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Egg shelf life, welfare, and physiological responses in jumbo quail hens fed a diet containing raw or heat-treated cassava tuber meal

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Abstract

The study assessed the effect of total replacement of maize with raw or heat-treated cassava tuber meal (CTM) on egg shelf life, welfare indicators, and physiological and carcass traits of laying jumbo quail hens. Two hundred and forty, six-week-old quail hens (191 ± 16.60 g initial body weight) were reared in 60 cages (four hens/cage) to produce 12 replicates per dietary treatment. The diets were a standard layer diet (CON) and CON where maize was completely replaced with raw (CTMR), autoclaved (CTMA), boiled (CTMB), and oven-dried CTM (CTMO). On day 5 of storage, birds on CON produced eggs with the highest breaking strength and the lowest was from CTMR group. Similarly, birds fed CTMR had the lowest albumen height on day 5. On day 10, eggs from birds on CTMA had the lowest albumen pH and the highest was from CON. There were no dietary effects on egg weight, yolk height, haemato-biochemical parameters, and visceral morphometry of the hens. A Kruskal–Wallis H test revealed no treatment effects on gait and feather scores but showed an effect on latency-to-lie test. Birds fed CON had larger carcass weights than those fed CTMR and CTMB. Diet CON caused lower 24-h breast meat pH than CTMB. Although hens fed with the maize-control diet showed optimal egg quality and carcass performance, autoclaving and oven-drying treatments were more effective than the raw or boiled CTM treatments. The treatments did not compromise welfare, blood parameters, or visceral morphometry of the hens.

Keywords: egg quality, energy sources, layers, blood parameters, welfare

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Introduction

Quail farming is becoming increasingly popular due to attributes such as fast growth rates, less space requirement, shorter production cycles, and high laying abilities (Jeke *et al*., 2018; Batool *et al*., 2023). Although chicken eggs have the highest per capita consumption, quail eggs are recognised as a healthy delicacy in many African and Asian countries, making them a valuable commodity in niche markets (Akinwumi *et al*., 2019). Nutritionally, quail eggs are rich in protein, vitamins (A, B complex, C, and D), lipids and minerals (Tunsaringkarn *et al*., 2013). Moreover, the eggs' protein profile has high concentrations of lysine, leucine, and valine (Bayomy *et al*., 2017; Ali & Abd El-Aziz, 2019), which are essential amino acids required for normal physiological functions. The eggs also contain bioactive substances such as ovomucoid, cystatin, and lysozymes, which have beneficial health effects (Ali & Abd El-Aziz, 2019). Additionally, the bioactive compounds in quail eggs can mitigate ailments or diseases (Jeke *et al*., 2018). Augustine *et al*. (2018) reported that quail eggs are used in the cosmetic

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industry to produce various (skin or hair) products that are more potent than products made from chicken eggs. Thus, large-scale layer quail production can be a viable strategy to reduce the high demand for chicken eggs. However, upscaling of high-input production systems is currently limited by the availability of quality feeds as well as high feeding costs, which present sustainability challenges (Mnisi *et al*., 2021).

The South African poultry industry is currently facing several socio-economic and environmental sustainability challenges that negatively affect industrial operations and long-term viability (Kleyn & Ciacciariello, 2021). From an economic perspective, the use of expensive feed ingredients such as maize in animal feeds, which is also a staple food for many Africans, increases feeding costs. This is because the high competition between humans and animals for maize increases its selling price. Furthermore, high maize yield is mainly dependent on the use of arable land, high rainfalls, good soil fertility, and application of chemical fertilizers (Cullis *et al*., 2019), hence South Africa relies on foreign countries to satisfy local demand for maize.

Although maize is a primary energy source in poultry diets (Ravindran, 2013; Thirumalaisamy *et al*., 2016), its prices remain volatile in the markets, making it an unsustainable ingredient for use in high-input layer quail production (Mnisi *et al*., 2021). Alternatively, cassava (*Manihot esculenta Crantz*), which is a root vegetable crop that is locally available, highly adaptable, and easy to cultivate, can be utilised as an alternative source of energy in the formulation of layer quail diets (Kanyinji & Moonga, 2014; Mnisi *et al*., 2023). Cassava tuber meal (CTM) has a high metabolizable energy value (16.2 MJ/kg) and is less prone to market price fluctuations, making it a more stable energy source for use in layer quail diets (Kanyinji & Moonga, 2014). In South Africa, the per capita consumption of cassava tubers is low, indicating that its use in layer quail feeds would not pose competition with humans. However, cassava contains secondary metabolites, such as hydrogen cyanide (HCN), non-starch polysaccharides, and phytates (Ogunsipe *et al*., 2022), which can reduce nutrient digestibility and production in layer quail (Apata & Babalola, 2012). For example, the presence of cyanogenic glycosides in cassava produces hydrocyanic acid, which is harmful to poultry when consumed in high concentrations (Omosuli *et al*., 2017; Yulvianti & Zidorn, 2021). Generally, this compound is non-toxic, but produces toxic HCN when glycosides react with linamarase upon tissue damage (McMahon *et al*., 2022). Cyanide reduces the bird's ability to absorb iron and zinc (Bayata, 2019). Nonetheless, different varieties of cassava have varying concentrations of HCN, with the bitter varieties having a higher HCN level than sweet varieties (Panghal *et al*., 2019). Bitter or unprocessed varieties can lead to respiratory problems, toxicity, paralysis, and mortality in poultry (Ramteke *et al*., 2019). Consequently, various thermal or heat treatment methods such as autoclaving, boiling, or oven-drying can be adopted to negate the antinutritional effects of HCN and maintain the feeding value and safety of cassava products.

Autoclaving reduces the concentration of antinutrients resulting in increased nutrient utilisation (Udensi *et al*., 2005). Abbas & Ahmad (2018) observed an improvement in nutrient bioavailability, texture, and palatability of autoclaved CTM, indicating a potential reduction in secondary metabolites. Additionally, boiling improves the utility of cassava, by eliminating nearly 90% of free cyanide and 55% of bound cyanide within 15 and 25 min, respectively (Ngiki *et al*., 2014). Importantly, these methods can preserve the relative stability of water-soluble vitamins (vitamin C and B-complex) and therefore enhance nutrient utilisation. Andama & Oloya (2017) showed that rapid air-drying reduced the total cyanide content of peeled cassava root chips by 10–30%. No studies have attempted to enhance the energy value of CTM as a substitute for maize in diets of laying jumbo quail. Thus, we investigated the effects of total substitution of maize with raw or heat-treated CTM on egg shelf-life indicators, welfare parameters, haemato-biochemistry, visceral morphometry, and carcass characteristic of laying jumbo quail hens.

Materials and Methods

All study procedures (feeding, handling, and slaughter protocols) were reviewed and approved (NWU-00814-22-A5) by the Ethics Committee for Animal Production studies in the North-West University (Mafikeng, South Africa). The study was carried out during winter (-2 and 22 °C) of 2023 in the University farm (25°40.459′ S, 26°10.56′ E; Mafikeng, South Africa). Fresh cassava tubers were harvested from a small-scale farmer in Masibekela village (25.8692° S, 31.8136° E) in Mpumalanga, South Africa. The tubers were transported by road to Molelwane farm where they were peeled and sliced using hand knives, sun-dried to constant weight and divided into four equal sample groups. The first group was not subjected to heat processing (remained raw), the second group was boiled (86–100 °C) for 40 min using tap water on a conventional stove (Ismaila *et al*., 2018), the third group was autoclaved (3870E Heidolph, Tuttnaeur model, New York, USA) at a temperature of 121 °C (7 psi) for 15 min, and the last group was oven-dried at 55 °C for 3 h (Dahal & Tamang, 2021). After processing, the samples were dried at room temperature (22–25 °C) until a constant weight was achieved and thereafter ground using a cutting mill fitted with a 2-mm sieve (Retsch Cutting Mill SM 100, Germany) in preparation for diet formulation and analysis.

The National Research Council (NRC, 1994) nutritional guidelines were followed to formulate experimental diets (Table 1) in accordance with the nutrient requirements of layer *Coturnix* quail. The diets were: a conventional layer mash diet without CTM (CON) and a conventional layer mash diet in which maize was completely replaced with raw (CTMR), boiled (CTMB), autoclaved (CTMA), and oven-dried CTM (CTMO).

Table 1 Composition of ingredients (g/kg *as fed* basis) for the formulated diets fed to layer jumbo quail

	[1]Diets	
Ingredients	**CON**	**CTMd**
Cassava tuber meal	0.000	437.3
Yellow maize (8,5%)	437.3	0.000
Soya oilcake (44%)	350.6	350.6
Sunflower oilcake (38%)	68.4	68.4
[2]Premix	3.00	3.00
DL-Methionine	1.40	1.40
Fine salt	1.60	1.60
Lysine-HCL	1.00	1.00
Monodicalcium phosphate	56.1	56.1
Sodium bicarbonate	15.4	15.4
Soya oil	65.1	65.1
Zinc Bacitracin	0.100	0.100
Total	1000	1000

[1]Diets: CON = a conventional layer mash diet without cassava tuber meal; CTMd = a conventional layer mash diet where maize is completely replaced with cassava tuber meal.
[2]Premix: vitamin A (11,000 IU), vitamin B_6 (5.1 mg), vitamin D_3 (2,500 IU), vitamin E (25 IU), vitamin B_1 (2.5 mg), vitamin B_2 (4.5 mg), vitamin K3 (2.0 mg), pantothenic acid (10 mg), folic acid (0.7 mg), biotin (0.12 g), copper sulphate (8.0 mg), niacin (30 mg), potassium iodide (0.34 mg), ferrous sulphate (80 mg), zinc sulphate (79 mg), magnesium sulphate (100 mg), and sodium selenite (0.25 mg)
All raw materials (except for cassava) were purchased from Simplegrow Agric Services and Nutroteq (Gauteng, South Africa).

The CTM samples and diets were then analysed (Table 2) for dry matter, organic matter, ether extract, crude protein, and crude fibre following the protocols from the Association Official Analytical Chemists (AOAC, 2005). Crude fibre, acid detergent fibre (ADF) and neutral detergent fibre (NDF) were assayed by means of an ANKOM^{DELTA} Fibre Analyser (ANKOM Technology, NY, USA) following the detergent methods by Van Soest *et al.* (1991). Ether extract (EE) was assayed using ethyl ether by means of an ANKOM XT-20 Extractor (ANKOM Technology, NY, USA). Gross energy (GE) was analysed at NutriLab of the University of Pretoria (Gauteng, South Africa); amino acids (AA) were quantified by means of a Waters Acquity Ultra Performance Liquid Chromatograph (AccQ-Tag Ultra; Waters Corporation, Milford, USA) at Stellenbosch University (Western Cape, South Africa).

A modified method for determining the free and potential cyanide was used to measure the HCN content of the CTM samples (Dahal & Tamang, 2021). This method uses endogenous linamarase enzyme to break down linamarin and release free cyanide, which is subsequently quantified using a colorimetric reaction with picrate solution. The absorbance readings were then taken at 535 nm and the concentration of HCN was determined as:

$$HCN\ content\ (mg/kg)\ = \frac{(Y \times 50 \times 1000)}{(5 \times sample\ weight)} \qquad (1)$$

Where, Y = 0.0304x + 0.0054; (R^2 = 0.969) and x = absorbance

Table 2 Gross energy, proximate constituents (g/kg DM, unless stated otherwise) and essential amino acids (g /100 g DM) of dietary treatments

	CTM Samples[1]				Diets[2]				
Components	**RCAS**	**ACAS**	**BCAS**	**OCAS**	**CON**	**CTMR**	**CTMA**	**CTMB**	**CTMO**
Gross energy (kcal/kg)	3716	3685	3685	3698	4072	4079	4007	4151	3955
Dry matter	425	535	578	652	911	908	906.8	901	912
Crude protein	65.1	48.2	60.7	57.8	225	236	230	238	236
Organic matter	401	509	549	624	822	812	807	805	816
Neutral detergent fibre	236	150	152	165	115	92.4	107	104	106
Acid detergent fibre	107	75.1	71.1	63.3	54.0	55.9	67.7	62.5	66.3
Crude fat	85.0	89.8	86.6	52.9	284	227	252	265	266
Crude fibre	150	108	94.4	122	103	94.5	118	117	116
Histidine	0.07	0.06	0.08	0.08	0.70	0.65	0.63	0.38	0.55
Arginine	0.17	0.11	0.15	0.20	1.69	1.74	1.60	1.35	1.71
Threonine	0.08	0.08	0.08	0.08	0.94	0.94	0.92	0.73	0.89
Lysine	0.14	0.07	0.13	0.10	1.38	0.93	1.30	2.11	1.16
Methionine	0.08	0.0	0.07	0.07	0.44	0.51	0.43	0.52	0.58
Valine	0.12	0.11	0.12	0.11	1.17	1.06	1.02	0.96	1.06
Isoleucine	0.09	0.09	0.10	0.10	1.03	0.94	0.93	0.89	0.96
[3]HCN (mg/kg)	6.24	3.62	4.98	3.16	24.7	34.7	25.5	45.8	28.1

[1]Samples: RCAS = raw cassava tuber meal; ACAS = autoclaved cassava tuber meal; BCAS = boiled cassava tuber meal; OCAS = oven-dried cassava tuber meal
[1]Diets: CON = a conventional layer mash diet without cassava tuber meal; CTMR = a conventional layer mash diet where maize is completely replaced with raw cassava tuber meal; CTMA = a conventional layer mash diet where maize is completely replaced with autoclaved cassava tuber meal; CTMB = a conventional layer mash diet where maize is completely replaced with boiled cassava tuber meal; CTMO = a conventional layer mash diet where maize is completely replaced with oven-dried cassava tuber meal
[3]HCN: Hydrogen cyanide

Two hundred and forty, five-week-old Jumbo quail hens were bought from Quailish Farm in Pretoria (Gauteng, South Africa). The hens were crated and driven to the University farm. On arrival, the hens were provided electrolytes and vitamins as stress alleviators via drinking water for the first 3 days and thereafter accustomed to the cages and experimental diets for a week before experimental measurements were taken. At 6 w, the hens were weighed (191 ± 16.6 g body weight) and randomly dispersed into 60 cages (experimental units) to which the experimental diets were assigned, producing 12 replicate cages per diet. Each cage (34 cm length × 27.5 cm width × 30 cm height) carried four hens and polythene sheets were used as bedding. Clean water and experimental diets were provided *ad libitum* using drinkers (1 L) and feeders (500 g) on a daily basis. Regular washing and refilling of drinkers and feeders were done to avoid contamination. The average humidity (60%) and temperature (30 °C) of the quail house were monitored by means of a hygrometer. The temperature was maintained using electric bulbs and heaters, with natural daylight being the source of light.

A total of 120 eggs per dietary treatment were collected on day 42 of the trial and stored at room temperature (22–25 °C) and evaluated on days 1, 5 and 10 of storage. Measurements of egg weight (EWT) were made using a digital scale (HM-200, A & D Co., Ltd., Tokyo, Japan). Egg breaking strength was measured using a digital egg tester (DET-6500, Nabel Co., Ltd., Kyoto, Japan). After breaking the eggs on a flat surface, albumen height and yolk height were measured using an Electronic Digital Calliper. Albumen pH was determined by separating the albumen and yolk of an egg and measured using a pH meter that was fitted with a penetrating electrode (Eutech pH25, Eutech Instruments, Spain). The meter was cleaned and wiped for every measurement.

Two hens in a cage were randomly selected to conduct a latency-to-lie test (LTL) on day 52 of the trial. The hens were each placed in a container filled with water (32 °C) to a 3-cm level (Berg & Sanotra, 2003). The time (s) taken by the bird to lie down and touch the water was recorded. If the bird remained upright for 10 min, then the test was terminated and the legs were declared healthy. Feather score was assessed using two hens in a cage as described by Gyles *et al*. (1962). A three-point arbitrary scale was used to score each bird based on how much skin showed through pressed feathers; the scale was: 1) represented complete feather coverage; 2) represented a relatively small amount of skin visibility; and 3) represented poor feather coverage. Thereafter, each hen was gently stroked by hand from anterior to posterior of the keel to determine gait score. A five-point scale described by Kestin *et al*. (1992) ranging from 0 (normal movement) to 5 (walking difficulty) was used.

At 14 w of age, all the hens were stunned and sacrificed at a nearby poultry abattoir. Blood drippings from the jugular vein of 24 hens per treatment were harvested using sterilized individual containers and hastily collected (4 mL) using syringes into sterilized tubes for analysis of haemato-biochemical indices. Whole blood analysis for platelets, red blood cells, lymphocytes, white blood cells, monocytes, and neutrophils was performed within 24 h of blood collection using an Automated LaserCyte Haematology Analyzer (IDEXX Laboratories S.A. Pty, Ltd, Midrand, South Africa). For serum biochemistry, clotted blood samples were centrifuged (Hermle Labortechnik GmbH, Wehingen, Germany) at 1000 × *g* for 15 min (Washington & Hoosier, 2012). The serum was thereafter analysed for total protein, glucose, total bilirubin, albumin, cholesterol, amylase, alkaline phosphatase (ALP), symmetric dimethylarginine (SDMA), and alanine transaminase (ALT) using an automated Vet Test Chemistry Analyser (IDEXX Laboratories S.A. PTY, Midrand, South Africa).

All carcasses were used for post-mortem measurements. Hot and cold carcass weights (HCW and CCW) were measured immediately and 24 h (stored in a cold room) after slaughter, respectively. The carcasses were then dissected into different portions (breast, wing, thigh, drumstick, and back length) and measured. Keel bone size was also determined by measuring its length and width using an Electronic Digital Calliper (150 mm). A digital scale (HM-200, A & D Co., Ltd, Tokyo, Japan) was used to measure the weights of the proventriculus, cleaned ventriculus, liver, and small and large intestines. The length of the small intestine as well as the large intestines were measured using a tape measure (cm).

The pH of breast meat from all carcasses was measured using a pH meter with a spear-type electrode (Corning Glass Works, Medfield, MA) immediately and 24 h after slaughter. Standard pH solutions were used to recalibrate the meter after measuring each replicate cage. A colorimeter (45/0 BYK-Gardener GmbH, Germany) was used to record breast meat *L** (lightness), *a** (redness), and *b** (yellowness) both immediately and 24 h post-slaughter (Thema *et al*., 2022).

Data for egg shelf life, haemato-biochemistry, visceral morphometry, and carcass traits were analysed using the procedure of the general linear model of SAS 9.4 (2013), where diet was the only considered variable. The probability of difference option in the least square means (LSMeans) statement of SAS was used to separate the LSMeans, and significance was declared at P <0.05. A Kruskal–Wallis test was used to analyse the effect of dietary treatments on welfare indicators (LTL, and feather and gait scores) of the quail.

Results

Table 3 shows that shelf-life indicators such as egg weight, albumen height, and yolk height were not affected by the treatments for the entire storage duration. However, treatment effects (P <0.05) were observed on day 5 for egg breaking strength and albumen height and on day 10 for albumen pH. The CON diet caused the highest (P <0.05) breaking strength on day 5, whereas the lowest strength was observed in the CTMR group. Both diets promoted similar (P >0.05) egg breaking strength to CTMA, CTMO, and CTMB. Furthermore, eggs from birds fed diet CTMR had higher (P <0.05) albumen height on day 5 compared to eggs from CTMA. Eggs from birds on diet CTMA had the lowest (P <0.05) albumen pH on day 10, whereas those from diet CON had the highest albumen pH. Diets CTMB, CTMO and CTMR promoted similar (P >0.05) albumen pH.

Table 3 Effect of complete replacement of maize with raw or heat-treated cassava tuber meal on egg shelf-life indicators (means ± standard error) of jumbo quail hens over a storage period of 10 d

	[1]Experimental diets					
	CON	**CTMR**	**CTMA**	**CTMB**	**CTMO**	***P*-value**
Egg weight (g)						
Day 1	11.2±0.371	10.5±0.371	11.1±0.371	11.0±0.371	10.9±0.371	0.772
Day 5	10.8±0.423	10.3±0.423	11.4±0.423	10.9±0.423	10.6±0.423	0.494
Day 10	11.2±0.454	10.4±0.454	10.5±0.454	10.6±0.454	10.2±0.454	0.665
Egg breaking strength (N)						
Day 1	1.19±0.090	1.07±0.090	1.18±0.090	1.02±0.090	1.09±0.090	0.627
Day 5	1.42±0.103[a]	0.93±0.103[b]	1.31±0.103[ab]	1.23±0.103[ab]	1.31±0.103[ab]	0.018
Day 10	1.09±0.088	1.17±0.088	1.25±0.088	1.18±0.088	1.12±0.088	0.725
Albumen height (mm)						
Day 1	3.53±0.046	3.58±0.046	3.64±0.046	3.65±0.046	3.69±0.046	0.143
Day 5	3.65±0.021[b]	3.70±0.021[a]	3.59±0.021[b]	3.65±0.021[b]	3.68±0.021[b]	0.003
Day 10	3.66±0.049	3.56±0.049	3.55±0.049	3.57±0.049	3.60±0.049	0.545
Yolk height (mm)						
Day 1	9.49±0.231	9.71±0.231	9.70±0.231	9.26±0.231	9.97±0.231	0.275
Day 5	8.82±0.202	9.17±0.202	8.83±0.202	8.97±0.202	8.87±0.202	0.721
Day 10	9.04±0.254	8.71±0.254	8.83±0.254	9.08±0.254	8.76±0.254	0.779
Albumen pH						
Day 1	8.83±0.358	9.98±0.358	9.75±0.358	8.78±0.358	9.54±0.358	0.068
Day 5	9.47±0.272	8.68±0.272	9.67±0.272	9.31±0.272	9.55±0.272	0.101
Day 10	9.86±0.294[a]	9.54±0.294[ab]	7.81±0.294[c]	9.27±0.294[ab]	8.37±0.294[bc]	<0.0001

[1]Diets: CON = a conventional layer mash diet without cassava tuber meal; CTMR = a conventional layer mash diet where maize is completely replaced with raw cassava tuber meal; CTMA = a conventional layer mash diet where maize is completely replaced with autoclaved cassava tuber meal; CTMB = a conventional layer mash diet where maize is completely replaced with boiled cassava tuber meal; CTMO = a conventional layer mash diet where maize is completely replaced with oven-dried cassava tuber meal

A Kruskal–Wallis H test showed that there were no statistically significant dietary effects on gait and feather scores of jumbo quail hens (Table 4). However, there was a treatment effect on latency-to-lie test [$X^2(2) = 10.18$; $P = 0.0375$].

Table 4. Effect of total replacement of maize with raw or heat-treated cassava tuber meal on medians of welfare indicators of jumbo quail hens

	[1]Experimental diets					
	CON	**CTMR**	**CTMA**	**CTMB**	**CTMO**	***P*-Chi-square**
Gait score	33.7	29.4	28.7	31.9	26.8	0.720
Feather score	31.0	28.5	29.8	30.3	33.0	0.976
Latency-to-lie (sec)	30.2	32.1	36.3	35.4	18.5	0.0375

[1]Diets: CON = a conventional layer mash diet without cassava tuber meal; CTMR = a conventional layer mash diet where maize is completely replaced with raw cassava tuber meal; CTMA = a conventional layer mash diet where maize is completely replaced with autoclaved cassava tuber meal; CTMB = a conventional layer mash diet where maize is completely replaced with boiled cassava tuber meal; CTMO = a conventional layer mash diet where maize is completely replaced with oven-dried cassava tuber meal

Table 5 reveals that there were no treatment effects ($P > 0.05$) on haematological and serum biochemical parameters of laying Jumbo quail hens.

Table 5 Effect of complete replacement of maize with raw or heat-treated cassava tuber meal on blood parameters (means ± standard error) of 14-week-old Jumbo quail hens

	[1]Diets					
[2]Parameters	**CON**	**CTMR**	**CTMA**	**CTMB**	**CTMO**	***P*-value**
Neutrophils (%)	9.49±5.33	5.51±5.33	8.18±5.33	28.17±5.84	10.04±5.33	0.053
Lymphocytes (%)	140±14.08	120±14.08	140±14.08	127±15.42	155±14.08	0.473
Monocytes (%)	52.0±15.48	38.1±15.48	41.2±15.48	51.7±16.96	57.0±15.48	0.9
Glucose (mmol/L)	249±16.90	234±16.90	242±16.90	196±18.52	241±16.90	0.253
Calcium (mmol/L)	15.1±0.81	14.6±0.81	14.6±0.81	12.9±0.89	14.5±0.81	0.445
Total protein (g/L)	4.74±0.24	4.95±0.24	4.26±0.24	3.97±0.27	4.57±0.24	0.067
SDMA (µg/dL)	12.8±1.11	11.7±1.11	10.5±1.11	10.7±1.21	10.4±1.11	0.499
Phosphorus (mmol/L)	6.34±0.66	7.47±0.66	6.22±0.66	7.64±0.73	5.78±0.66	0.232
Albumin (g/L)	1.45±0.09	1.52±0.10	1.40±0.09	1.76±0.10	1.43±0.10	0.071
Cholesterol (mmol/L)	125±10.1	124±10.1	112±10.1	119±11.0	115±10.1	0.873
Globulin (g/L)	3.29±0.20	3.43±0.20	2.85±0.20	2.65±0.21	3.13±0.20	0.059
ALB/GLOB	0.45±0.02	0.44±0.02	0.49±0.02	0.45±0.02	0.48±0.02	0.234
ALT (U/L)	11.2±1.84	15.0±1.84	10.0±1.84	10.0±1.84	12.2±1.84	0.295
Amylase (U/L)	221±25.9	232±25.9	262±25.9	249±28.4	227±25.9	0.796
Total bilirubin (mmol/L)	0.32±0.07	0.42±0.07	0.23±0.07	0.32±0.07	0.30±0.07	0.523

[1]Diets: CON = a conventional layer mash diet without cassava tuber meal; CTMR = a conventional layer mash diet where maize is completely replaced with raw cassava tuber meal; CTMA = a conventional layer mash diet where maize is completely replaced with autoclaved cassava tuber meal; CTMB = a conventional layer mash diet where maize is completely replaced with boiled cassava tuber meal; CTMO = a conventional layer mash diet where maize is completely replaced with oven-dried cassava tuber meal
[2]Paremeters: ALT = alanine Transaminase; ALB/GLOB = albumen/globulin ratio; SDMA = symmetric dimethylarginine

Table 6 indicates no treatment effects ($P > 0.05$) on visceral morphometry and carcass traits, except on HCW, CCW, wing and back length of Jumbo quail hens. Quail hens fed with CON diet had larger ($P < 0.05$) HCW and CCW than those fed with diets CTMR and CTMB, but all three diets were similar ($P > 0.05$) to diets CTMO and CTMA. Diets CTMO and CTMB resulted in hens with heavier ($P < 0.05$) wing weights than diets CON and CTMA. Nonetheless, all diets were statistically the same ($P > 0.05$) as diet CTMR in terms of wing weights. Quail birds fed diet CTMA had the shortest ($P < 0.05$) back length, whereas birds fed CTMB and CTMO had the longest ($P < 0.05$) back length. However, all three diets were similar ($P > 0.05$) to diets CTMR and CON.

Table 6 Effect of complete replacement of maize with raw or heat-treated cassava tuber meal on carcass characteristics and visceral morphometry (means ± standard error) of 14-week-old jumbo quail hens

	[1]Diets					
[2]Parameters	CON	CTMR	CTMA	CTMB	CTMO	*P*-value
HCW (g)	168±4.13[a]	147±4.13[b]	163±3.97[ab]	149±4.32[b]	154±4.13[ab]	0.003
CCW (g)	167±4.16[a]	146±4.16[b]	161±4.00[ab]	148±4.35[b]	153±4.16[ab]	0.004
Thigh (g)	6.1±0.22	6.9±0.22	6.7±0.21	6.8±0.23	6.9±0.22	0.074
Wing (g)	4.1±0.12[b]	4.5±0.12[ab]	4.8±0.12[b]	4.7± 0.13[a]	4.6±0.12[a]	0.007
Drumstick (g)	4.1±0.12	4.3±0.12	4.1±0.12	4.3±0.13	4.3±0.12	0.629
Breast (g)	17.4±0.45	18.3±0.45	18.2±0.44	19.1±0.47	18.4±0.45	0.144
Back (cm)	10.1±0.16[ab]	10.0±0.16[ab]	9.5±0.15[b]	10.4±0.16[a]	10.2±0.16[a]	0.007
Thigh (cm)	4.4±0.06	4.6±0.06	4.5±0.06	4.4±0.06	4.5±0.06	0.684
Drumstick (cm)	55.3±0.93	54.7±0.93	53.4±0.90	52.5±0.98	52.9±0.93	0.186
Wing (cm)	10.8±0.12	10.6±0.12	10.9±0.12	10.6±0.12	10.5±0.12	0.151
Keel length (mm)	46.5±1.64	48.0±1.64	49.5±1.58	48.0±1.72	49.7±1.64	0.619
Keel diameter (mm)	20.8±0.80	20.2±0.80	19.4±0.77	18.6±0.84	18.8±0.80	0.265
Liver (g)	3.66±0.13	3.67±0.13	3.90±0.12	3.99±0.13	3.65±0.13	0.206
Gizzard (g)	2.77±0.09	2.88±0.09	2.73±0.09	2.79±0.09	2.89±0.09	0.614
Proventriculus (g)	0.82±0.06	0.86±0.06	0.88±0.05	0.91±0.06	0.84±0.06	0.785
Small intestine (g)	6.28±0.26	6.30±0.26	6.33±0.25	6.71±0.27	5.90±0.26	0.332
Large intestine (g)	1.56±0.47	2.79±0.47	1.88±0.46	2.17±0.50	2.43±0.47	0.405
Small intestine (cm)	73.9±2.40	70.8±2.40	70.8±2.30	70.7±2.50	74.5±2.40	0.644
Large intestine (cm)	14.0±0.88	14.7±0.88	15.7±0.84	15.3±0.92	12.8±0.88	0.146

[1]Diets: CON = a conventional layer mash diet without cassava tuber meal; CTMR = layer mash where maize is completely replaced with raw cassava tuber meal; CTMA = layer mash diet where maize is completely replaced with autoclaved cassava tuber meal; CTMB = layer mash diet where maize is completely replaced with boiled cassava tuber meal; CTMO = layer mash diet where maize is completely replaced with oven-dried cassava tuber meal [2]Parameters: HCW = hot carcass weight; CCW = cold carcass weight

Table 7 shows that there were no dietary effects ($P > 0.05$) on breast meat quality parameters measured immediately and 24 hours post-slaughter, except for 24-hour breast meat pH. Diet CON caused lower ($P < 0.05$) breast meat pH after 24 hours of slaughter than diet CTMB, but both groups had a similar meat pH to CTMA, CTMO and CTMR groups.

Table 7 Effect of complete replacement of maize with raw or heat-treated cassava tuber meal on breast meat quality attributes in Jumbo quail hens

	[1]Diets					
	CON	CTMR	CTMA	CTMB	CTMO	*P*-value
Immediately after slaughter						
pH	5.74±0.05	5.76±0.05	5.78±0.04	5.78±0.05	5.83±0.05	0.654
*L** (lightness)	45.2±0.82	45.8±0.82	46.0±0.78	45.2±0.85	43.8±0.82	0.309
*a** (redness)	8.10±0.28	7.96±0.27	7.85±0.26	7.86±0.29	8.01±0.27	0.961
*b** (yellowness)	1.03±0.12	1.01±0.12	0.68±0.12	0.89±0.13	1.12±0.12	0.090
24 hours after slaughter						
pH	5.90±0.05[b]	5.97±0.05[ab]	5.96±0.05[ab]	6.14±0.05[a]	6.00±0.05[ab]	0.023
*L** (lightness)	43.0±0.44	42.5±0.44	42.7±0.43	41.8±0.46	42.6±0.44	0.502
*a** (redness)	10.6±1.01	8.54±1.01	8.67±0.97	8.60±1.05	8.57±1.01	0.521
*b** (yellowness)	2.51±0.27	1.65±0.27	2.11±0.26	1.47±0.28	1.59±0.27	0.050

[1]Diets: CON = a conventional layer mash diet without cassava tuber meal; CTMR = layer mash diet where maize is completely replaced with raw cassava tuber meal; CTMA = layer mash diet where maize is completely replaced with autoclaved cassava tuber meal; CTMB = layer mash diet where maize is completely replaced with boiled cassava tuber meal; CTMO = layer mash diet where maize is completely replaced with oven-dried cassava tuber meal

Discussion

Indicators of egg shelf life in layers are important to evaluate the effectiveness of dietary treatments to maintain or prolong the storage period of eggs. However, there is limited research on the shelf life parameters of quail eggs, as most research efforts are directed to chicken eggs. Contrary to external quality attributes, an eggs' internal quality begins to deteriorate immediately after being laid (Kumari *et al*., 2020). Thus, while nutritional and management factors of hens are important to maintain the internal quality of eggs, the handling and storage of eggs affect the ultimate quality of saleable eggs. Consequently, the most important factors influencing egg quality during storage are relative humidity and temperature (Menezes *et al*., 2012). This study indicates that complete replacement of maize with CTM in the diet of laying jumbo quail hens had no influence on most shelf-life attributes throughout the 10-day storage period. However, a notable difference was seen on albumen pH on at day 10 of storage, where eggs from quail fed the control diet had a substantially higher albumen pH compared to eggs from the CTMA treatment, which had a lower albumen pH. The increase in albumen pH could be due to the loss of carbon dioxide from the egg through the shell pores (Eke *et al*., 2013). The lower egg breaking strength from the CTMR group when compared to the CON group at day 5 of storage could be attributed to the nutritional superiorities of maize to supply all the nutrients required to form a stronger egg shell. However, it is surprising that the CTMR group had a long albumen height on day 5 of storage, which indicated better internal quality.

There is limited research on welfare parameters of quail birds (Mnisi *et al*., 2021; Mnisi *et al*., 2023). The treatment effect observed on latency-to-lie (LTL) test, which indicates that the time taken by the hens to lie down, could be associated with the impact of the diets on their leg development and health. Birds reared on CTMO diet lay down sooner than birds on the other treatments, indicating a possibility of poor leg health. On the other hand, birds fed CTMC resulted in the longest LTL, which points to optimal leg health (Thema *et al*., 2022). This could have been influenced by the nutritional changes of the diets in response to the different heat processing methods. The gait and feather scores were not affected by the treatments, signifying that the addition of CTM did not compromise the walking ability and feather coverage of the hens.

Blood parameters are typically used as physiological indicators to determine a bird's nutritional status, health and wellbeing, and diseases (Akinosun *et al*., 2020). Additionally, blood indices serve as a pathological reflector of an animal's status before or after exposure to illnesses (Etim *et al*., 2014). There were no treatment variations on haematological parameters, which implies that the health of the hens' blood cells was not compromised by the replacement of maize with raw or thermally-processed CTM. Similarly, there were no dietary effects on serum indices such as glucose, cholesterol, and amylase, which was expected, given that the diets were isoenergetic. Moreover, serum values obtained from this study were comparable to the values reported by Scholtz *et al*. (2009) for Japanese quail fed a maize–soybean diet. Moreover, the addition of raw or heat-treated CTM had no impact on liver enzymes (ALP and ALT), suggesting that even raw cassava can be used to replace maize without affecting the functions of the liver (Washington & van Hoosier 2012). However, this may be possible if sweet varieties of cassava are used since they are reported to have low concentrations of secondary metabolites (Ogunsipe *et al*., 2022). Serum components such as blood urea nitrogen, uric acid, and creatinine are indicators of optimal renal function (Donsbough *et al*., 2010), thus the lack of dietary alterations further confirms that cassava does not cause kidney failure and can, therefore, be used as a suitable energy source for laying jumbo quail.

The digestive system facilitates the body's ability to absorb nutrients for normal physiological, metabolic, and anatomical requirements. Healthier birds have desirable intestinal morphology that allows them to efficiently break down and absorb nutrients (Yang *et al*., 2009). The visceral organs of the hens were not negatively affected by the diets containing either raw or heat-treated CTM in place of maize, signifying that cassava posed no challenge to the birds that required digestive anatomical coping mechanisms. This could also explain the absence of differences in egg weights since eggs are developed and carried by the lower digestive tract. Contrary to our findings, Ojediran *et al*. (2023) found that birds raised on diets containing processed cassava root meal had lower proventriculus weights compared to birds fed the maize-control diet. This could imply efficient digestion in quail fed with the cassava-containing diet since the proventriculus initiates feed digestion. Furthermore, the treatments had no influence on the liver of the hens, suggesting that the presence of HCN in cassava did not require severe detoxification (Dewi & Normasari, 2021), which further explains the absence of dietary effects on liver enzymes.

Carcass traits are crucial economic indicators even in layer quail farming because they influence the profitability of the business. This is because the price of quail products and overall revenue of a quail business are influenced by reductions in carcass weights (López-Pedrouso *et al*., 2019). In this study, autoclaved and boiled CTM improved the back length and wing weights of the hens better than the maize-based diet. Quail fed the maize-control diet had heavier carcass weights than those on CTMR and CTMB, which could be due to nutritional superiorities of maize when compared to cassava. These results support the findings of Ojediran *et al*. (2023), who observed lower carcass weights in quail fed a CTM-based diet than that fed a maize-control diet. Edache *et al*. (2013) found that Japanese quail fed a diet containing 420 g/kg CTM had lower carcass weights than those fed a standard control diet. Thus, the similarities among the CON, CTMA, and CTMO groups in terms of carcass weights signify that autoclaving and oven-drying are suitable strategies to enhance the nutritional merits of cassava and ensure economic viability in layer quail.

Meat quality attributes are important in broiler birds, however, there is now a growing demand for spent birds due to the texture of their meat. Unfortunately, there are limited research studies that examine the meat quality attributes of layer hens, heeding a call for future research. This study shows that meat pH measured 24 hour postmortem was affected by the dietary treatments but was within the range reported by Deminicis *et al*. (2022) for normal meat. However, birds fed with CTMB had meat pH values above the reported range, which is an effect that requires further research. According to Deminicis *et al*. (2022), a normal pH of a quail breast should range between 5.7 and 6.0. Thus, the negative effect of the boiling treatment on meat pH warrants further investigation. Furthermore, Mir *et* al. (2017) reported that meat colour attributes depend on myoglobin content, whereby poor oxygenation of myoglobin is associated with high pH, leading to dark meat. Lipid oxidation products react with myoglobin, denaturing it and reducing its ability to hold oxygen, leading to browning reactions which contributes to a less red or more brown meat colour (Mancini & Hunt, 2005). However, the redness values reported in this study corroborate the observations of Genchev *et al*. (2010), who reported that a normal redness of quail meat should range between 5.1 and 14. However, the lack of dietary alterations on meat pH immediately after slaughter and on overall meat colour, suggest that feeding diets with raw or heat-treated CTM does not compromise the appearance and stability of spent Jumbo quail meat. Similarly, Abu *et al*. (2015) observed that the inclusion of 200 g/kg cassava peel meal did not alter broiler meat colour.

Conclusion

Although Jumbo quail hens reared on the maize-based control diet showed optimal egg quality attributes during storage and better carcass performance, the autoclaving and oven-drying treatments enhanced carcass weights better than raw or boiled cassava tuber meal treatments. Nonetheless, the incorporation of raw or thermally-processed cassava tuber meal did not alter welfare and blood parameters, visceral morphometry, and breast meat quality attributes of the spent quail hens. It can be concluded that total replacement of maize with raw or heat-treated cassava tuber meal produced suboptimal performance metrics in laying Jumbo quail. Thus, future studies can investigate partial replacement levels coupled with autoclaving or oven-drying treatments to achieve optimal results.

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Author's contributions

AG conducted the feeding trial, curated data, and wrote the initial draft; CMM conceptualised the study, performed the statistical analysis, and finalised the manuscripts. Both authors have read and approved the final version of the manuscript.

Conflict of interest declaration

The authors have no conflict of interest to declare.

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