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COMPARATIVE STUDY ON THE CHARACTERISTICS OF EGG SHELLS OF SOME BIRD SPECIES

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ABSTRACT. Egg shells of francolin, duck and turkey were compared for their physical and chemical characteristics. The range of weight of eggs and shells, respectively, were 25.2-74.9 and 5.23-9.40 g. Protein content was between 65.2-73.1 g/100 g; crude fat ranged from 2.54-8.54 g/100 g; crude fibre was low with value range of 0.04-1.14 g/100 g; ash content range was 3.44-7.56 g/100 g. Total and essential amino acids, respectively, were between 189-353 and 98.1-188 mg/g and threonine was limiting. Gross energy ranged from 1556-1687 kJ/100 g. High concentrations of minerals were detected.

KEY WORDS: Bird species, Egg shell samples, Chemical composition

INTRODUCTION

A report prepared by ADAS Consulting Ltd., UK for DEFRA-MPEP Branch (The UK Egg Products Industry) [1] 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 of 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 of 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 [1]. 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 [1]. 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 egg 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 [1]. 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); it's 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 [1].

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Ihekoronye and Ngoddy [2] 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 [2] based on wet weight.

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 nutrients of egg shells back to the animals portends that the nutritional composition of the egg shells should be evaluated to see which of the birds egg under study (francolin, duck and turkey) would likely to serve the best purpose in the feed formulation.

EXPERIMENTAL

Collection and treatment of samples

The francolin eggs were collected in the month of November in the bush (it is a taboo to real the bird at home) while the eggs of local duck and white plumage turkey were directly obtained from poultry keepers. Five eggs were involved in each study and they were collected at once. 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 [3] methods, while nitrogen was determined by the micro-Kjeldahl method [4] and the percentage of nitrogen was converted to crude protein by multiplying with 6.25. Both carbohydrate and organic matter were determined by difference.

The crude fat values were used to calculate the theoretical total fatty acids by multiplying with a conversion factor of 0.945 (for poultry) [5]. 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 [6].

Mineral analysis

Minerals were analysed using the solution obtained by dry ashing the samples at 550 ^{o}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, Zn, Cu, Mn, Fe, Co and Ni were determined with a Buck atomic absorption spectrophotometer. Na and K were measured with a Corning 405 flame photometer [3]. The detection limits had previously been determined using the methods of Varian Techtron [7]. The limit of detection is the concentration in solution of an element which can be detected with a 95 per cent 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 [3]. All chemicals used were of British Drug House (BDH) analytical grade.

Amino acid analysis

Details of the procedure had been given earlier [8]. To determine the amino acids, about 30 mg of defatted shell sample was weighed into glass ampoules, 7 mL of 6 M HCl added and oxygen expelled by passing nitrogen into samples. The glass ampoules were sealed with a flame and heated at 105±5 ^{o}C for 22 h. The ampoules were 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 costs of these specific analyses. The method of amino acid analysis was by ion-exchange chromatography (IEC) [9] using the Technicon Sequential Multisample (TSM) Amino Acid Analyser (Technicon Instruments Corporation, New York).

Estimation of quality of protein

The amino acid score was calculated by the following formula [10]:

$$\text{Amino acid score} = \frac{\text{Amount of amino acid per test protein [mg/g]}}{\text{Amount of amino acid per protein in reference pattern [mg/g]}}$$

Calculation of the total essential amino acid (TEAA) to the total amino acid (TAA), i.e. (TEAA/TAA); total sulfur 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.* [11], 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 [12]:

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

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 amino acid in the mixture.

Statistical analysis

The proximate composition, amino acid composition and the essential amino acid scores were each subjected to the F test analysis setting the confidence level at $p < 0.05$ [13].

RESULTS AND DISCUSSION

Table 1 shows the whole egg weight and other measurements of the bird species. The duck has the highest value of total egg weight (74.9 g), egg length (7.40 cm), all on the average basis of five eggs involved in each case. The most varied range of values was in edible egg among all the samples while the least varied was the breadth of the egg for all the samples. Measurements of length and breadth gave part of the physical characteristics of the shells.

Table 1. Whole egg weight, size and other measurements of the bird species.

Parameter		Francolin	Duck	Turkey
Total weight (g)		25.2	74.9	70.9
	Range (g)	23.5-27.1	62.3–76.8	62.3–79.5
Length (cm)		4.18	7.40	6.50
	Range (cm)	4.0-4.3	6.2–8.1	6.2–6.8
Breadth (cm)		3.42	4.10	4.70
	Range (cm)	3.1–3.8	4.0–4.5	4.5–4.9
Edible egg (g)		19.9	64.6	62.7
	Range (g)	18.3–21.6	54.0–67.3	54.0–71.4
Shell weight (g)		5.23	9.40	8.20
	Range (g)	4.18–5.68	8.35–9.54	8.13–8.35

Organic matter was the most highly concentrated in the shell samples with an average range of 92.4–96.6 g/100 g and closely followed by the crude protein with a range of 65.2–73.1 g/100 g. The egg shells were low in crude fat content, calculated fatty acid, available carbohydrate content and crude fibre content but high in the gross energy. The total ash in the francolin was higher than in the duck with the likelihood that mineral levels in the francolin egg shell would be higher than in the duck. The results are given in Table 2.

Table 2. Proximate composition (g/100 g) of the egg shells of the bird species.

Parameter	Francolin	Duck	Turkey
Total ash	5.80	3.44	7.56
Moisture content	5.64	3.49	4.55
Crude protein	73.1	65.2	71.9
Crude fat	2.54	5.32	8.54
Organic matter	94.2	96.6	92.4
Crude fibre	0.04	0.11	1.14
Available carbohydrate	12.8	22.5	6.31
Calculated gross energy (kJ/100 g)	1556	1687	1645
Fatty acid (crude fat x 0.945)	2.41	5.02	8.07

Table 3 contains the mineral composition of the egg shells. Phosphorus was the most highly concentrated mineral in all the samples. Among other major minerals, the trends were: in francolin, potassium > calcium > sodium > magnesium; in duck, potassium > magnesium > sodium > calcium; in turkey, magnesium > potassium > calcium > sodium. Among the trace metals, iron > zinc in all the samples while copper, manganese, cobalt and nickel were not detected in all the samples.

The amino acid profile for the samples is shown in Table 4. Aspartic acid was the most concentrated in the francolin while glutamic acid was the most concentrated in the duck and the turkey. The most concentrated essential amino acid in all the samples was leucine. Tryptophan was not determined. Table 5 shows that the total amino acid content in francolin was 189 mg/g protein, in duck it was 224 mg/g protein and in turkey it was 353 mg/g protein while the corresponding essential amino acid (with histidine) were: 98.1 mg/g protein (or 51.9 %), 131 mg/g protein (or 58.3 %) and 188 mg/g protein (or 53.2 %). The TSAA levels were 7.7 mg/g protein (francolin), 9.0 mg/g protein (duck) and 12.4 mg/g protein (turkey) while the corresponding Cys/TSAA (%) were: 45.5, 38.9 and 52.4. Table 5 also shows that the predicted protein efficiency ratio was least in francolin (0.47) and best in turkey (1.44) and the calculated isoelectric point (pI) showed that the precipitation of the protein of the shells can all occur at the acid pH level of 1.1–2.0. Table 6 depicts the essential amino acid scores of the samples.

Table 3. Mineral composition (mg/100 g) of the egg shells of the bird species.

Parameter	Francolin	Duck	Turkey
Sodium	61.8	51.3	41.6
Potassium	68.3	59.8	51.7
Calcium	61.9	42.3	50.1
Magnesium	60.0	52.2	61.4
Copper	ND[A]	ND	ND
Manganese	ND	ND	ND
Cobalt	ND	ND	ND
Nickel	ND	ND	ND
Iron	8.03	10.2	9.96
Zinc	3.83	4.65	3.73
Phosphorus	133	117	151

[A]ND = Not detected. Detection limit of: Cu = 0.05 mg/100 g; Mn = 0.1 mg/100 g; Co = 0.5 mg/100 g; Ni = 0.2 mg/100 g.

Table 4. Amino acid profile (mg/g) of the egg shells of the bird species.

Amino acid	Francolin	Duck	Turkey
Lysine [A]	9.9	12.4	22.5
Histidine	6.0	7.1	10.4
Arginine[A]	17.9	21.3	31.0
Aspartic acid	26.0	20.6	49.9
Threonine[A]	4.1	7.0	8.1
Serine	10.6	10.2	13.6
Glutamic acid	21.5	26.0	57.0
Proline	2.9	5.4	3.1
Glycine	10.1	8.0	11.3
Alanine	5.9	10.1	3.4
Cystine	3.5	3.5	6.5
Valine[A]	10.1	12.7	11.4
Methionine[A]	4.2	5.5	5.9
Isoleucine[A]	11.0	10.8	20.6
Leucine[A]	23.1	34.6	46.8
Tyrosine	10.5	9.7	20.5
Phenylalanine[A]	11.8	19.4	31.1
Tryptophan	-[B]	-	-

A = Essential amino acids. B = Not determined.

The results in Table 1 when compared with literature values showed that duck with an average egg weight of 74.9 g is closely related to the value of 78.0 g in Mallard bird whereas the egg shell percentage in duck (12.6 %) was close to the value of 12.5 % in Coturnix Mourning species, 11.6 % in turkey was close to 10.6 % in Mallard and 20.8 % in francolin was close to 15.2 % in Starling bird [14].

The protein levels in Table 2 were all high. This will supplement very well the protein level of any feed formulation where the shells are used. The protein levels here were much better than the cheliped (muscle and exoskeleton together) in male and female fresh water crabs with values of 20.5 g/100 g (male) and 18.4 g/100 g (female) [15]. The carapace of crabs has been used in snail food formulation (about 15 %) to cover the calcium requirements of growing snails [16]; the shells can improve tremendously the protein intake of animal feeds where the shells are involved in their formulation. The low pI values will quickly assist in the production of protein isolate from the egg shells. It is interesting here to note that the protein level in francolin shell was 73.1 g/100 g > 71.9 g/100 g (turkey) > 65.2 g/100 g (duck). The gross energy obtained from the shells which ranged from 1.56-1.69 MJ/100 g were close to values from cereals with values of 1.3-1.6 MJ/100 g [17] showing that the shells would serve as reasonable sources of energy.

The values of calcium and magnesium in the shells as shown in Table 3 were very close and relatively high. Egg shell waste falls within the category of waste food, drink or materials used in or resulting from the preparation of food or drink and could, subject to adequate scrutiny, be suitable for land spreading. The total neutralising value (lime) is almost the same as ground chalk or limestone tonne for tonne [18]. The calcium/phosphorus ratio of 0.33-0.47 will assist the absorption of calcium in the feed formulation and this can be improved in the presence of higher calcium than phosphorus in the feed. Animals have a high tolerance for zinc, but very high levels may depress feed intake or induce copper deficiency; the current zinc levels (3.73-4.65 mg/100 g dry weight) were not high to cause any deleterious effect. The iron level was good in all the shells but particularly in the duck.

In Table 4, turkey egg shell had the highest levels of the following essential amino acids: lysine, histidine, arginine, threonine, methionine, isoleucine, leucine, phenylalanine and second highest in valine; this means turkey egg shell is the most suitable shell in animal feed preparation. This trend was also followed in the second position by the duck egg shell with the exception of isoleucine. The better levels of the essential amino acids in the turkey and duck shells could be due to their enhanced better home feeding since their crude protein levels were both lower than in the francolin.

Calculations in Table 5 showed that the essential amino acids with or without histidine were more concentrated than in the non-essential amino acids: in francolin, 51.9 % (with histidine) and 48.7 % (no histidine); in duck, 58.3 % (with histidine) and 55.2 % (no histidine); in turkey, 53.2 % (with histidine) and 50.2 % (no histidine); their corresponding non-essential amino acids were: 48.1 %, 41.7 % and 46.8 %. Also the Cys/TSAA% were: 45.5 (francolin), 38.9 (duck) and 52.4 (Turkey). The fact that the essential amino acids were higher in percentage values than the non-essential amino acids is good for the shells in formulating animal feed [16]. The value of Cys/TSAA % less than 50.0 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 %) [19]. However, the value of 52.4 % in turkey resembles the situation in many vegetable proteins whose values are greater than 50.0 %, e.g. in coconut endosperm (62.9 %) [20], it also ranged between 58.9-72.0 % in guinea corn grains [21]. The percentage of Cys in TSAA had been set at 50 % in rat, chick and pig diets [7]. Cystine has positive effects on mineral absorption, particularly zinc [22, 23]. Under this situation, it means that the turkey egg shell is the best. The predicted protein efficiency ratio (P-PER) showed that turkey egg shell would be better utilised than any of the other two shells.

Table 6 shows that threonine is the limiting essential amino acid in all the samples with values of 0.10 (francolin) 0.18 (duck) and 0.20 (Turkey). In order to correct for the day's needs for the essential amino acids in the shells it would be: 100/10 or 10 times as much egg shell protein in francolin, 100/18 or 5.56 times as much shell protein in duck and 100/20 or 5.0 times as much shell protein in turkey. Threonine is in number three of the essential amino acid often acting in a limiting capacity (others are lysine, methionine + cystine and tryptophan) [24].

The F test results showed that there is a significant difference ($p < 0.05$) in the amino acid scores between turkey and francolin; in the total amino acid composition, significant differences existed between turkey and francolin and between turkey and duck.

Table 5. Some specially calculated parameters in the egg shells of the bird species.

Parameter	Francolin	Duck	Turkey
Calcium/phosphorus ratio	0.47	0.36	0.33
Sodium/potassium ratio	0.90	0.85	0.80
Total amino acid	189	224	353
Total acidic amino acid	47.5	46.6	107
Total aromatic amino acid	22.3	29.1	51.6
Total essential amino acid (with histidine)	98.1	131	188
(without histidine)	92.1	124	177
Total non-essential amino acid	91.0	93.5	165
Total neutral amino acid	108	137	182
Total sulfur amino acid (TSAA)	7.7	9.0	12.4
Cystine/TSAA (%)	45.5	38.9	52.4
Total basic amino acid	33.8	40.8	63.9
Leucine/isoleucine ratio	2.1	3.2	2.3
Predicted protein efficiency ratio	0.47	1.00	1.44
Isoelectric point (pI)	1.10	1.23	2.00

Table 6. Essential amino acid scores of the egg shells in the bird species.

Amino acid	Francolin	Duck	Turkey
Lysine	0.18	0.23	0.41
Threonine	0.10	0.18	0.20
Valine	0.20	0.25	0.23
Methionine + cystine	0.22	0.26	0.35
Isoleucine	0.28	0.27	0.52
Leucine	0.33	0.49	0.67
Phenylalanine + tyrosine	0.37	0.49	0.86
Total	0.25	0.33	0.50

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 current report has shown the nutritional qualities of the egg shells of three bird species. (francolin, duck and turkey) and shown that they are good sources of protein, energy, minerals and many essential amino acids that will make them serve effectively in feed formulations for animals.

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