact of these biofortified common beans in Burundi and other targeted populations.

Abbreviations

AACC
American Association of Cereal Chemists
ADG
Average Daily Gain
AMVA
American Veterinary Medical Association
ANOVA
Analysis of variance
AOAC
Association of Analytical Chemists
Avg.
Average
Bwt.
Body weight
CF
Crude Fat
CP
Crude Protein
DFI
Dietary Feed Intake
DL
methionine
DM
Dry Matter
FCR
Feed Conversion Ratio
Fe
Iron
FI
Feed intake
HB
Fe- Index for iron absorption
Hb
Hemoglobin
HME
Hemoglobin Maintenance Efficiency
HSD
Honestly Significant Difference
ILRI
International Livestock Research Institute
ISABU
Institut des Sciences Agronomiques du Burundi
KALRO
Kenya Agricultural and Livestock Research Organization
KEMRI
Kenya Medical Research Institute
NACOSTI

National Commission for Science, Technology and Innovation
PABRA
Pan African Bean Research Alliance
PA
Phytic acid
PICS
Purdue Improved Crop Storage
P
phytate
SAS
Statistical Analysis System
TCA
Trichloroacetic acid
Zn
Zinc

Declarations

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MWM: Conceptualization, Formal Analysis, Methodology, Resources, Investigation, Data Curation, Writing - Original Draft, LWM: Supervision, Conceptualisation, Methodology, Resources, Writing - Review and Editing, RF: Supervision, Conceptualisation, Methodology, Resources, Writing-Review and Editing, NWW: Methodology, Data Curation, Writing - Review and Editing, CNM: Conceptualisation, Resources, Investigation, LKW: Data Curation, Writing - Review and Editing, PN: Resources, Investigation, SMM: Supervision, Conceptualisation, Methodology, Resources, Writing - Review and Editing.

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6.5.1 Ethics approval

This study was conducted in strict accordance with the recommendation in the guide for the care and use of Laboratory Animals of the National Institute of Health, the International Livestock Research Institute (ILRI) and to the American Veterinary Medical Association (AMVA). The research was reviewed and approved by the Egerton University Research and Ethics Committee (EU/RE/DVC/009 Approval No. EUREC/APP/149/2021) and the National Commission for Science, Technology and Innovation, Kenya under License No: NACOSTI/P/21/14502.

6.5.2 Consent for publication

Not applicable

6.5.3 Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

6.5.4 Competing interests

The authors declare that they have no competing interests.

References

1. FAO, IFAD, UNICEF, WFP, WHO. The State of Food Security and Nutrition in the World 2024. FAO; IFAD & UNICEF; WFP; WHO. ; (2024). Available from: <https://openknowledge.fao.org/handle/20.500.14283/cd1254en>
2. Fungo, R. et al. Biofortified Beans: An Agricultural Investment for Nutrition, Income and Food Security in Burundi. SDC Burundi Policy Brief. Kampala; (2020). Mar Available from: <https://hdl.handle.net/10568/109119>
3. Stevens, G. A. et al. Micronutrient deficiencies among preschool-aged children and women of reproductive age worldwide: a pooled analysis of individual-level data from population-representative surveys. *Lancet Glob Health*. **10** (11), e1590–e1599. [https://doi.org/10.1016/S2214-109X\(22\)00367-9](https://doi.org/10.1016/S2214-109X(22)00367-9) (2022).
4. Benson, A. E. et al. Management of iron deficiency in children, adults, and pregnant individuals: evidence-based and expert consensus recommendations. *Lancet Haematol*. **12** (5), e376–e388. [https://doi.org/10.1016/S2352-3026\(25\)00038-9](https://doi.org/10.1016/S2352-3026(25)00038-9) (2025).
5. Bouis, H., Low, J., Mcewan, M., Tanumihardjo, S. & Biofortification Evidence and lessons learned linking agriculture and nutrition [Internet]. (2013). Available from:
6. Bechoff, A. Micronutrient (provitamin A and iron/zinc) retention in biofortified crops. *Afr. J. Food Agric. Nutr. Dev*. **17** (02), 11893–11904. <https://doi.org/10.18697/ajfand.78.HarvestPlus04> (2017).
7. Buruchara, R. P. A. B. R. A., Narrative Cumulative & (PABRA). Report 2015–2018. Pan-Africa Bean Research Alliance. (2019). Mar <https://cgspace.cgiar.org/server/api/core/bitstreams/ba11396b-c975-4aee-92ab-3518835f19d8/content>
8. Haas, J. D. et al. Consuming Iron Biofortified Beans Increases Iron Status in Rwandan Women after 128 Days in a Randomized Controlled Feeding Trial. *J. Nutr*. **146** (8), 1586–1592. <https://doi.org/10.3945/jn.115.224741> (2016).
9. Tako, E., Bar, H. & Glahn, R. The Combined Application of the Caco-2 Cell Bioassay Coupled with In Vivo (Gallus gallus) Feeding Trial Represents an Effective Approach to Predicting Fe Bioavailability in Humans. *Nutrients* **8** (11), 732. <https://doi.org/10.3390/nu8110732> (2016).
10. Katungi, E. et al. Impacts of improved bean technology adoption among smallholder farmers in Burundi SDC FLAGSHIP PROJECT END LINE REPORT 2020 [Internet]. 2020 [cited 2022 Sep 6]. Available from: <https://hdl.handle.net/10568/109119>
11. Connorton, J. M., Balk, J. & Rodríguez-Celma, J. Iron homeostasis in plants – a brief overview. *Metallomics* **9** (7), 813–823. <https://doi.org/10.1039/c7mt00136c> (2017).

12. Blair, M. W. & Izquierdo, P. Use of the advanced backcross-QTL method to transfer seed mineral accumulation nutrition traits from wild to Andean cultivated common beans. *Theor. Appl. Genet.* **125** (5), 1015–1031. <https://doi.org/10.1007/s00122-012-1891-x> (2012).
13. Katungi, E. & Farrow Geagrofia, A. Common bean in Eastern and Southern Africa: a situation and outlook analysis [Internet]. (2009). Available from: <https://www.researchgate.net/publication/228601612>
14. Wiesinger, J. A. et al. An In Vivo (*Gallus gallus*) Feeding Trial Demonstrating the Enhanced Iron Bioavailability Properties of the Fast Cooking Manteca Yellow Bean (*Phaseolus vulgaris* L.). *Nutrients* **11** (8), 1768. <https://doi.org/10.3390/nu11081768> (2019).
15. Hummel, M. et al. Iron, zinc and phytic acid retention of biofortified, low phytic acid, and conventional bean varieties when preparing common household recipes. *Nutrients* **12** (3). <https://doi.org/10.3390/nu12030658> (2020).
16. Tako, E., Blair, M. W. & Glahn, R. P. Biofortified red mottled beans (*Phaseolus vulgaris* L.) in a maize and bean diet provide more bioavailable iron than standard red mottled beans: Studies in poultry (*Gallus gallus*) and an in vitro digestion/Caco 2 model. *FASEB J.* **26** (S1). <https://doi.org/10.1186/1475-2891-10-113> (2012).
17. Asif, M. et al. Reduction of Phytic Acid and Enhancement of Bioavailable Micronutrients in Common Beans (*Phaseolus vulgaris* L.) in Changing Climatic Scenario. In: Developing Climate Resilient Grain and Forage Legumes. Singapore: Springer Nature Singapore; 59–76. https://doi.org/10.1007/978-981-16-9848-4_3 (2022).
18. Miller, L. V., Krebs, N. F. & Hambidge, K. M. Mathematical model of zinc absorption: effects of dietary calcium, protein and iron on zinc absorption. *Br. J. Nutr.* **109** (4), 695–700. <https://doi.org/10.1017/S000711451200195X> (2013).
19. Petry, N., Boy, E., Wirth, J., Hurrell, R. & Review The Potential of the Common Bean (*Phaseolus vulgaris*) as a Vehicle for Iron Biofortification. *Nutrients* **7** (2), 1144–1173. <https://doi.org/10.3390/nu7021144> (2015).
20. Muroki, M. W., Waswa, L. M., Fungo, R., Kabwama, A. & Mahungu, S. M. Cooking properties and nutrient retention of biofortified common bean (*Phaseolus vulgaris*) varieties: The case of Burundi. *Front. Sustain. Food Syst.* **7**. <https://doi.org/10.3389/fsufs.2023.1062978> (2023).
21. Biama, P. K., Faraj, A. K., Mutungi, C. M., Osuga, I. N. & Kuruma, R. W. Nutritional and Technological Characteristics of New Cowpea (*Vigna unguiculata*) Lines and Varieties Grown in Eastern Kenya. *Food Nutr. Sci.* **11** (05), 416–430. <https://doi.org/10.4236/fns.2020.115030> (2020).
22. Pandey, A. K., Kumar, P. & Saxena, M. J. Feed Additives in Animal Health. In: (eds Gupta, C., Ramesh, S. & Lall, A.) *Rajiv, Nutraceuticals in Veterinary Medicine*. Cham: Springer International Publishing; 345–362. https://doi.org/10.1007/978-3-030-04624-8_23 (2019).
23. Li, P., He, W. & Wu, G. Composition of Amino Acids in Foodstuffs for Humans and Animals. In: (ed Guoyao, W.) *Amino acids in nutrition and health* Advances in Experimental Medicine and Biology. TX, USA: Springer, Cham; 189–210. https://doi.org/10.1007/978-3-030-74180-8_11 (2021).
24. Muremera, C., Ambula, M. & King'ori, A. Performance of improved indigenous grower chicken in Kenya fed enzyme-treated Moringa (*M. oleifera*) leaf meal-based diets. *Int. J. Veterinary Sci. Anim. Husb.* **7** (3), 48–52. <https://doi.org/10.22271/veterinary.2022.v7.i3a.423> (2022).
25. AOAC. *Official Methods of Analysis (Association of Official Analytical Chemists) International* 18th edn (AOAC International, 2007).
26. Horwitz, W. & Latimer, G. W. *Association of official analytical chemists* (Gaithersburg, 2000).
27. Makkar, H. P. S., Siddhuraju, P. & Becker, K. Phorbol Esters. In: *Plant Secondary Metabolites*. Totowa, NJ: Humana; 101–105. (2007).
28. Tako, E., Rutzke, M. A. & Glahn, R. P. Using the domestic chicken (*Gallus gallus*) as an in vivo model for iron bioavailability. *Poult. Sci.* **89** (3), 514–521. <https://doi.org/10.3382/ps.2009-00326> (2010).
29. Kakagida, A. Y., Isa, M. M., Anka, A. B., Mohammed, A. A. & Shinkafi, M. S. Carcass Evaluation in Broiler Feed Diets Containing Graded Levels of Locust Beans (*Parkia biglobosa*) Seed Meal in North Western Zone of Sokoto, Nigeria. *Asian J. Biochem. Genet. Mol. Biology.* 1–10. <https://doi.org/10.9734/ajbgmb/2021/v7i130163> (2021).
30. Clark, S. & Duncan, A. M. The role of pulses in satiety, food intake and body weight management. *J. Funct. Foods.* **38**, 612–623. <https://doi.org/10.1016/j.jff.2017.03.044> (2017).
31. Chávez-Mendoza, C. & Sánchez, E. Bioactive Compounds from Mexican Varieties of the Common Bean (*Phaseolus vulgaris*): Implications for Health. *Molecules* **22** (8), 1360. <https://doi.org/10.3390/molecules22081360> (2017).
32. Chen, P. X. et al. Characterization of free, conjugated and bound phenolics and lipophilic antioxidants in regular- and non-darkening cranberry beans (*Phaseolus vulgaris* L.). *Food Chem.* **185**, 298–308. <https://doi.org/10.1016/j.foodchem.2015.03.100> (2015).
33. Petry, N., Boy, E., Wirth, J. P., Hurrell, R. F. & Review The potential of the common bean (*Phaseolus vulgaris*) as a vehicle for iron biofortification. *Nutrients* **7** (2), 1144–1173. <https://doi.org/10.3390/nu7021144> (2015).
34. Hummel, M. et al. Iron, zinc and phytic acid retention of biofortified, low phytic acid, and conventional bean varieties when preparing common household recipes. *Nutrients* **12** (3). <https://doi.org/10.3390/nu12030658> (2020).
35. Tako, E. & Glahn, R. P. White Beans Provide More Bioavailable Iron than Red Beans: Studies in Poultry (*Gallus gallus*) and an in vitro Digestion/Caco-2 Model. *Int. J. Vitam. Nutr. Res.* **80** (6), 416–429. <http://doi.org/10.1024/0300-9831/a000028> (2010).

36. Tako, E., Blair, M. W. & Glahn, R. P. Biofortified red mottled beans (*Phaseolus vulgaris* L.) in a maize and bean diet provide more bioavailable iron than standard red mottled beans: Studies in poultry (*Gallus gallus*) and an in vitro digestion/Caco-2 model. *Nutr. J.* **10** (1), 113. <https://doi.org/10.1186/1475-2891-10-113> (2011).
37. de Oliveira, A. P. et al. Effect of cooking on the bioaccessibility of essential elements in different varieties of beans (*Phaseolus vulgaris* L.). *J. Food Compos. Anal.* **67**, 135–140. <https://doi.org/10.1016/j.jfca.2018.01.012> (2018).
38. Coelho, R. C., Barsotti, R. C. F., Maltez, H. F., Lopes Júnior, C. A. & de Barbosa, H. Expanding information on the bioaccessibility and bioavailability of iron and zinc in biofortified cowpea seeds. *Food Chem.* **347**, 129027. <https://doi.org/10.1016/j.foodchem.2021.129027> (2021).
39. Faizal, F. A., Ahmad, N. H., Yaacob, J. S., Abdul Halim Lim, S. & Abd Rahim, M. H. Food processing to reduce antinutrients in plant-based foods. *Int. Food Res. J.* **30** (1), 25–45. <https://doi.org/10.47836/ifrj.30.1.02> (2023).
40. Mugabo, E., Afoakwa, E. O., Annor, G. & Rwubatsa, B. Effect of pretreatments and processing conditions on anti-nutritional factors in climbing bean flours. *Int. J. Food Stud.* **6** (1), 34–43. <https://doi.org/10.7455/ijfs/6.1.2017.a4> (2017).
41. Ojo, M. A. Tannins in Foods: Nutritional Implications and Processing Effects of Hydrothermal Techniques on Underutilized Hard-to-Cook Legume Seeds-A Review. *Prev. Nutr. Food Sci.* **27** (1), 14–19. <https://doi.org/10.3746/pnf.2022.27.1.14> (2022).
42. Fabbri, A. D. T. & Crosby, G. A. A review of the impact of preparation and cooking on the nutritional quality of vegetables and legumes. *Int. J. Gastron Food Sci.* **3**, 2–11. <https://doi.org/10.1016/j.ijgfs.2015.11.001> (2016).
43. Carbas, B. et al. Nutrients, Antinutrients, Phenolic Composition, and Antioxidant Activity of Common Bean Cultivars and their Potential for Food Applications. *Antioxidants* **9** (2), 186. <https://doi.org/10.3390/antiox9020186> (2020).

Figures

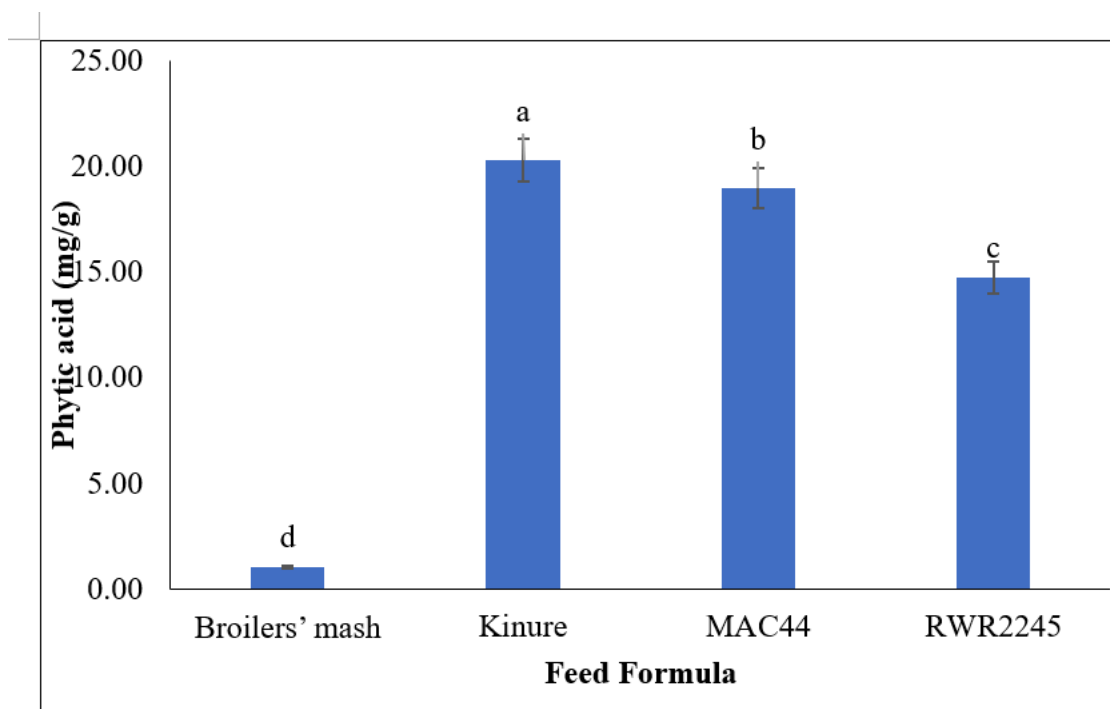


Figure 1

Phytic acid concentration (mg/g) in broiler chicken feed formulated using different dry common bean varieties (Kinure, MAC44, RWR2245)