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NUTRIENT CONTENT OF QUAIL EGGS AS INFLUENCED BY DIETS ENHANCED WITH WATERLEAF MEAL

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ABSTRACT

This study assessed the nutritive value of quail eggs as influenced by diets enhanced with waterleaf meal. Three diets tagged W0 (100% layer's feed), W20 (80% layers' feed plus 20% waterleaf) and W40 (60% layers' feed plus 40% waterleaf) were used for this study. Ninety quails were used for the trial. They were divided into three groups of 30 birds each which were further divided into 10 birds per replicate. Each group was randomly allotted to the three diets in a Completely Randomized Design experiment which lasted 3 weeks. Three eggs per treatment per week or 9 eggs per group were collected for analysis of proximate composition, minerals, cholesterol and cholesterol fractions. Data was analysed using One-way Analysis of Variance in Statistical Package for Social Sciences. Tukey Post-Hoc test was used to separate significant means at 5% level of significance. Results show that percent DM, CP, EE, ash and NFE as well as GE (kcal/g) ranged from 28.16–28.39, 14.64–14.93, 10.23–10.39, 2.40–2.56, 0.67–0.74 and 1.97–1.99, respectively. The Na, K, Ca, P, Mg, Fe, Zn, Se, I, Cu and Mn values (mg/100g) ranged from 144.67–145.08, 138.78–139.04, 64.52–65.08, 178.68–179.13 and 78.48–79.35, 3.40–3.64, 0.47–0.54, 0.014–0.018, 0.88–0.94, 0.32–0.37 and 0.68–0.75, respectively. The contents for total cholesterol, HDL, LDL and VLDL (mg/100g), lipid peroxidation (MDA/g) and triglycerides (mg/100g) ranged from 871.70–986.37, 418.80–526.40, 238.50–347.67, 18.67–19.40 13.66–14.53 and 75.15–195.77, respectively. There were significant differences ($p<0.05$) among treatment means for all the parameters studied. Inclusion of waterleaf in quail diets increased ash and most minerals in the eggs as well as reduce the cholesterol and peroxidation of egg fats, potentially minimizing the risk for atherosclerosis in the quail egg consumers. We recommend inclusion of 20% waterleaf in quail diets to enhance the egg nutritive value.

Keywords: Poultry, Eggs, Forage, Cholesterol, Minerals, Proximate Composition.

1. INTRODUCTION

The cost of poultry feed is increasing and becoming unaffordable to smallholder farmers, even as poultry feed constitutes 60–70% of the total cost of producing eggs, especially, under intensive production systems (Wongnaa *et al*., 2023). Hence, farmers that are unable to afford this high operational cost, but want to increase their profits or stay in business, need to find ways to cut their costs of production. The easiest and most impactful way to cut costs of animal production is through reducing the cost of feed (Mallick *et al*., 2020). Intensive commercial feeding of poultry usually depends mostly on compounded rations. However, with the emergence of organic poultry production, feeding chickens with only compound feed and rearing them indoors has become a questionable feeding strategy that negatively affects their welfare because chickens naturally forage; feeding on insects and fodder under extensive or semi-extensive systems (Tufarelli *et al*., 2018). Therefore, due to animal welfare and cost concerns as well as consumer trend moving towards organically produced eggs and meat, the organic egg market share is rising. This trend implies that there is an increased need for the regulation of the organic poultry sector to reduce sharp practices in organic labelling. Hence, the European Union, through the EU Organic Regulatory Board stipulates that for poultry to be certified organic, roughage, fodder (fresh or dried) must be added to poultry ration besides giving them access to pasture to complement their dietary needs and express their natural behaviour (Crawley, 2015). Hence, about 20% of the daily dry matter intake of layers can be sourced from forage (Crawley, 2015).

Several studies have demonstrated that inclusion of forage in the feed of poultry could reduce the cost of concentrate feeds by as much as 30% (Fanatico, 2003). The forage can be fed to birds by allowing them free range for some part of the day or cutting the forage and feeding to the birds under intensive system. However, there are serious risks associated with free ranging poultry, especially quails. Those include threats posed by predators and managerial hiccups that may impact costs significantly (Amato and Castellini, 2022; Crandall *et al*., 2009). In addition, allowing quails freedom of movement means offering them opportunity to escape captivity because at this stage in their domestication process, quails, unlike chickens, are not docile enough to return to the coop after pasturing. To obviate these risks and still provide the quails access to forage, most farmers opt for cutting the forage and feeding to the birds reared intensively. One of such forages with the potential to be fed to quails is waterleaf (*Talinum triangulare*), a short-term permanent vegetable crop with succulent stems and green leaves that grows fast. Waterleaf is rich in calcium, magnesium, potassium, ascorbic acid and vitamin B6 (Ikewuchi *et al*., 2017; Igwe *et al*., 2015). Poultry easily eat waterleaf because it is succulent and poor in fibre (Ekine *et al*., 2020).

Waterleaf has been fed to broiler and layer chickens as leaf meals or aqueous extract, to improve their performance and minimize the cost of their feeding (Sanda, 2015; Ekine *et al*., 2020; Nworgu *et al*., 2014). But supplementing layer quail feed with waterleaf and the effect such a practice would have on quail egg and egg quality is poorly researched. There is need for a study of the effect of supplementing layer quail feed with fresh waterleaf meal on quail eggs and their quality. Results might be used to improve the quality of quail eggs and enhance the performance of layer quails.

2. MATERIALS AND METHODS

2.1 Study location

The study was conducted at the University of Port Harcourt Teaching and Research Farm, Choba, Rivers State, Nigeria. The location is 16 meters above sea level and located at latitude 4°47’21" North and longitude 6°59'55" East of the Equator. It is in the humid rainforest zone of Nigeria with prolonged precipitation which spans from March to November while the dry season is short. The mean annual temperature in this area ranges from 25–28°C while the relative humidity is higher than 80% (Ijeomah *et al*., 2013).

2.2 Experimental quails and waterleaf

The experiment used 90 unsexed Japanese quails aged three weeks and purchased from a quail supplier in Aluu, Port Harcourt, Nigeria. The experimental waterleaf was grown at the University of Port Harcourt Teaching and Research Farm, Choba, Rivers State. Waterleaf was propagated using the stems which were sown on raised beds sized 2.0m x 5.0m and spaced 15cm x 15cm. Poultry faecal droppings were used as manure. All the waterleaf cultivation practices were according procedures proffered by (Ndaeyo, 2013). The waterleaf was first harvested at 4 weeks after planting and daily thereafter for use in the experiment. Daily, freshly harvested waterleaf was air-dried overnight to wilt before feeding to the quails.

2.3 Experimental design

There were three experimental groups in the study. Group 1 was the Control labelled W0 while groups 2 and 3 were labelled W20 and W40. In W0, the birds were fed 100% concentrate feed without waterleaf. In W20, the quails were fed 80% concentrate feed and 20% water leaf, while in W40, the quails were fed 60% concentrate feed and 40% water leaf. The ninety quails were first divided into three groups of 30 birds each. The 30 birds were weighed initially. The initial weights of the three groups were balanced to avoid initial bias. Each of the group of 30 birds were then randomly allocated to the three dietary treatments (W0, W20 and W40). Furthermore, within each group, the 30 quails were further subdivided into three replicates of 10 quails each. Completely Randomized Design (CRD) was the experimental design used for the study. The experimented lasted three weeks.

2.4 Diets and feeding

An analysis of the commercial layer diets showed they contained 22% crude protein 2800kcal ME/kg. The layer mash was offered from 6th week to the end of the study at three weeks later (9th week of age). The wilted waterleaf was chopped with a kitchen knife and mixed with the feed at the ratio of 0% waterleaf to 100% concentrate feed (W0 or control); 20% waterleaf to 80% concentrate feed (W20); and 40% waterleaf to 60% concentrate feed (W40) before feeding to the quails. The quails were fed twice daily at 8.00 hours and 17.00 hours. Clean fresh water was offered *ad-libitum*. The feeders were an 8-hole flip-top plastic type necessary to stop the spilling and wasting of feed by the quails.

2.5 Management and housing

The quails were housed in wire cages that were cleaned and disinfected regularly. Two weeks before the quails arrived, the house was washed and fumigated with boiled water and Hypo solution. The feeders and drinkers were washed and disinfected in the same solutions.

2.6 Collection of egg samples

The birds started laying at 6 weeks of age. From the 7th week of age (4 weeks of experiment) three eggs per replicate and 9 eggs per treatment group were collected per week for nutritional analysis. Egg collection was done for three weeks and stopped when the birds were 9 weeks old.

2.7 Nutritional analysis of eggs

The eggs were analysed for DM, GE and proximate composition (CP, ash and EE) while NFE was calculated by difference. The DM was determined by drying the samples to a constant weight in an oven set at 105°C. The crude protein content was determined by the Kjeldahl method while EE was determined by the Soxhlet method. The ash content was determined by ashing the samples overnight in an oven at 550°C. The determinations and calculations were according to AOAC (2005). Also, some minerals (Ca, P, Na, K, Mg, Mn, Fe, I, Se, Cu, and Zn) were analysed according to the methods of AOAC (2005). Flame photometer 410 was used for sodium, spectrophotometer for phosphorus while Perki-Elmer Atomic Absorption Spectrophotometer 2380 was used for Ca, Mn, Fe and Zn. Three eggs were broken and homogenized for 1gram of the whole egg sample was weighed into a 100ml conical flask, containing 20ml of buffer and a pH of 6.3. Next, 50ml of 0.75% LDL detergent solution was added and shaken to solubilize the cholesterol from a non-LDL particle. The cholesterol was precipitated to form a clear colourless suspension. Furthermore, 50ml of 0.25% LDL detergent solution was added to the mixture to produce a blue colour. The increase in absorbance of the blue colour solution at a wavelength of 540nm is directly proportional to the concentration of LDL. LDL in mg/100g = increase in Absorbance x Df. The same process was followed for HDL analysis.

2.8 Data analysis

The data was analysed with the Statistical Package for Social Sciences (SPSS) using descriptive statistics and One-way Analysis of Variance (ANOVA) to spot significant differences in the means. Tukey Post-Hoc test was used to separate significant means at 5% level of significance. Data was presented in tables.

3. RESULTS

3.1 Proximate composition of eggs from quails fed diets supplemented with waterleaf

Table 1 shows the proximate composition of eggs from quails fed diets supplemented with waterleaf meal. The results indicate that percent DM, CP, EE, ash and NFE as well as GE (kcal/g) values ranged from 28.16–28.39, 14.64–14.93, 10.23–10.39, 2.40–2.56, 0.67–0.74 and 1.97–1.99, respectively. There were significant differences ($p<0.05$) among treatment means for proximate composition indices and gross energy. Group W0 had the highest ($p<0.05$) DM, CP, EE and GE values while group W20 recorded the least ($p<0.05$) values for these indices. Nevertheless, the CP and EE values of group W40 were not different ($p>0.05$) from that of group W0. Furthermore, group W20 recorded the highest ($p<0.05$) ash and NFE values while group W0 recorded the least ($p<0.05$). But, the ash and NFE values of group W40 were not different ($p>0.05$) from those of group W0.

Table 1: Proximate composition of eggs from quails fed diets supplemented with waterleaf

	Waterleaf inclusion levels (%)				
Components (%DM)	**W0**	**W20**	**W40**	***±SEM***	***p-value***
DM	28.39[a]	28.16[c]	28.30[b]	0.034	0.000
CP	14.93[a]	14.64[b]	14.83[a]	0.046	0.001
EE	10.39[a]	10.23[b]	10.34[a]	0.024	0.000
Ash	2.40[b]	2.56[a]	2.45[b]	0.025	0.002
NFE	0.67[b]	0.74[a]	0.68[b]	0.012	0.005
GE (kcal/g)	1.99[a]	1.97[c]	1.98[b]	0.003	0.000

[a b c] Means in same row with different superscripts are significantly different ($p<0.05$); DM=dry matter; CP=crude protein; EE=ether extract; NFE=nitrogen free extract; GE=gross energy.

3.2 Macro-minerals in eggs from quails fed diets supplemented with waterleaf

The macro-minerals in eggs from quails fed diets supplemented with waterleaf are presented in Table 2. Results indicate that the Na, K, Ca, P and Mg values (mg/100g) ranged from 144.67–145.08, 138.78–139.04, 64.52–65.08, 178.68–179.13 and 78.48–79.35, respectively. There were significant differences ($p<0.05$) among treatment means for all the macro-minerals studied. All the macro-minerals contents of group W0 were the least ($p<0.05$) while those of group W20 were the highest ($p<0.05$).

Table 2: Macro-minerals in eggs from quails fed diets supplemented with waterleaf

	Waterleaf inclusion levels (%)				
Minerals (mg/100g)	**W0**	**W20**	**W40**	***±SEM***	***p-value***
Na	144.67[c]	145.08[a]	144.77[b]	0.062	0.000
K	138.78[c]	139.04[a]	138.91[b]	0.038	0.000
Ca	64.52[c]	65.08[a]	64.64[b]	0.085	0.000
P	178.68[c]	179.13[a]	178.87[b]	0.065	0.000
Mg	78.48[c]	79.35[a]	78.61[b]	0.136	0.000

[a b c] Means in same row with different superscripts are significantly different ($p<0.05$); Na=sodium; K= potassium; Ca= calcium; P= phosphorus; Mg=

magnesium

3.3 Micro-minerals in eggs from quails fed diets supplemented with waterleaf meal

Table 3 shows the micro-minerals in eggs from quails fed diets supplemented with waterleaf. Results show that the Fe, Zn, Se, I, Cu and Mn contents in mg/100g ranged from 3.40–3.64, 0.47–0.54, 0.014–0.018, 0.88–0.94, 0.32–0.37 and 0.68–0.75, respectively. There were significant differences ($p<0.05$) among treatment means for all the micro-mineral contents. Group W20 had the highest ($p<0.05$) values for all the micro-minerals while group W0 had the least ($p<0.05$) values. However, the selenium value for group W40 was not different from those of group W0 and W20 while the least iodine value of group W0 was not different ($p>0.05$) from that of group W40. Also, the group W40 value of copper was not different ($p>0.05$) from those of groups W0 and W20.

Table 3: Micro-minerals in eggs from quails fed diets supplemented with waterleaf

	Waterleaf inclusion levels (%)				
Minerals (mg/100g)	**W0**	**W20**	**W40**	***±SEM***	***p-value***
Fe	3.40^{c}	3.64^{a}	3.58^{b}	0.036	0.000
Zn	0.47^{c}	0.54^{a}	0.50^{b}	0.011	0.000
Se	0.014^{b}	0.018^{a}	0.016^{ab}	0.001	0.046
I	0.88^{b}	0.94^{a}	0.90^{b}	0.009	0.003
Cu	0.32^{b}	0.37^{a}	0.34^{ab}	0.008	0.009
Mn	0.68^{c}	0.75^{a}	0.72^{b}	0.011	0.000

^{abc} Means in same row with different superscripts are significantly different ($p<0.05$); Fe= iron; Zn= zinc; Se= selenium; I= iodine; Cu= copper; Mn= manganese

3.4 Cholesterol and its fractions in eggs from quails fed diets supplemented with waterleaf

Table 4 shows cholesterol and cholesterol fractions in eggs from quails fed diets supplemented with waterleaf. Results indicate that the contents for total cholesterol, HDL, LDL, VLDL (mg/100g), lipid peroxidation (ngMDA/g) and triglycerides (mg/100g) ranged from 71.70–86.37, 418.80–526.40, 238.50–347.67, 18.67–19.40 13.66–14.53 and 75.15–195.77, respectively. There were significant differences ($p<0.05$) among treatment means for cholesterol and all the values for cholesterol fractions. Group W0 had the highest ($p<0.05$) values for all the parameters except triglycerides while group W20 was least ($p<0.05$) for all the parameters except triglycerides. Group W20 recorded the highest value for triglycerides while group W40 had the least ($p<0.05$) value for this parameter.

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Table 4: Cholesterol and its fractions in eggs from quails fed diets supplemented with waterleaf

	Waterleaf inclusion levels (%)				
Fractions	**W0**	**W20**	**W40**	**±*SEM***	***p-value***
Total cholesterol (mg/100g)	86.37[a]	71.70[b]	58.57[c]	17.267	0.000
HDL (mg/100g)	526.40[a]	418.80[c]	521.37[b]	17.529	0.000
LDL (mg/100g)	347.67[a]	238.50[c]	342.80[b]	17.803	0.000
VLDL (mg/100g)	19.40[a]	18.67[b]	19.27[a]	0.120	0.001
Lipid peroxidation (ngMDA/g)	14.53[a]	13.66[c]	14.47[b]	0.140	0.000
Triglycerides (mg/100g)	92.90[b]	195.77[a]	75.15[c]	18.799	0.000

[abc] Means in same row with different superscripts are significantly different ($p<0.05$); HDL= high density lipoprotein; LDL= low density lipoprotein; VLDL= very low-density lipoprotein

4. DISCUSSION

The DM contents were within the range reported for whole raw quail egg (Ossamulu *et al*., 2023; Tokusoglo, 2006). The DM reduced with dietary inclusion of waterleaf meal. As waterleaf level increased from 20 to 40%, DM also increased. Differences could, however, not be attributed any specific reason. The CP contents of the eggs were marginally higher than values reported for quails (Tokusoglo, 2006; Ossamulu *et al*., 2023; Odafe–Shalome and Owen, 2020). Though 79-88% of amino acids (methionine and lysine) in forages consumed by poultry can be digested (Linden, 2013), several studies have been unable to prove that inclusion of forages in diets increase crude protein content of eggs (Steenfeldt and Hammershoj, 2015; Zheng *et al*., 2019). Therefore, differences could not be due to dietary inclusion of waterleaf. Nevertheless, the CP values decreased at 20% waterleaf but equalled that of the control at 40% of waterleaf. This further indicates that waterleaf inclusion could not meaningfully affect protein content of eggs. The EE contents were slightly lower than values reported for some quail eggs (Tokusoglo, 2006; Song *et al*., 2000) but similar to others (Ossamulu *et al*., 2023; Odafe–Shalome and Owen, 2020; Song *et al*., 2000). Therefore, differences could not be explained by waterleaf inclusion. The EE value increased with increase in dietary waterleaf inclusion for quails. Though there is abundant evidence that feed, especially some oil-based diets, influence the fatty acids, especially omega 3 fatty acid content in eggs (Rehault-Godbert *et al*., 2019; Franco *et al*., 2020), increases in EE has not been attributed to any forage. Hence, waterleaf could not have been responsible in this context too. The ash contents were lower than values reported for some quails in Nigeria (Odafe–Shalome and Owen, 2020) but higher than for quails reported elsewhere (Ossamulu *et al*., 2023; Tokusoglo, 2006). Hence, no particular trend was observed in this study. However, the ash content of the eggs increased at 20% of waterleaf but fell to the same level as the control when waterleaf was increased to 40% level. This indicates that inclusion of waterleaf at 20% level could increase the ash content of the eggs. The NFE value of the eggs increased at 20% of waterleaf but reduced to the same level as the control when waterleaf level was further increased to 40%. The pattern was similar to that for ash with similar implications. The GE value of the eggs was reduced when waterleaf was included at 20% but further increase in waterleaf to 40% increased the GE level though still below that of the control. This shows that inclusion of waterleaf in the diets of quails minimized the energy content of the eggs.

The Na, K, Ca, P and Mg contents of the eggs increased ($p<0.05$) when 20% of waterleaf was added to the diets but reduced, though still higher than that of control, when the waterleaf content of the diets was increased to 40%. Studies have shown that free range (Heflin *et al*., 2018) and organic (Kucukyilmazm *et al*., 2012) poultry management whereby birds eat forage can affect the mineral content of poultry eggs. It implies that the increase in the macro-mineral contents could be attributed to inclusion of the waterleaf. Furthermore, Linden (2013) reported that the main contribution of forages to poultry nutrition is in the area of minerals and vitamins. In the reported studies, forage-fed birds had lower P and Zn contents but higher Mg. In the present study all the examined minerals were enhanced with inclusion of waterleaf. Differences could be due to management system (Heflin *et al*., 2018) because in the present study, the birds were reared in cages and the forage cut and fed to them unlike when the birds are allowed to roam and feed on forage by themselves.

The micro-mineral content of the quail eggs shows that the Fe, Zn, Se, I, Cu and Mn contents increased ($p<0.05$) with addition of 20% dietary waterleaf. However, at 0% and 40% waterleaf inclusion, the Fe, Zn, I and Mn decreased while Se and Cu remained high. The increases agree with literature (Heflin *et al*., 2018; Kucukyilmaz *et al*., 2012) and implies that Fe, Zn, Se, I, Cu and Mn contents in quail egg might be enhanced by addition of 20% waterleaf to the diet. The patterns of change where some increase, decrease or remain unchanged also agrees with reports by Heflin *et al*. (2018) where only Mn increased while others remain unchanged. Compared to literature values (Ali and Abd El-Aziz, 2019; FDC, 2019), the Fe, I and Mn values were high while those of Cu, Se and Zn were low. Differences could be attributed to the breed and type of feed as those affect the mineral content of eggs (Franco *et al*., 2020).

Total cholesterol decreased with increase in waterleaf inclusion level. Compared to literature value of 73.45mg/100g (Tokusoglu, 2006), the total cholesterol of W0 was high while those of W20 and W40 were low. This agrees with Linden (2013) that forage consumption by poultry can minimize the cholesterol content in their eggs, thus implying that inclusion of waterleaf might reduce cholesterol content in quail eggs.

The HDL and LDL contents of the eggs decreased ($p<0.05$) with inclusion of waterleaf in quail diets. This pattern was different from that reported by Kismiati *et al*. (2020) whereby the HDL increased as the LDL decreased. Decrease in HDL unlike LDL, is undesirable from the nutrition point of view (Khalifa and Noseer, 2019). However, because both of those parameters decreased at the same time and in the same pattern, the effect of one may have counterbalanced the other.

The inclusion of 20% waterleaf in the diet of quails reduced the VLDL content of quail eggs. Compared to literature (Ogunwole *et al*., 2015), the VLDL values in this study were low. This could be attributed to inclusion of waterleaf as Linden (2013) reported decrease in cholesterol fractions due to consumption of forages by chickens. VLDL supplies body tissues with triglycerides and high levels of VLDL could form plaques on the walls of the arteries to limit blood flow (Lopez-Jimenez, 2024). This implies that inclusion of waterleaf in the diets of quails could reduce the risk of atherosclerosis in people consuming such eggs.

Compared to literature (Romero *et al*., 2022), the lipid peroxidation values in this study were high. The higher values could not be attributed to any obvious reason. Nevertheless, lipid peroxidation values decreased as waterleaf was included in quail diets with W20 being the least ($p<0.05$). Lipid peroxidation is the oxidation of polyunsaturated fatty acids by free radical to distort the assembly of the membrane leading to changes in Fe transport and inhibition of metabolic processes (Catala and Diaz, 2017). This implies that inclusion of waterleaf in quail diets could minimize the peroxidation of the fatty acids in the quail egg thus elongating the shelf life of the egg. This agrees with Linden (2013) that forages positively impact the unsaturated fatty acids in poultry eggs.

Inclusion of waterleaf in the diets of quails at 20% level increased the triglycerides but at 40% reduced them. Compared to literature (Ogunwole *et al*., 2015), the triglyceride values in this study were high. High levels of triglycerides in the body could result in heart disease and stroke (Morris, 2021). However, complex metabolism of egg triglycerides in the blood of humans means that the negative effects in consuming the eggs might be farfetched.

5. CONCLUSION

Evidence from the study indicates that inclusion of waterleaf in quail diets could increase the values for ash and most minerals as well as reduce the cholesterol and peroxide values with potential for positive effect on the risk for atherosclerosis in consumers of the eggs. It is recommended that waterleaf could be included in quail diets at 20% level to improve the nutritive value of the eggs.

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