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Effects of supplementing fermented water hyacinth (*Eichhornia crassipes*) meal in hy-Line brown hens on oviduct morphometric characteristics, internal egg quality and shelf life

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The study investigated the effects of partially replacing soybean meal with fermented water hyacinth meal (FWHM) on oviduct morphometry and egg quality in Hy-Line hens. Ninety-six eighteen-week-old hens were randomly assigned to one of three dietary treatments: 0% (Control), 2.5%, or 5% FWHM. Following a 2-week dietary adaption period, hens were fed the experimental diets for twelve weeks. Internal egg quality, and eggshell traits were measured at regular intervals during the feeding trial, with freshly laid eggs stored at 4°C (39.2°F) under approximately 70-80% relative humidity and equilibrated at room temperature (21-25°C) for one hour prior to analysis to minimize condensation and thermal shock effects. Oviduct morphometric measurements were obtained at the end of the study, to evaluate dietary effects. Most oviduct morphometric parameters were unaffected by FWHM supplementation ($P > 0.05$). Notable exceptions were higher infundibulum weight in the 5% FWHM group (1.65 $\pm$ 0.46 g) compared to the 2.5% group (1.10 + 0.34 g; $P < 0.05$), and lower magnum weight in the 5% FWHM group (27.84 $\pm$ 7.24 g) relative to Control (38.28 $\pm$ 7.01 g; $P < 0.05$). Internal egg quality and eggshell characteristic traits: yolk and albumen weights, Haugh unit and eggshell thickness were largely unchanged. Yolk pH was lower in the 2.5% (6.63 $\pm$ 0.09) and 5% (6.60 $\pm$ 0.10) FWHM groups versus Control (6.70 $\pm$ 0.13; $P < 0.05$), while yolk diameter slightly decreased in FWHM-fed hens. These findings indicate that FWHM can be included in laying hen diets at levels up to 5% without adversely effecting reproductive tract development or core egg quality, while modulating yolk traits that may enhance egg shelf life.

KEYWORDS

egg quality, fermented water hyacinth meal (FWHM), hy-line brown hens, oviduct morphometry, shelf life

1 Introduction

As the demand for poultry products continues to rise globally, there is growing pressure on industry to find sustainable, affordable, and nutritionally adequate feed alternatives to support optimal bird health and productivity. The poultry sector has seen rapid growth in response to increasing global demand for affordable animal protein sources such as eggs and chicken meat (Moreki et al., 2025; Govoni et al., 2021; Nkukwana, 2018; Magdelaine et al., 2008). This trend is expected to intensify with projected global population growth and rising per capita income (Erdaw and Beyene, 2022; Henchion et al., 2021; Kleyn and Ciacciariello, 2021; United Nations, Department of Economic and Social Affairs (UN DESA), 2019). Soybean meal (SBM), widely used as a protein-rich feed component in poultry diets (Govoni et al., 2021; Nkukwana, 2018), presents challenges due to rising prices (Ayoola et al., 2024), inconsistent availability, and growing competition from human consumption industries, where soybeans plays a pivotal role in the production of soy-based foods and plant-based meat substitutes (Qin et al., 2022; Zhang et al., 2021; Joshi and Kumar, 2015). Its increasing global demand has also been linked to environmental concerns such as deforestation, biodiversity loss, and greenhouse gas emissions, especially in regions like South America (Gollnow and Lakes, 2014; Castanheira and Freire, 2013). Moreover, climate-related threats to soybean yield in sub-Sahara Africa raise concerns about long-term feed security in the region (Affoh et al., 2022).

Considering these challenges, attention has shifted toward locally available, underutilized resources that could partially replace SBM in poultry diets. One such is water hyacinth (*Eichhornia crassipes*), an invasive aquatic weed that causes significant ecological and economic disruption through obstruction of waterways and degradation of aquatic habitants (Gebremeskel, 2024). While often regarded as a nuisance, its biomass contains moderate protein (10-25% dry matter) and high fibre content (20-30%), offering potential nutritional value if properly processed (El-Sayed, 2003; Dharmawati et al., 2024). However, raw water hyacinth is limited by its high fibre content and the presence of antinutritional factors. Fermentation has been proposed as a cost-effective biotechnological approach to enhance its nutritional profile and improved nutrient utilization in layer hens (Antonio et al., 2025). Water hyacinth contains approximately 2 – 5% soluble carbohydrates, 10 – 16% crude protein, and 20–30% crude fiber, with metabolizable energy (ME) of 8.4 – 8.6 MJ/kg DM (2000–2050 kcal/kg DM) which is sufficient to support microbial fermentation without adding other energy sources (Hossain et al., 2015). Studies have shown that fermentation can significantly reduce compounds such as phytic acid, saponins, and phenolics, while improving nutrient digestibility and amino acid profiles of feedstuff (Kabir et al., 2023; Malik et al., 2024). These biochemical changes are particularly relevant to laying hens, whose oviduct development and egg quality traits can be influenced by feeding fermented miscellaneous feedstuff (Lu et al., 2023). Improved phosphorus availability and calcium retention, both of which can be enhanced by fermentation, are also essential for eggshell formation and oviduct health (Wengerska et al., 2022; Lu et al., 2023). Additionally, fermentation generates bioactive peptides and antioxidants that may improve intestinal health, egg quality, and modulate endocrine function. This can lead to upregulation of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), both of which are vital for follicular development, ovulation, and sustained laying performance (Lu et al., 2023). The study aims to investigate the effects of supplementing fermented water hyacinth (*Eichhornia crassipes*) meal in Hy-Line brown hens on oviduct morphometric characteristics, internal egg quality and shelf life.

2 Materials and methods

2.1 Study site

The study was conducted at the University of Zimbabwe Livestock science bioassay, located in Harare Zimbabwe. The University of Zimbabwe is situated 8.5km northeast of Harare Central business district, between longitude 31.0533° E and latitude 17.7883° S. The site is 1,483 meters above sea level. The University is in agro-ecological region IIb with an average rainfall of about 870 mm per annum. The site has a subtropical climate; the 24-hour average temperature of the year is 25.7°C, with maximum temperature ranging between 22 and 30°C. The least amount of precipitation occurs in the month of September (1mm) while the greatest amount of precipitation occurs in January with an average of 140mm.

2.2 Sourcing and processing of water hyacinth

Water hyacinth was harvested from Darwendale dam in Norton. A permit (Permit No. 23 (1) (C) (II) 64/2024) to harvest the water hyacinth was granted by the Zimbabwe Parks and Wildlife Authority (ZIMPARKS). The harvested plants were thoroughly washed to remove any dirt and debris. Subsequently, the plants were chopped into smaller (0.5 – 1cm), uniform pieces to facilitate fermentation.

2.3 Fermentation process

Fermentation process was conducted in clean, sterilized plastic containers. Chopped water hyacinth (0.5 – 1cm) was packed into the containers, leaving headspace for gaseous exchange. A starter culture consisting of commercial baking yeast (*Saccharomyces cerevisiae*) and fermented milk was inoculated into the mixture, introducing yeast and lactic acid bacteria (LAB), *Lactococcus lactis* species. Inoculation with LAB was performed at approximately 10 g of fermented milk/kg of water hyacinth (containing $\geq 10^9$ CFU LAB/g), followed by anaerobic fermentation. The combination of these two microorganisms was expected to enhance fermentation efficiency and produce desirable fermentation products. The containers were then sealed tightly to create an anaerobic

environment, which was essential for fermentation. The sealed containers were incubated at a temperature range of 24 - 35°C for 14 days. The fermentation process was monitored daily for signs of fermentation, such as gas production and a change in odour. The pH of the fermented water hyacinth was measured periodically to assess the progress of fermentation.

2.4 Drying of fermented water hyacinth biomass and feed chemical analysis

To preserve the nutritional value, enzymes, and beneficial probiotics produced during fermentation, the fermented water hyacinth biomass was dried using a solar drier. The solar drier was designed like a greenhouse commonly used in horticulture, with a transparent plastic roof and walls to allow for maximum sunlight penetration, and vents and chimneys to enhance air circulation and temperature control. Initially, excess moisture was removed from the fermented biomass by wilting in direct sunlight for 6–8 hours, with the material spread in a thin layer approximately 2–3 cm thick on clean polytene sheets. This preliminary drying aimed to reduce the moisture content to around 60 -70%. The pre-treated biomass was then loaded onto drying trays, ensuring uniform spreading at a thickness of 1.5–2 cm to ensure adequate air circulation and even drying. Drying was done at a controlled temperature between 25°C - 35°C to prevent denaturation of enzymes and probiotics. Temperatures above 60°C (140°F) were avoided, as they could deactivate beneficial microorganisms. The drying process was monitored regularly using a digital moisture meter, aiming for a final moisture content of 10 - 15% (dry basis). Once dried, the biomass was stored in airtight bags or containers to maintain freshness and prevent rehydration as described by Emshaw et al. (2023). Proximate analyses were conducted to determine the basic nutritional composition of the fermented, unfermented water hyacinth and feed samples were done following be methods of The Association of Official Analytical Chemists (AOAC), 1990. The tannin content of the water hyacinth was assayed using the protein precipitation/binding assay (Makkar et al., 1993).

2.5 Experimental design and animal management

A completely randomized design (CRD) was employed. A total of ninety-six Hy-line brown layer hens at 18 weeks of age were randomly allocated to three dietary treatments in a completely randomized design with 8 replicate pens per treatment and each pen was considered an experimental unit. Hens were allowed a 2-week acclimatization period to the diets and housing conditions before commencing the twelve-week experimental period in which all measurements and observations were performed. Birds were housed in battery cages measuring 50cm by 50cm, and each cage housed 4 birds providing approximately 625cm^2 of floor space per hen. Birds were housed in a naturally ventilated, open-sided poultry house, with functional ventilator fans used to enhance air movement. The microclimatic conditions were maintained within acceptable limits for laying hens, with ambient temperatures ranging from 25 to 30°C, with relative humidity maintained between 60-75% RH and an average air speed of approximately 2.0 m/s at bird level. Fresh droppings were collected and removed daily from the cages to prevent accumulation, minimize ammonia buildup, maintain air quality and good hygienic conditions throughout the study. Experimental diets were Control: Basal diet (0% FWHM); Treatment 1 (T1): Basal diet + 2.5% FWHM; Treatment 2 (T2): Basal diet + 5% FWHM. All birds were fed a laying ration in mesh form containing 2650–2700 kilocalories of metabolizable energy (ME) per kilogram from the point of lay. Tables 1, 2 summarizes the feed ingredients and chemical composition of experimental diets offered to layer hens. Feed was measured and offered twice daily in the morning and at midday; clean, fresh water was readily available *ad-libitum*. The birds were fed a total of 130g per bird/day, divided into two equal meals to stimulate appetite and encourage complete consumption of all feed components, particularly fine premix particles containing vitamins and minerals, preventing selective feeding and ensuring balanced nutrient intake for optimal health and performance (Hy-Line International, 2023). Daily feed refusals in each replicate were carefully collected, weighed, and recorded before the subsequent feed offer. A total day length of 16 hours (including natural and artificial lighting) was maintained throughout the experimental period: natural daylight lasted 12 hours, while artificial lighting was provided from 04:00 to 06:00 h (pre-dawn) and from 18:00 to 21:00 h (post-sunset). All birds were vaccinated against Infectious Bronchitis (IB) and Newcastle Disease (ND) according to the standard vaccination schedule for laying hens. All vaccination procedures were done according to guidelines in Cobb Vaccination Procedure guide (2010). All experimental procedures that were used in the research project were approved under the Zimbabwe Animal Act, Chapter19:12,1963, License number L729 (Scientific Experiments on Animal Act,1963;03 2025-18).

2.6 Laying performance, internal egg quality, eggshell characteristics and egg shelf life

The number of eggs produced was recorded daily including those that were broken. Laying performance was determined using the following formulas.

Internal egg quality and eggshell characteristics were examined daily for the duration of the feeding trial and were calculated following established methods and formulas outlined by Yörük et al. (2004) and Alikhanov et al. (2021).

Egg shelf life was determined by monitoring changes in key physical and chemical properties over a period of one month and egg quality test were done weekly. Freshly laid eggs were stored at 4°C (39.2 °F) under refrigerated conditions; relative humidity was not actively controlled but was approximately 70 - 80% based on refrigerator specifications. Eggs were stored in closed egg cartons

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TABLE 1 Formulations and chemical analysis of experimental diets.

Ingredient/ Inclusion %	0% FWHM	2.5% FWHM	5% FWHM
White maize, crushed	54	54	54
Wheat bran	16	16	16
Soybean meal	24.5	22	20
Fermented water hyacinth	0	2.5	5
Limestone flour	2	2	2
Rock salt	1	1	1
Vitamin mineral mix	2.5	2.5	2.5
Total	100	100	100
Chemical Analysis (%DM)			
Crude Protein	15.36	16.66	16.87
Crude Fiber	2.97	2.88	3.06
Ash	9.31	8.26	10.57
Ether Extract	3.65	3.83	3.5
Moisture	8.33	9.5	8.81
Calcium	3.55	3.13	3.78
Phosphorus	0.4	0.44	0.38
Calculated Analysis (Total Amino Acids and Metabolizable Energy)			
Metabolizable Energy (MJ/ Kg)	11.5	11.4	11.3
Methionine	0.41	0.41	0.41
Lysine	0.84	0.84	0.84
Threonine	0.61	0.61	0.61
Meth + Cystine	0.75	0.75	0.75
Tryptophan	0.19	0.19	0.19

TABLE 2 Chemical and heavy metal analysis of fermented water hyacinth meal.

Parameter	% DM
Crude Protein	15.36
Crude Fiber	17.62
Ash	18.57
Ether Extract	2.68
Moisture	12.26
Calcium	1.51
Phosphorus	0.28
Heavy Metal Analysis	mg/Kg
Lead	13.34
Cadmium	0.48
Arsenic	ND
Chromium	1.90

(cardboard), which are commonly used under refrigerated conditions due to their hygroscopic nature, allowing them to buffer moisture and minimize surface condensation at moderate to high relative humidity. Prior to analysis, eggs were allowed to equilibrate at room temperature (21 to 25°C) for one hour before measurement to eliminate the effects of condensation or thermal shock. The most important indicators include yolk and albumen weight, yolk pH, yolk index, Haugh Unit (HU), and egg weight loss. Egg weight (g) was recorded using a digital precision balance (Precisa 1212 M SCS, Precisa Instruments, Dietikon, Switzerland). Yolk diameter (mm) was measured using a digital Vernier calliper (QLR VCL150, Pierre Vernier, France). Yolk colour was determined using the Roche Yolk Colour Fan (DSM Nutritional Products, Kaiseraugst, Switzerland). Yolk pH was assessed using a calibrated digital pH meter (pH 8+ DHS, XS Instruments, Modena, Italy), with electrodes rinsed among samples to avoid cross-contamination. Shell thickness (mm) was measured at the blunt end, equator, and pointed end using a screw micrometre (9SM127M, James Watt, London, UK), and the average was recorded. Albumen height (mm) was measured using a tripod micrometre, and Haugh units (HU) were computed using the formula:

$$HU = 100 \times \log_{10}(H + 7.57 - 1.7W^{0.37}).$$

where H is albumen height and W is egg weight.

All measurements were conducted under standardized laboratory conditions with consistent lighting and were performed by the same trained personnel to minimize observer bias. Instruments were calibrated weekly using standard weights and buffers (Ikusika et al., 2025).

2.7 Slaughter procedures of the laying hens

At the end of the feeding trial, hens in all cages were fasted for a period of 13 hours to allow the clearing of the crop (Disetlhe, 2017). Thereafter, the hens were taken to a slaughterhouse at the University of Zimbabwe for slaughter. Birds were grouped according to dietary treatment at the slaughterhouse. They were then put into a metal rack with cones that held them upside down for stunning. Chickens were slaughtered by cutting the jugular vein with a sharp knife and were bled for up to 2 minutes. Subsequently, chickens followed the standard operating procedures for slaughter before they were manually de-feathered. When feathers were removed, carcass evisceration and hot carcass weight measurement was done at the slaughterhouse, while the oviduct was harvested as explained by Mohammadpour et al. (2012) for morphometrical examination.

2.8 Oviduct morphology and characteristics

The carcasses and internal organs from different treatments were identified by placing them in tagged plastics bags for each

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TABLE 3 Effect of fermented water hyacinth meal in diet on oviduct morphometric characteristics.

	Dietary treatments			
	Control 0% FWHM	2.5% FWHM	5% FWHM	
Parameter	Mean ± SD	Mean ± SD	Mean ± SD	Significance
Oviduct Length (mm)	75.00 ± 8.93	74.80 ± 7.07	77.80 ± 3.58	
IL (mm)	12.33 ± 2.24	11.50 ± 1.78	10.60 ± 2.84	
ML (mm)	39.44 ± 4.48	42.90 ± 7.45	40.20 ± 3.88	
ISL (mm)	14.78 ± 2.99	13.70 ± 3.56	16.20 ± 2.97	
SGL (mm)	12.22 ± 2.11	11.80 ± 2.57	13.00 ± 3.02	
Oviduct weight (g)	67.08 ± 9.05	59.80 ± 10.50	60.54 ± 11.90	
IW (g)	1.46 ± 0.25^{b}	1.10 ± 0.34^{a}	1.65 ± 0.46^{c}	**
MW (g)	38.28 ± 7.01^{c}	34.75 ± 7.91^{b}	27.84 ± 7.24^{a}	*
ISW (g)	7.15 ± 1.45	5.70 ± 1.60	6.99 ± 1.89	
SGW (g)	18.99 ± 2.16	17.24 ± 2.32	17.28 ± 1.78	

^{a}, ^{b}, means in the same row with different superscripts are significantly different at p <.05. SD, Standard deviation; IW ,Infundibulum weight; IL, Infundibulum length; MW, Magnum weight; ML, Magnum length; IW, Isthmus weight; IL, Isthmus length; SGW, Eggshell gland weight; SGL, Eggshell gland length; SD, Standard deviation.

treatment. The eviscerated carcasses and the reproductive organs were examined. The oviducts were separated into different segments namely, infundibulum, magnum, isthmus and eggshell gland. The weight of each part of the oviduct was measured by an electronic weighing scale. The length of each part of the oviduct was measured by vernier caliper as described by Hlokoe et al. (2025).

2.9 Statistical analysis

Statistical analysis was performed using Stata version 17 (StataCorp LLC, College Station, TX USA). The Shapiro-Wilk test was performed to check if the data followed a normal distribution prior to analysis. Data on endpoint measures were subjected to one-way analysis of variance (ANOVA) under the General Linear Model (GLM) framework. The model assessed the main effect of dietary treatment on each response variable. Where statistically significant treatment effects were detected ($P < 0.05$), *post hoc* comparisons were carried out using the Bonferroni correction to adjust for multiple comparisons and reduce the likelihood of Type I error.

For egg quality changes during storage, linear and polynomial (quadratic) regression models were fitted to describe trends over time, with storage time as the independent variable and dietary treatment as a grouping factor. Regression coefficients and R^2 values were used to quantify the rate and pattern of change in yolk and albumen traits across treatments.

The regression models were specified as:

Linear: $y = \beta_0 + \beta_1 x$.

Polynomial: $y = \beta_0 + \beta_1 x + \beta_2 x^2$.

where y represents the dependent variable (egg quality trait), x represents the storage time, β_0 is the intercept, β_1 the linear coefficient, β_2 and the quadratic coefficient.

The statistical model for ANOVA was as follows:

$Yij = \mu + Ti + \varepsilon ij$.

Where: Yij = observation (oviduct morphology and internal egg quality), μ = population mean constant common to all observations, Ti = effect of diet, and εij = random error term.

For all tests, the level of significance was set at ($P < 0.05$).

3 Results

3.1 Effect of fermented water hyacinth meal in diet on oviduct morphometric characteristics

The oviduct morphometric characteristics of hens fed diets containing fermented water hyacinth meal (FWHM) are presented in Table 3. The results show that there were no significant differences ($P > 0.05$) among dietary treatments for most oviduct parameters, including oviduct length (OL), infundibulum length (IL), magnum length (ML), isthmus length (ISL), eggshell gland length (SGL), oviduct weight, isthmus weight (ISW), and eggshell gland weight (SGW).

However, significant differences were observed in infundibulum weight (IW) and magnum weight (MW). The infundibulum weight was significantly higher ($P < 0.05$) in the 5% FWHM group compared to the 2.5% FWHM group, while the magnum weight was significantly lower in the 5% FWHM group compared to the control.

3.2 Effects of fermented water hyacinth meal on laying performance of hy-line brown hens

The effects of dietary inclusion of fermented water hyacinth meal (FWHM) on the laying performance of Hy-Line Brown hens are summarized in Table 4. There were no statistically significant

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TABLE 4 Effects of fermented water hyacinth meal on laying performance.

	Dietary treatments		
	Control - 0% FWHM	2.5% FWHM	5% FWHM
Parameter	Mean ± SD	Mean ± SD	Mean ± SD
Cum. Eggs/Hen Housed	46.98 ± 2.13	46.63 ± 2.21	44.78 ± 3.32
Hen Week Lay %	82.41 ± 3.73	81.81 ± 3.88	78.57 ± 5.82
Eggs/Hen/Week	6.71 ± 0.30	6.66 ± 0.31	6.40 ± 0.47

[a], [b], means in the same row with different superscripts are significantly different at P <.05.

differences (P > 0.05) among the treatment groups for cumulative eggs per hen housed, hen-week lay percentage, or eggs per hen per week.

Hens in the control group (0% FWHM) recorded a cumulative egg production of 46.98 ± 2.13 eggs per hen, which was marginally higher than that of the 2.5% FWHM (46.63 ± 2.21) and 5% FWHM (44.78 ± 3.32) groups (P = 0.2183). Similarly, the hen-week lay percentage declined slightly with increasing FWHM inclusion, from 82.41 ± 3.73% in the control to 81.81 ± 3.88% and 78.57 ± 5.82% in the 2.5% and 5% FWHM groups, respectively (P = 0.2185). Weekly egg production per hen followed the same trend, with values of 6.71 ± 0.30, 6.66 ± 0.31, and 6.40 ± 0.47 eggs for the 0%, 2.5%, and 5% FWHM treatments, respectively (P = 0.2176).

3.3 Effects of fermented water hyacinth meal on internal egg quality and eggshell characteristics

The effects of fermented water hyacinth meal (FWHM) inclusion on internal egg quality and eggshell characteristics were assessed, and the results are summarized in Table 5. Inferential statistical analysis using one-way ANOVA revealed no significant differences between treatment groups for yolk weight (YW) and albumen weight (ALW) (P > 0.05). The observed mean YW values ranged from 13.63 ± 0.94 g (5% FWHM) to 14.73 ± 1.37 g (Control), while mean ALW values ranged 31.94 ± 2.86 g (Control) to 33.84 ± 6.71 g (2.5% FWHM). A statistically significant effect of FWHM inclusion was observed on yolk pH (YP) (P > 0.05). *Post-hoc* analysis (Bonferroni test) showed that the mean YP was significantly lower in the 2.5% FWHM treatment (6.62 ± 0.091) and the 5% FWHM treatment (6.60 ± 0.098) compared to the Control group (6.70 ± 0.14). Yolk color (YC) did not exhibit significant variation across treatment groups (P = 0.0554), with mean values ranging from 14.32 ± 0.67 (Control) to 14.79 ± 0.56 (2.5% FWHM), yolk diameter showed significant differences across treatment groups. The Control group had significantly larger mean YD (42.69 ± 2.68 mm) compared to the 2.5% FWHM (40.65 ± 2.04 mm) and 5% FWHM (41.03 ± 1.76 mm) treatment groups. No significant differences were detected among treatment groups for yolk height (YH) and yolk index (YI) (P > 0.05). Mean YH values were 13.88 ± 1.76 mm (Control), 13.37 ± 2.14 mm (2.5% FWHM), and 13.45 ± 1.41 mm (5% FWHM). All group exhibited a mean YI of 0.33 ± 0.04.

Haugh unit (HU) values, an indicator of albumen quality, did not differ significantly between treatment groups (P > 0.05). Mean HU values were 66.07 ± 4.79 (2.5% FWHM) and 67.73 ± 5.58 (Control). Statistical analysis revealed that FWHM inclusion did not significantly affect eggshell weight (SW) and eggshell thickness (ST) (P > 0.05). Mean SW values ranged from 7.53 ± 0.79 g (Control) to 7.79 ± 0.92 g (2.5% FWHM), and mean ST values ranged from 0.59 ± 0.11 mm (2.5% FWHM) to 0.62 ± 0.13 mm (5% FWHM).

3.4 Effects of fermented water hyacinth meal on egg shelf life

The effects of dietary inclusion of fermented water hyacinth meal (FWHM) on egg quality during storage are summarized in Table 6. Yolk weight declined significantly over storage in the control (0.0% FWHM; P = 0.009) and 2.5% FWHM (P = 0.010) groups, while no significant change was observed in the 5.0% group (P = 0.537). Regression analysis revealed a strong linear decline in yolk weight for the 0.0% and 2.5% groups (R^2 > 0.80), while a cubic trend was evident for the 5.0% group (Figure 1A). Yolk diameter increased progressively with storage time (P = 0.002), with consistently higher values in the 2.5% and 5.0% groups relative to the control. Yolk height was significantly affected by treatment (P = 0.042), remaining higher in the 2.5% and 5.0% groups across most weeks. The yolk index declined with storage (P = 0.022), with the most pronounced reduction in the 2.5% group. The relationship between yolk index and shelf time was quadratic, increasing initially, peaking, and then decreasing as storage progressed (Figure 1B). Yolk pH increased significantly with storage time (P = 0.002). At week 1, values ranged from 6.45 to 6.88, but by week 4, they had exceeded 8.0 in the control and 2.5% FWHM groups, while the 5.0% group maintained lower values (7.29). Regression analyses showed higher rates of pH increase in the 2.5% group (0.583 pH units/week; R2 = 0.896), followed by the control (0.460; R2 = 0.920) and the 5.0% group (0.243; R2 = 0.852) (Figure 2B).

Albumen weight increase during storage (P < 0.001), ranging from 31.9 g at week 1 (control) to 45.7 g at week 4 (control). Treatment effects became evident in later weeks, with the 5.0% FWHM group consistently maintaining higher albumen weights than the control. Regression analysis revealed strong linear increases in albumen weight for all treatments, with gradients of 4.83, 2.10, and 4.38 g/week for the 0.0%, 2.5% and 5.0% FWHM groups, respectively (Figure 2A).

Haugh Unit values declined progressively with storage duration, approaching statistical significance (P = 0.052). Regression analysis showed linear decreases, with steeper declines in the 2.5% group (-3.85 HU/week; R2 = 0.784) compared to the control (-2.80; R2 = 0.789) and 5.0% group (-1.67; R2 = 0.852) (Figure 3A). Based on USDA grading, week 1–2 egg from the 5.0% group remained in grade AA, while weeks 3–4 dropped to grade A. In contrast, control eggs declined to grade B by week 3, while the 2.5% group maintained grade A until week 4.

Egg weight loss increased with storage time, although treatment differences were not significant (P = 0.070). Regression analysis indicated that the rate of loss was higher in the 2.5% FWHM (-0.50 g/week) and 0.0% (0.40 g/week) groups compared to the 5.0% group (-0.215 g/week) (Figure 3B). Weight loss was most pronounced during the early storage weeks.

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TABLE 5 Effects of fermented water hyacinth meal on internal egg quality and eggshell characteristics.

	Dietary treatments			
	0% FWHM	2.5% FWHM	5% FWHM	
Parameter	Mean ± SD	Mean ± SD	Mean ± SD	Significance
Yolk Weight (mm)	1.73 ± 1.37	14 ± 1.48	13.63 ± 0.94	
Albumen Weight (mm)	31.94 ± 2.86	33.84 ± 6.71	32.80 ± 4.47	
Yolk pH	6.70 ± 0.14[b]	6.62 ± 0.091[a]	6.60 ± 0.098[a]	*
Yolk Colour (Roche scale)	14.32 ± 0.67	14.79 ± 0.56	14.38 ± 0.61	
Eggshell Weight (g)	7.53 ± 0.79	7.79 ± 0.92	7.73 ± 0.70	
Eggshell Thickness (mm)	0.60 ± 0.12	0.59 ± 0.11	0.62 ± 0.13	
Yolk Diameter (mm)	42.69 ± 2.68[b]	40.65 ± 2.04[a]	41.03 ± 1.76[ab]	*
Yolk Height (mm)	13.88 ± 1.76	13.37 ± 2.14	13.45 ± 1.41	
Yolk Index	0.33 ± 0.04	0.33 ± 0.04	0.33 ± 0.04	
Hugh Unit	67.73 ± 5.58	66.07 ± 4.79	66.53 ± 4.00	

[a, b] means in the same row with different superscripts are significantly different at $p < 0.05$.

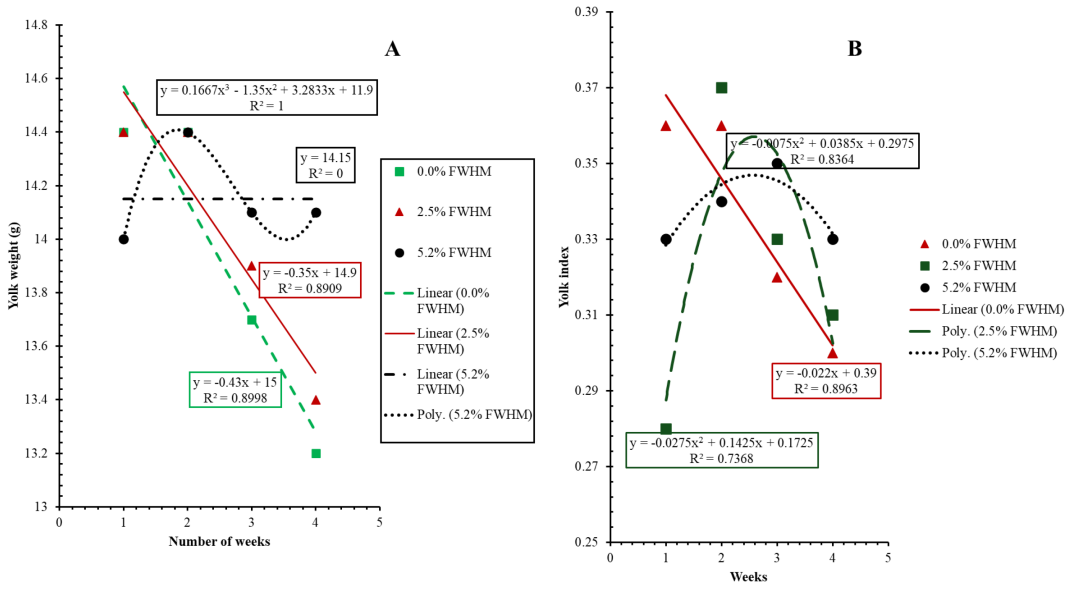


FIGURE 1
(A) changes of yolk weight with time. **(B)** changes of yolk index with time.

4 Discussion

4.1 Effect of fermented water hyacinth meal in diet on oviduct morphometric characteristics

Quantitative analyses showed that layer hen oviduct parameters, including oviduct length, infundibulum length, magnum length, isthmus length, eggshell gland length, oviduct weight, isthmus weight, and eggshell gland weight, were not significantly affected by dietary treatments, implying that FWHM can be integrated into layer diets at levels up to 5% without eliciting adverse effects on oviduct development and function. These findings are consistent with previous studies that have reported no adverse effects of fermented feed ingredients on reproductive tract morphology and function in laying hens, since egg weight and laying performance was improved by feeding fermented feeds (Chen et al., 2023; Guo et al., 2022; Engberg et al., 2009). However, significant differences were observed in infundibulum weight (IW) and magnum weight (MW). These findings suggest that FWHM may have a dose-dependent effect on oviduct morphology, particularly in the infundibulum and magnum regions. Lu et al. (2023) reported increased oviduct weight in hens fed

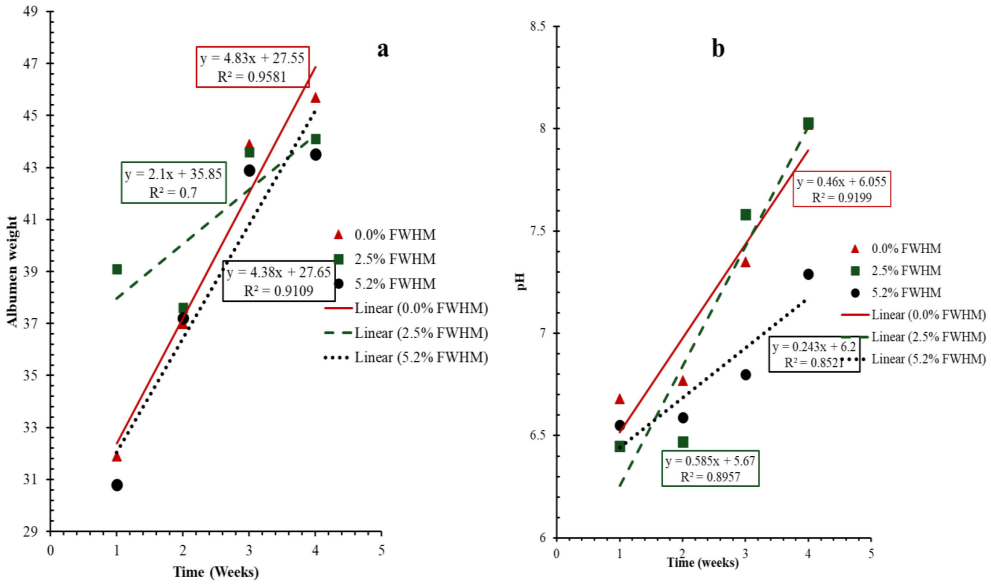


FIGURE 2
The relationship between shelf time and **(a)** albumen weight **(b)** pH.

miscellaneous fermented feed and attributed these findings to possible upregulation of reproductive endocrine function, specifically the coordinated action of FSH, LU and estrogen.

Fermented feed in laying hen diets can increase the mRNA expression of the hormone receptors in the ovary hence increased hormone sensitivity which can affect oviduct development (Jiang et al., 2024). The increased IW in the 5% FWHM treatment group may indicate enhancement of oviduct function, potentially leading to improved egg production or quality, as suggested by Etches (1996). Conversely, the decrease in MW in the 5% FWHM group

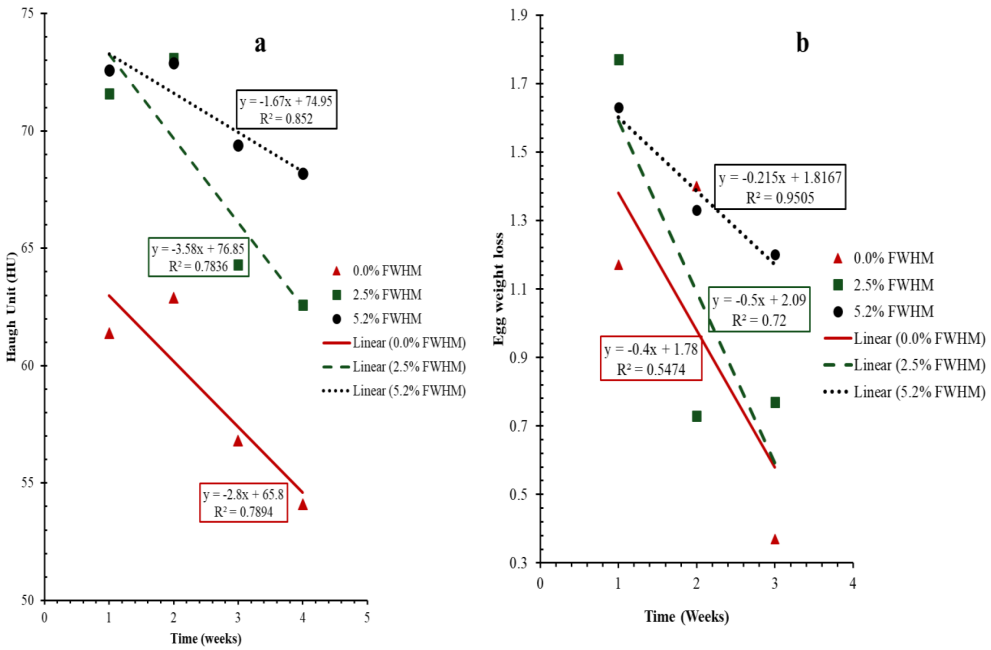


FIGURE 3
The relationship between shelf time with **(a)** Haugh unit **(b)** egg weight loss.

TABLE 6 Effects of fermented water hyacinth meal on egg quality changes during storage.

Parameter/Week	Week 1	Week 2	Week 3	Week 4
Yolk weight (g)	0%: 14.4 ± 0.33^{a} 2.5%: 14.4 ± 0.22^{ab} 5%: 14.0 ± 0.38b	0%: 14.4 ± 0.27^{a} 2.5%: 14.4 ± 0.28^{a} 5%: 14.4 ± 0.19	0%: 13.7 ± 0.56^{b} 2.5%: 13.9 ± 0.46^{b} 5%: 14.1 ± 0.54	0%: 13.2 ± 0.12^{b} 2.5%: 13.4 ± 0.15^{b} 5%: 14.1 ± 0.49
Yolk diameter (mm)	0%: 33.5 ± 0.23^{a} 2.5%: 43.1 ± 0.36 5%: 41.9 ± 1.10^{a}	0%: 36.3 ± 5.98^{ab} 2.5%: 44.3 ± 2.18 5%: 44.4 ± 1.06^{b}	0%: 37.2 ± 4.81^{ab} 2.5%: 41.0 ± 7.31 5%: 44.2 ± 1.05^{b}	0%: 42.9 ± 2.31^{b} 2.5%: 44.3 ± 3.67 5%: 44.5 ± 1.49^{b}
Yolk height (mm)	0%: 11.9 ± 1.28 2.5%: 12.5 ± 1.09^{a} 5%: 13.9 ± 1.00^{a}	0%: 13.0 ± 2.34 2.5%: 16.4 ± 1.04^{b} 5%: 15.3 ± 1.02^{b}	0%: 11.6 ± 0.95 2.5%: 13.7 ± 2.95^{ab} 5%: 15.3 ± 0.69^{b}	0%: 12.9 ± 3.00 2.5%: 13.6 ± 1.17^{ab} 5%: 14.6 ± 0.80^{ab}
Yolk index	0%: 0.36 ± 0.04 2.5%: 0.28 ± 0.02^{a} 5%: 0.33 ± 0.02	0%: 0.36 ± 0.10 2.5%: 0.37 ± 0.02^{b} 5%: 0.34 ± 0.02	0%: 0.32 ± 0.05 2.5%: 0.33 ± 0.01^{ab} 5%: 0.35 ± 0.01	0%: 0.30 ± 0.08 2.5%: 0.31 ± 0.04^{a} 5%: 0.33 ± 0.03
Albumen weight (g)	0%: 31.9 ± 4.25^{a} 2.5%: 39.1 ± 10.6 5%: 30.8 ± 4.29^{a}	0%: 37.0 ± 7.62^{ab} 2.5%: 37.6 ± 7.18 5%: 37.2 ± 5.98^{b}	0%: 43.9 ± 2.08^{bc} 2.5%: 43.6 ± 1.50 5%: 42.9 ± 1.14^{c}	0%: 45.7 ± 1.05^{c} 2.5%: 44.1 ± 0.99 5%: 43.5 ± 1.24^{c}
Yolk pH	0%: 6.68 ± 0.06^{a} 2.5%: 6.45 ± 0.39^{a} 5%: 6.55 ± 0.05^{a}	0%: 6.77 ± 0.10^{a} 2.5%: 6.47 ± 0.14^{a} 5%: 6.59 ± 0.16^{a}	0%: 7.35 ± 0.58^{b} 2.5%: 7.58 ± 0.60^{b} 5%: 6.80 ± 0.52^{a}	0%: 8.02 ± 0.14^{c} 2.5%: 8.03 ± 0.47^{b} 5%: 7.29 ± 0.47^{b}
Haugh Unit	0%: 61.4 ± 1.96^{a} 2.5%: 71.6 ± 0.96^{ab} 5%: 72.6 ± 1.03	0%: 62.9 ± 6.23^{a} 2.5%: 73.1 ± 4.25^{a} 5%: 72.9 ± 1.06	0%: 56.8 ± 2.29^{ab} 2.5%: 64.3 ± 5.29^{bc} 5%: 69.4 ± 5.88	0%: 54.1 ± 1.18^{b} 2.5%: 62.6 ± 6.23^{c} 5%: 68.2 ± 7.04
Egg weight loss (g)	0%: 1.17 ± 0.75 2.5%: 1.77 ± 0.60 5%: 1.63 ± 0.99	0%: 1.40 ± 0.17 2.5%: 0.73 ± 0.49 5%: 1.33 ± 0.12	0%: 0.37 ± 0.12 2.5%: 0.77 ± 0.42 5%: 1.20 ± 0.09	0%: Nd 2.5%: Nd 5%: Nd

Superscript letters. (^{a}, ^{b}, ^{c}) indicate statistically significant differences between treatments within each week ($P < 0.05$). Nd, Not determined; Units, Yolk weight & Albumen weight (g); Yolk diameter & height (mm); Yolk pH (unitless); HU, Haugh Unit; Egg weight loss (g).

may affect albumen synthesis or secretion by reducing the production of proteins such as ovalbumen, ovo-transferrin, and ovomucoid, which make up the albumen. Considering prior work (Cole, 1938; Wyburn et al., 1970; Moynihan and Edwards, 1975) linking magnum morphology to albumen conditions, this reduction in MW could potentially affect egg quality, possibly through a decrease in the total surface epithelium area available for secretion within the magnum as suggested by the relationship between magnum fold height and Haugh unit (HU) values in the cited study. However, Haugh unit values in the present study confirm that FWHM does not compromise albumen quality.

4.2 Effects of fermented water hyacinth meal on laying performance

The present study demonstrates that dietary inclusion of fermented water hyacinth meal (FWHM) at 2.5% and 5% did not affect cumulative egg production, hen-week lay percentage, or weekly egg output in Hy-Line Brown hens. Guo et al. (2022) indicated that fermented fed or unconventional protein sources can partially replace soybean meal in layer diets without compromising productivity (Pirgozliev et al., 2023; Abdel-Wareth et al., 2024). The absence of significant variation among treatments suggests that FWHM is a viable alternative feed ingredient when used at moderate inclusion levels. The beneficial effects observed are likely attributable to the fermentation process, which improves protein digestibility and reduces anti-nutritional factors such as oxalates, tannins, and phytates (Zhou et al., 2023). Additionally, microbial biotransformation (Van Hylckama Vlieg et al., 2011) during fermentation may enhance nutrient availability and support gut health, thereby contribute ng to the maintenance of laying performance (Katu et al., 2025). However, the slight numerical reduction in performance at 5% FWHM inclusion level may reflect the limitations imposed by residual fiber (Singh and Kim 2021) and secondary plant compounds, which can affect nutrient utilization at higher levels (Abdel-Moneim et al., 2020).

The incorporation of FWHM into poultry diets holds significant promise, particularly in resource-constrained contexts where feed cost and ingredient availability remain critical constraints and a way of valorizing invasive aquatic weed (Makkar and Ankers, 2014). Fermented water hyacinth meals present a cost-effective and ecologically responsible option for enhancing poultry feed systems without compromising productivity.

4.3 Effects of fermented water hyacinth meal on internal egg quality and eggshell characteristics

This study evaluated the effects of dietary inclusion of fermented water hyacinth meal (FWHM) on internal egg quality parameters and eggshell characteristics in Hy-Line laying hens. A significant reduction in yolk pH was observed in hens fed diets containing 2.5% and 5% FWHM compared to the control group, with values ranging

from 6.60 to 6.70. These values remain within the established normal range for fresh egg yolk pH (6.0 – 6.8), as reported in previous studies on egg quality dynamics (Feddern et al., 2017).

A lower yolk pH in layer birds fed dietary treatment with FWHM suggested an improved egg quality and stability and it accords with (Cedro et al., 2009). A more acidic yolk environment has been linked to reduced bacterial proliferation, particularly that of neutrophilic species like Salmonella spp (McWahorter et al., 2021), and decreased lipid oxidation rates, contributing to enhanced egg safety and extended shelf life (Cedro et al., 2009). The ability of FWHM to modulate yolk pH could be associated with its prebiotic influence on the gut microbiota of laying hens (Dai et al., 2022). Fermentation processes and prebiotic fibers in FWHM alter microbial populations in the gastrointestinal tract, enhancing nutrient absorption and producing microbial metabolites such as short-chain fatty acids (SCFAs), which are known to influence metabolic pathways and potentially influence yolk composition (Park et al., 2016). Similar findings have been reported in studies examining the effects of fermented feed ingredients on poultry health and quality parameters (Park et al., 2016; Choi et al., 2018; Guo et al., 2022).

Conversely, eggshell characteristics such as eggshell weight, eggshell thickness, and Haugh units were not significantly affected by dietary inclusion of FWHM up to 5% inclusion, aligning with findings by Lu et al. (2008), who reported similar results when feeding water hyacinth to ducks. This suggests that FWHM does not negatively affect eggshell integrity or albumen quality at moderate inclusion levels. These results agree with the findings from prior research on unconventional feed ingredients, where no adverse effects on egg and eggshell quality were observed (Engberg et al., 2009; Park et al., 2016; Tian et al., 2022; Zhou et al., 2023). However, studies using higher concentrations of fermented plant-based ingredients have shown varied effects on eggshell parameters, highlighting the importance of inclusion level and feed processing methods in determining overall egg quality parameters. For instance, Yang et al. (2022) observed that an 8% inclusion of fermented corn by-products in laying hens' diets led to decreased eggshell strength and shape index compared to control groups.

Notably, yolk diameter significantly decreased among hens fed FWHM-supplemented diets. Yolk diameter is influenced by lipid and protein deposition during oocyte maturation, particularly through the hepatic synthesis and oocyte uptake of very low-density lipoprotein (VLDL) and vitellogenin (Schneider, 2016). The reduction observed in the FWHM groups may indicate alterations in nutrient partitioning, possibly due to changes in digestibility or absorption associated with FWHM inclusion due to altered gut morphology (Zhang et al., 2023). Similar alterations in yolk proportions have been reported in hens fed non-conventional feed ingredients, suggesting dietary modulation of egg formation processes (Kowalska et al., 2021). Investigating the effects of FWHM on lipid and protein metabolism in laying hens would be valuable in elucidating the underlying mechanisms contributing to these changes.

The findings of this study demonstrate that dietary inclusion of FWHM up to 5% significantly lowers yolk pH, potentially enhancing egg safety and shelf life, without negatively influencing eggshell characteristics or albumen quality. However, the observed reduction in yolk diameter suggests the need for further investigation into the role of FWHM in nutrient utilization and egg formation.

4.4 Effects of fermented water hyacinth meal on egg quality changes during storage

The present study demonstrated that storage markedly influenced egg quality traits, and the effects varied depending on the level of fermented water hyacinth meal (FWHM) in the diet. Across treatments, yolk weight generally decreased with storage time, consistent with earlier reports that storage accelerates water migration from the albumen into the yolk, leading to dilution and shrinkage of yolk solids (Lee et al., 2016; Akter et al., 2014). Interestingly, this decline was significant in eggs from hens fed 0% and 2.5% FWHM, but not in the 5% FWHM treatment group, which instead displayed a cubic relationship. This suggests that higher FWHM inclusion may alter the dynamics of water and solute redistribution between yolk and albumen during storage. Rather than a steady loss, the yolk may undergo temporary stabilization or recovery phases before further decline, possibly linked to compositional or structural changes induced by FWHM in lipids, proteins, or membrane integrity.

The yolk index declined progressively with storage time, consistent with the reduction in yolk height and weakening vitelline membrane (Tabidi, 2011; Drabik et al., 2021). Notably, only the 2.5% FWHM group showed a significant response, with transient increase before decline, implying short-term membrane stabilization. This slower rate of deterioration indicates that moderate FWHM inclusion may prolong egg shelf life by enhancing yolk structural integrity.

With respect to albumen quality, albumen weight increased over time across all treatments, consistent with moisture redistribution during storage. The rate of increase was slowest in the 2.5% FWHM treatment, which may imply reduce water migration into the yolk fraction compared to the control and the 5% treatment. In contrast, albumen pH, an indicator of chemical changes associated with egg freshness (Drabik et al., 2021), rose most rapidly in the 2.5% group, due to CO_2 loss through eggshell pores (Eke et al., 2013; Kumari et al., 2020). This suggests that moderate FWHM inclusion may modify buffering capacity or ionic composition, thus accelerating pH changes independently of water redistribution.

The Haugh Unit (HU), a standard measure of internal egg quality (Jones, 2012; Khaleel, 2019; Maggonage et al., 2024), decrease linearly with storage time in all treatments, consistent with deterioration of albumen height during storage (Tabidi, 2011).

Egg from the 5% FWHM treatment retained higher HU values for longer, maintaining Grade AA and Grade A quality up to week 4. In contrast, control eggs dropped to Grade B by week 4. This finding is particularly relevant from a market perspective, as consumers and retailers prefer eggs with higher HU values. The improved HU stability in FWHM-fed groups suggests a potential role of bioactive compounds or antioxidants present in water hyacinth or derived from the fermentation process which could delay albumen protein degradation and maintain albumen viscosity.

Egg weight loss also followed typical storage patterns (Yamak et al., 2021), with greater losses occurring in early weeks. Notably, weight loss was lowest in the 5% FWHM group, which may point to eggshell quality improvements or reduced permeability. This observation is noteworthy, as eggshell properties are closely linked to mineral metabolism, particularly calcium and phosphorus dynamics (Neijat et al., 2011; Yan et al., 2023). In a companion study (Antonio et al., 2025), phosphorus retention was significantly improved in FWHM-fed birds, suggesting a possible nutritional mechanism whereby enhanced phosphorus utilization contributes to superior eggshell mineralization and barrier function. While these findings are consistent, confirmatory analyses of eggshell mineral composition and microstructure would be required to establish a direct causal link.

The present findings demonstrate that dietary fermented water hyacinth meal (FWHM) exerts measurable influences on the dynamics of egg quality decline during storage, with dose-dependent effects. At 2.5% inclusion, FWHM transiently stabilized yolk index and moderated water redistribution between yolk and albumen, while at 5% inclusion eggs exhibited superior Haugh Unit retention, reduced evaporative weight loss, and improved eggshell barrier function. These responses are consistent with potential roles of fermentation-derived bioactive compounds in slowing albumen protein degradation, as well as enhanced phosphorus utilization supporting eggshell mineralization. Collectively, these findings show that 5% FWHM inclusion can prolong egg shelf life by stabilizing albumen and yolk quality while enhancing eggshell integrity.

5 Conclusion

This study demonstrates that dietary inclusion of fermented water hyacinth meal (FWHM) up to 5% in laying hen diets does not significantly alter most oviduct morphometric characteristics, productive performance or core indicators of egg quality and shelf life. The stability of parameters such as oviduct length, dimensions, and overall oviduct weight suggests that FWHM does not impair reproductive tract development or function. However, significant changes in infundibulum and magnum weights indicate possible dose-dependent effects, which may influence albumen synthesis and warrant further investigation. However, Haugh unit values confirm that FWHM does not compromise albumen quality or eggshell structure. Nevertheless, the observed reduction in yolk diameter suggests a possible shift in nutrient partitioning or deposition, meriting further exploration of the metabolic effects of FWHM inclusion.

The significant reduction in yolk pH observed in the FWHM-fed groups, though within the normal physiological range may reflect a more stable biochemical environment, potentially enhance microbial resistance and extend egg shelf life. Collectively, the findings support the use of FWHM as a sustainable and nutritionally viable feed ingredient at inclusion levels up to 5%. However, subtle shifts in infundibulum and magnum weights highlight the need for additional research to optimize dietary formulations and better construe the physiological mechanisms and processes involved.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by National Animal Research Ethics Commitiee (NAREC). Zimbabwe Animal Act, Chapter19:12,1963, License number L729 (Scientific Experiments on Animal Act,1963;03 2025-18). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

RN: Visualization, Writing – original draft, Formal analysis, Funding acquisition, Project administration, Conceptualization, Methodology, Supervision, Writing – review & editing. TC: Investigation, Writing – review & editing, Writing – original draft, Formal analysis, Visualization, Methodology. JB: Project administration, Data curation, Funding acquisition, Resources, Writing – original draft, Writing – review & editing, Investigation, Validation. JA: Validation, Methodology, Visualization, Formal analysis, Data curation, Investigation, Conceptualization, Writing – review & editing, Funding acquisition, Writing – original draft.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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