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Chemical composition of Guinea fowl (*Numida meleagris*) egg shells

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ABSTRACT

The study reported was based on the chemical evaluation of the egg shells of guinea fowl. The egg shell percentage was 16.7; proximate levels were (g/100 g): protein 3.34, fat 1.50, ash 8.33, soluble carbohydrate 90.0 and gross energy 1489 (kJ/100 g) and the utilisable energy due to protein was 34.1 %. Sample was high in Na, K, Ca, Mg, Zn but low in Mn, Fe, P and Ca/Mg. Many essential acids were of high concentration: Lys, His, Arg (the most concentrated acids, 52.0 mg/g), Thr, Val, Leu, Ile and Phe. Limiting amino acid score showed that Ser shared the position with Tyr (0.14) in hen's egg composition, it was Met+Cys (0.26) in the provisional score comparison and Leu (0.31) in the comparison with pre-school child requirement.

Key words: Guinea fowl, egg shells, chemical composition.

INTRODUCTION

These noisy birds look like a bunch of AWOL army helmets as they run across the yard. They are said to be good for controlling the lyme disease-bearing deer tick[1]. They certainly range well and eat lots of small things. In fact if you keep bees, you don't really want to keep guineas. They'll stand by the hive and snap up the bees as they come. Guineas often lay their eggs out in the fields and hatch their young by themselves. If you do find the eggs and wish to incubate them, the time period is 26 to 28 days and you treat them like chicken eggs. Young guineas are called "keets". Being native to dry areas of Africa, they are very susceptible to dampness during their first two weeks, and can die from following the mother through dewy grass. After two weeks of age, they are probably the hardiest of all domestic land fowls[2].

A report by ADAS Consulting Ltd., UK for DEFRA-MPEP Branch (The UK egg products Industry) [3] highlighted the difficulties which the disposal of egg shells presents to UK egg processors. In the report, it was estimated that 10,000-11,000 tonnes of egg shell has to be disposed off each year by egg processors and producers of hard cooked eggs. Similar issues affect UK hatcheries for both egg and poultry meat production where again the quantity of egg shell and other hatchery waste to be disposed off is considerable. It is estimated that this amounts to some 360 tonnes per annum for egg laying birds and 4,800 tonnes per annum for broilers[3]. The disposal of egg shells and hatchery waste is not only a problem for the UK industry, although the problem is alleviated in many other countries where it is an acceptable practice to feed treated egg shell back to animals as a source of calcium and this is a

very efficient option for the disposal of egg shells. Egg shell waste primarily contains calcium, magnesium carbonate (lime) and protein[3]. In order to maximise the recycling opportunities for egg shells, the material could be incinerated independently of other wastes. The calcium/ magnesium content of the shells will be converted into calcium/magnesium oxide and the resultant burnt lime could be used as a liming agent. Egg shell membrane contains around 10 % collagen, including the most common Type 1 collagen and the unusual Type 10 collagen. The collagen from the shell membrane is very useful in the medical area, where purified collagen can sell for up to US$1000 per gram. Collagen is used for skin grafts, dental implants, angioplasty sleeves, cornea repair, plastic surgery, treatment of osteoporosis and pharmaceuticals as well as food castings and film emulsions[3]. The membrane free shell powder can be used in the paper industry, or in agriculture as a lime substitute or calcium supplement. Other possibilities for utilising egg shell include: production of biodegradable plastics from egg shell membrane proteins; altering of food-borne bacterial pathogen heat resistance with an egg shell membrane bacteriolytic enzyme; as human dietary calcium supplement especially for post menopausal women. Egg shells also contain useful amounts of microelements such as strontium (Sr), fluorine (F) and selenium (Se); its membrane can be used as an adsorbent for the removal of reactive dyes from coloured waste effluents as well as to eliminate heavy metal ions from a dilute waste solution[3].

Ihekoronye and Ngoddy[4] had discussed the composition of the hen's egg made up of three major component parts: shell, the white or albumen and the yolk. The shell had been shown to be composed of cuticle, spongy calcareous layer and mammillary layer whereas the membrane was said to be made up of air cell, outer shell membrane and inner shell membrane. Also, the shell constituted about 95.1 % inorganic matter, 3.3 % protein and 1.6 % of the total hen's egg[4] based on wet weight. Adeyeye[5] had reported on the comparative study on the characteristics of egg shells of some bird species (francolin, duck and turkey) where proximate, minerals and amino acid profiles of the egg shells were reported.

Egg shell waste therefore does have a theoretical value as an animal feed or as a fertilizer or lime substitute. In many countries, it is an acceptable practice for egg shells to be dried and used as a source of calcium in animal feeds. The recycling of the animals portends that the nutritional composition of the egg shells should be evaluated to see which of the bird's egg shells would likely serve the best purpose in the feed formulation.

MATERIAL AND METHODS

Collection and treatment of samples

Numida meleagris eggs (5) were purchased in a market in Odo Ayedun-Ekiti, Ekiti State, Nigeria. The eggs were weighed whole, the length and breadth measured, cracked to remove the yolk and the albumen and weighed, and finally the shell was weighed. The shells were then oven-dried and ground to powder, sieved using 200 mm mesh and kept in freezer in McCartney bottles pending analysis. The experiments took two weeks to carry out.

Proximate analysis

Moisture, ash, crude fat and crude fibre were determined according to AOAC[6] methods while nitrogen was determined by the micro-Kjeldahl method[7] and the percentage of nitrogen was converted to crude protein by multiplying with 6.25. Both carbohydrate, organic matter and dry matter were determined by difference.

The crude fat value was used to calculate the theoretical total fatty acid by multiplying with a conversion factor of 0.945 (for poultry) [8]. The calorific values in kilojoules were calculated by multiplying the crude fat, protein and carbohydrate contents by the Atwater factor of 37, 17 and 17, respectively[9].

Mineral analysis

Minerals were analysed using the solution obtained by dry ashing the samples at 550 ^{0}C. The ash was dissolved in 10 % HCl (25 ml) and 5 % lanthanum chloride (2 ml), heated to boiling, filtered into 50 ml standard flask and made up to volume with distilled deionised water. Mg, Ca, Cu, Zn, Mn, Fe and Cr were determined with a Buck atomic absorption spectrophotometer. Na and K were

measured with a Corning 405 flame photometer[6]. The detection limits had previously been determined using the methods of Varian Techtron [10]. The limit of detection is the concentration in solution of an element which can be detected with a 95 per certainty. This is that quantity of the element that gives a reading equal to twice the standard deviation of a series of at least ten determinations at or near blank level. This means that at concentrations near the detection limit an element may be detected with reasonable statistical certainty. Phosphorus was determined using a Spectronic 20 colorimeter by the phosphovanado-molybdate method[6]. All chemicals used were of British Drug House (BDH) analytical grade.

Amino acid analysis

Details of the procedure had been given earlier[11]. To determine the amino acids, about 30 mg of defatted shell sample was weighed into glass ampoule, 7 ml of 6 M HCI added and oxygen expelled by passing nitrogen into sample. The glass ampoule was sealed with a flame and heated at 105±5 ^{0}C for 22 h. The ampoule was cooled, opened and the contents filtered to remove the humins, and the filtrate was evaporated to dryness at 40 ^{0}C under vacuum. The residue was dissolved with 5 ml acetate buffer (pH 2.0) and stored in the freezer. The period of analysis was 76 min, with gas flow rate of 0.50 ml/min at 60 ^{0}C and the reproducibility was ±3 %. The amino acid values were the average of two determinations. Tryptophan was not determined due to high cost of this specific analysis. The method of amino acid analysis was by ion-exchange chromatography (IEC) [12] using the Technicon Sequential Multisample (TSM) Amino Acid Analyser (Technicon Instruments Corporation, New York).

Estimation of quality of protein

The amino acid scores were calculated using three different methods:

(i) Calculating the amino acid score using the following formula:

Amino acid score = Amount of amino acid per test protein [mg/g]/Amount of amino acid per protein in reference pattern [mg/g]

(ii) Calculations based on the whole hen's egg[14];

(iii) Calculations based on the pre-school child (2-5 years) suggested requirements[15].

Calculation of the total essential amino acid (TEAA) to the total amino acid (TAA), i.e. (TEAA/TAA); total sulphur amino acid (TSAA); percentage cystine in TSAA (% Cys/TSAA); total aromatic amino acid (TArAA), etc; while the predicted protein efficiency ratio was determined using one of the equations developed by Alsmeyer et al[16], i.e.: P-PER = -0.468 +0.454 (Leu) -0.105 (Tyr). Theoretical estimation of isoelectric point (pI) can be carried out by the equation of the form[17]:

$$IPm=\sum_{i=1}^{n} iPixi$$

where IPm is the isoelectric point of the mixture of amino acids, IPi is the isoelectric point of the ith amino acid in the mixture and Xi is the mass or mole fraction of the ith acid in the mixture. The essential amino acid index was determined by the method of Steinke et al[18].

RESULTS AND DISCUSSION

Table 1 shows the whole egg weight and other measurements of the bird specie. The total egg weight (35.4 g), egg length (5.0 cm), all on the average basis was higher than the report for francolin: 25.2 g and 4.18 cm but much lower than duck and turkey: 74.9 g, 7.40 cm and 70.9 g, 6.50 cm respectively[5]. The edible portion of the guinea fowl egg was not as varied as those eggs mentioned in the above literature[5]. Measurements of length and breadth gave part of the physical characteristics of the shells; physical characteristics are important in the industrial manipulation of the egg.

Organic matter was highly concentrated in the shell (91.7 g/100 g) as we have in previous literature results in some egg shells (92.4-96.6 g/100 g) and closely followed by the available carbohydrate (90.0 g/100 g) in contrast to the literature results where protein took the second position (62.2-73.1 g/100 g) [5]. The calculated gross energy (kJ/100 g) of 1489 kJ/100 g was lower than in the three species with values of 1556-1687 kJ/100 g and the same observation followed in the fatty acid of 1.42 g/100 g which is lower than 2.41-8.07 g/100 g in literature [5]. The proximate composition of the sample is in Table 2.

Table 1: Guinea fowl egg characteristics (mean±SD)

Parameter	Value*
Total weight (g)	35.4±2.33 (31.8-38.2)
Length (cm)	5.0±0.34 (4.60-5.50)
Breadth (cm)	3.9±0.35 (3.60-4.50)
Shell (g)	5.9±1.11 (4.14-6.86)
% shell	16.7
Edible portion (g)	29.5±1.30 (27.7-31.3)
% edible portion	83.3

*Determination in quadruplicate

Table 2: Proximate composition (g/100 g) of the egg shells of guinea fowl

Parameter	Concentration
Total ash	8.33
Moisture content	2.36
Crude protein	3.34
Crude fat	1.50
Crude fibre	3.51
Dry matter	97.6
Carbohydrate (soluble)	90.0
Organic matter	91.7
Calculated gross energy (kJ/100 g)	1489
Fatty acid (crude fat x 0.945)	1.42

Table 3: Energy values as contributed by protein, fat and carbohydrate in guinea fowl egg shells

Parameter	Value
Total energy	1489
Proportion of total energy due to protein (PEP %)	3.81
Proportion of total energy due to fat (PEF %)	3.73
Proportion of total energy due to carbohydrate (PEC %)	92.5
Utilisable energy due to protein (UEDP %)	34.1

Table 4: Mineral composition (mg/100 g) of the egg shells of guinea fowl

Parameter	Concentration
Sodium (Na)	40.7
Potassium (K)	52.5
Calcium (Ca)	41.5
Magnesium (Mg)	59.5
Copper (Cu)	ND
Manganese (Mn)	0.01
Iron (Fe)	0.96
Zinc (Zn)	4.89
Chromium (Cr)	ND
Phosphorus (P)	4.79
[K/ (Ca+Mg)]	1.04 meq
Na/K	0.78
K/Na	1.29
Ca/Mg	0.70
Ca/P	8.66

ND = not detected. *milliequivalent.

Table 5: Amino acid profile of the egg shell of guinea fowl

Amino acid	Concentration
Lysine (Lys)*	30.3
Histidine (His)*	11.2
Arginine (Arg)*	52.0
Aspartic (Asp)	24.0
Threonine (Thr) *	21.5
Serine (Ser)	11.4
Glutamic (Glu)	36.0
Proline (Pro)	6.00
Glycine (Gly)	2.10
Alanine (Ala)	10.6
Cystine (Cys)	4.00
Methionine (Met)*	5.00
Valine (Val)*	33.0
Leucine (Leu)*	20.5
Isoleucine (Ile)*	23.0
Tyrosine (Tyr)	5.40
Phenylalanine (Phe)*	18.9
% N (fat free) × 6.25	18.1
Tryptophan	-[a]

*Essential amino acids. [a] = not determined.

The energy contributions of protein, carbohydrate and fat are shown in Table- 3. Carbohydrate contributed the largest percentage of 92.5. The utilisable energy due to protein was 34.1 % which is slightly high.

Table- 4 contains the mineral composition of the egg shells. Magnesium was the most concentrated mineral. The trend of concentration was Mg > K > Ca > Na > Zn > P > Fe > Mn. Copper and chromium were not detected. In francolin, duck and turkey egg shells were better than in guinea fowl in P, Fe, Ca, K and Na. The Ca/P and Ca/Mg weight ratios ranged between 8.66 and 0.70 respectively. These values were far from the standard value of 1.0 to effect effective absorption of calcium in the diet. The [K/ (Ca+Mg)] obtained was 1.04 milliequivalent; this is less than 2.2; hence, the sample could not lead to hypomagnesemia[19]. The K/Na was 1.29; it normally enhances the salt balance of the body fluid.

Table 6: Summary of some essential parameters of guinea fowl egg shells (mg/g crude protein)

Parameter	Value
Total amino acid (TAA)	305
Total essential amino acid (TEAA)	
-with His	173
-without His	162
% TEAA	
-with His	56.7
-without His	53.0
Total non- essential amino acid (TNEAA)	132
% TNEAA	43.3
Total acidic amino acid (TAAA)	60.0
% TAAA	19.7
Total basic amino acid (TBAA)	93.5
% TBAA	30.7
Total aromatic amino acid (TArAA)	35.5
% TArAA	11.6
Total neutral amino acid (TNAA)	151
% TNAA	49.7
Total sulphur amino acid (TSAA)	9.00
% TSAA	2.95
% Cys/TSAA	44.4

Table 7: Summary of some amino acid quality parameters of guinea fowl egg shells

Parameter	Value
Predicted protein efficiency ratio (P-PER)	0.41
Leucine/ isoleucine ratio (Leu/Ile)	0.89
Leu-Ile %	0.98
Isoelectric point (pI)	2.05
Essential amino acid index (EAAI)	0.53

The amino acid profile for the sample is shown in Table -5. Arginine was the most concentrated amino acid (AA). Glutamic and aspartic acids were the most concentrated AA in duck, turkey and francolin[5]. Arginine is an essential AA (EAA). Amino acids of the following were better than in francolin, duck and turkey: Lys, His, Arg, Thr, Pro, Ala and Val, out of these, five are essential amino acids. While our current total AA (TAA) was 305 mg/g crude protein (cp) (Table-6), it is 189 mg/g cp in francolin, it is 224 mg/g cp in duck and 353 mg/g cp in turkey[5] while the corresponding EAA (with His) were 173 mg/g cp (56.7 % in guinea fowl); 98.1 mg/g cp (51.9 %), 131 mg/g cp (58.3 %) and 188 mg/g cp (53.2 %). The TSAA in guinea fowl was 9.0 mg/g cp; 7.7 mg/g cp (francolin), 9.0 mg/g cp (duck) and 12.4 mg/g cp (turkey) while the corresponding Cys/TSAA (%) was: 44.4 (sample); 45.5, 38.9 and 52.4. The higher concentration of some EAA in the guinea fowl could be due to its unrestricted mode of feeding. Table-7 shows that the predicted protein efficiency ratio was 0.41 which was lower than the values in the three birds cited in literature with values of 0.47-1.44[5], however our current calculated isoelectric point (pI) fell within the literature for the birds which showed that the precipitation of the protein of the shells can all occur at the acid pH level of 1.1-2.0[5] whereas the present report was 2.05 (Table-7). The Leu/Ile was too low to cause any concentration antagonism in the diet containing the shells. The essential amino acid index was low but it is more important in man than in animals.

The fact that the EAA were higher than the non-essential AA (NEAA) is good for the shells in formulating animal feed[20]. The value of Cys/TSAA % less than 50 followed the trend in most animal and insect amino acids like in whole body crab (27.3 %), flesh of crab (30.4 %) and crab exoskeleton

(32.8 %) [21]. However, the percentage of Cys in TSAA had been set at 50 % in rat, chick and pig diets[12]. Cystine has positive effects on mineral absorption, particularly zinc[22, 23].

Table- 8 shows that Ser and Tyr shared the position of limiting AA (LAA) (0.14) in comparison of sample AA with whole hen's AA; with comparison with provisional AA score, the LAA was Met+Cys (0.26); with pre-school child suggested requirement, the LAA was Leu (0.31). In order to correct for the day's needs for the AA in the shells it would be: 100/14 or 7.14 times as much egg shell protein in guinea fowl. The LAA in the guinea fowl was better than all in francolin (Thr) (100/10 or 10 times), duck (100/18 or 5.56), turkey (100/20 or 5.0

Table 8: Amino acid scores of guinea fowl egg shells based on various standard

Amino acid	Comparison with Whole hen's egg	Provisional score	Pre-school child requirement
Lys	0.49	0.55	0.52
His	0.47		0.59
Arg	0.85		
Asp	0.22		
Thr	0.42	0.54	0.63
Ser	0.14		
Glu	0.30		
Pro	0.16		
Gly	0.07		
Ala	0.20		
Cys	0.22		
Met	0.16	0.26	0.36
Val	0.44	0.66	0.94
Ile	0.41	0.58	0.82
Leu	0.25	0.29	0.31
Tyr	0.14		
Phe	0.37	0.41	0.39
Total	0.31	0.49	0.53

times), for provisional EAA scoring pattern it was 100/26 or 3.85 times in the guinea fowl egg shell. However, Met+Cys is in the second most important position as an EAA as compared to Lys (first), Thr (Third) and Try[24] (forth).

Froning and Bergquist[25] had used ground egg shell (70 %), blended with technical albumin (8 %), maize (5 %), soy-bean meal (17 %) and propionic acid (0.15 %), extruded the blend, cooled and fed to laying hens as a protein and calcium supplement in a fully formulated diet. Hens fed the extrudate were not adversely affected in comparison to control birds (rate of lay, feed conversion, mortality, shell thickness and shell strength). Deshmukh and Patterson[26] had subjected chicks and shell waste to lactic acid fermentation; fermented product extruded and dried, and included as a feed ingredient in a feed evaluation trial for broiler chicks. Diets supplemented with hatchery by-products were comparable with control diets in terms of bird performance (body weight gain and feed conversion). Carcass yields were not adversely affected. These two examples showed how egg shells can be effectively used in feed formulation.

The present report has shown the nutritional qualities of the egg shells of guinea fowl and shown that they are good sources of carbohydrate, energy, minerals and many essential amino acids that will make them effective in food formations for animals.

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