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DIETARY INCLUSION OF VARYING LEVELS OF SUN – DRIED EDIBLE FROG (*Rana esculenta Linnaeus*) ON THE EGG COMPOSITION OF JAPANESE QUAIL (*Coturnix japonica Temminck & Schlegel*)

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ABSTRACT

Eggs are highly versatile food containing many essential nutrients. Information on egg composition and utilization of egg for food and other purposes have been limited mostly to chicken eggs. Chicken egg has been very well studied for its composition, however such information are not so abundantly documented in other poultry species. Hence, this study was to examine the differences in proximate composition of eggs of Japanese quails fed varying levels of frogmeal (*Rana esculenta*) as an alternative to fishmeal in their diet. The quails were raised under intensive system from day old to 8 weeks and balanced for weight with a sensitive digital scale and randomly allotted to 4 dietary treatments with 2 replicas of 8 birds per replicate in a completely randomized design. Sun-dried edible frog (frog meal) was included in the diet as replacement for fishmeal. The inclusion of frog meal for the treatments were at 0, 5, 10 and 15% levels and were fed ad-libitum, with experiments carried out in a modified cage-deep litter system and all analysis were carried out at P=0.05. At 10 weeks old, 3 quail egg samples (3 replicates) were randomly selected from each treatment for proximate analysis of eggs of the quails fed varying levels of edible frog meal. The result of T-test of significant difference between chemical composition and treatment of varying levels of edible frog in quail diet showed that there was a significant difference in crude protein chemical composition (t= 30.28, P<0.05), Crude fibre (t= 30.23, P<0.05), ash (t=7.00, P<0.05), Ether extract (t=66.82, P<0.05), Moisture content (t= 164.8, P<0.05) and Dry Matter (t= 89.07, P<0.05). The percentage crude protein content of eggs increased with corresponding percentage increase in frog meal in quail diet hence, quail eggs from the third treatment (10%) had the highest percentage of crude protein.

Keywords: Japanese quail, Frog meal, Egg composition, Diet

Introduction

Coturnix japonica eggs are characterized by a variety of color patterns. They range from snow white to completely brown. More commonly they are tan and dark brown speckled or mottled brown with a chalky blue covering. The average egg weighs about 10 g, and this is about 8% of the bodyweight of the quail hen. The egg of Japanese quail contains 158 Cal. of energy, 74.6% water, 13.1% protein, 11.2% fat, and 1.1% total ash. The mineral content of quail egg includes 0.59 mg calcium, 220 mg phosphorus and 3.8 mg iron. The vitamin content of quail egg is 300 I.u. of vitamin A, 0.12 mg of vitamin B1, 0.85 mg of vitamin B2 and 0.10 mg nicotinic acid (Shim 1984, Shim 2004). The chicken egg contains 149 kcal of energy, 75.33g water, 12.49g protein, 10.02g fat, 425mg cholesterol, and 1.22g carbohydrate. The mineral content of chicken egg includes 0.508mg riboflavin, 49mg calcium and 178mg phosphorus while the vitamin content is 635 I.U of vitamin A (USDA, 1976). A Japanese quail's egg weighs approximately 10g when compared to a small chicken's egg which weighs 55g and is approximately 8% of the body weight of a quail as against the chicken's egg which is 3% of the chicken's body weight. In many ways a quail's egg is more nutritious than a hen's egg (Suz, 2013).

Eggs are one of nature's highest quality sources of protein and indeed contain many important nutrients (Heqian *et al.*, 2018). They are a good source of high biological-value protein and they are easily digested (Evenopoel *et al.*, 1998). Therefore, they are valuable food for people who are recovering from illness. Eggs are composed of three main parts: Shell, Egg white and Egg yolk. The shell of an egg is porous to allow the developing chick to obtain oxygen. Other than oxygen, bacteria and odours can enter the egg. The pores also allow water and carbon dioxide to escape. The membrane that lines the inside of a shell acts as a filter to protect against bacteria. At one end of the egg, the membrane separates into an air space to supply the chick with oxygen. The shell is generally strong but the older birds tend to produce weaker shells. The shell's colour varies according to the breed of the bird. 88.5% of an egg is edible. There are two layers of egg white: the thick white layer (nearest to the yolk) and the thin white layer (nearest to the shell). The colour of the egg yolk is related to the diet of the bird and is due to the presence of carotenes and colouring added to a bird's feed. The nutritional value of the egg is not affected by the colour of the yolk (Belitz *et al.*, 2009). Table 1 below shows a comparison between the quail and chicken egg. However, these figures will alter slightly with the size of egg & food and conditions the birds are given (Suz, 2013).

Table 1: Comparison of Whole, Fresh, Raw Quail and Chicken Egg Nutrients

Nutrient	Unit	Quail egg value per 100g	Chicken egg value per 100g
Energy	Kcal	158	143
Protein	g	13.05	12.56
Total lipid (fat)	g	11.09	9.51
Carbohydrate, by difference	g	0.41	0.72
Sugars, total	g	0.40	0.37
Calcium	mg	64	56
Iron	mg	3.65	1.75
Magnesium	mg	13	12
Phosphorus	mg	226	198
Potassium	mg	132	138
Sodium	mg	141	142
Zinc	mg	1.47	1.29
Thiamin	mg	0.130	0.04
Riboflavin	mg	0.790	0.457
Niacin	mg	0.150	0.075
Vitamin B6	mg	0.150	0.170
Folate, DFE	mcg_DFE	66	47

Vitamin B12	µg	1.58	0.89
Vitamin A, RAE	mcg_RAE	156	160
Vitamin A	IU	543	540
Vitamin E	mg	1.08	1.05
Vitamin D	IU	55	82
Vitamin K	µg	0.3	0.3

Source: USDA (2012)

Eggs are considered to be among man's first foods as they are highly nutritious and easy to obtain. They are chockfull of proteins, minerals, and vitamins and are the favored breakfast food in most countries. The list of nutrients is rather impressive as it not only contains proteins, carbohydrates, healthy fats, vitamins and minerals, but also every single essential amino acid. The human body requires several different types of essential amino acids for almost all organ functions. While it is possible to include various foods in daily diet in order to obtain all the essential amino acids, an egg can simply be included in human diet to ensure that all amino acid requirements are met.

In order to understand this mystery of egg nutrient composition, it is important to differentiate between good (HDL) and bad (LDL) cholesterol. Eggs contain good cholesterol and some of the nutrients in eggs may actually help to decrease bad cholesterol levels. In addition, consuming foods that are high in cholesterol may increase cholesterol levels slightly; but consuming foods that are rich in saturated fats and trans fats will drastically increase cholesterol levels (DIETHEALTHCLUB, 2011).

Most calories from nutrients in eggs come from cholesterol contained in the egg yolk and only the whites can be used to drastically decrease calorie intake. The other nutrients from eggs include iron, zinc, phosphorous, potassium, and magnesium (DIETHEALTHCLUB 2011).

- Brain: Egg also contains choline which is an essential nutrient that supports brain development and function. This nutrient is also responsible for memory and muscle control and a severe choline deficiency can result in liver and kidney damage.
- Concentration: Eggs are a very healthy breakfast option for children as research shows that the regular consumption of eggs helps to increase attention span and has a positive influence on concentration abilities.
- Eyes: Eggs also contain certain antioxidants that help to protect the eyes and prevent cataracts later in life.
- Obesity: Obesity in children has become a common problem and nutritionists often recommend eggs for an obesity diet plan for children. This is because children who consume a single hard-boiled egg along with their breakfast are less likely to binge as the protein in the egg keeps them satisfied for a longer period.

It is extremely nutritious and in fact the amino acids are just one of the many nutrients in egg yolk. The egg yolk also contains Vitamins A, D, E, and K while the egg white contains sodium, potassium, and magnesium. The cholesterol content in egg yolk is very high and this is often a cause for concern. There are many doctors who insist that individuals with high cholesterol should avoid egg yolks, while other studies suggest that egg yolk may not have a negative impact on cholesterol levels (DIETHEALTHCLUB, 2011).

Eggs are highly versatile food containing many essential nutrients. Information on egg composition and utilization of egg for food and other purposes have been limited mostly to chicken eggs. Egg quality is composed of those characteristics of an egg that affects its acceptability to consumers such as cleanliness, freshness, egg weight, shell quality; yolk index, albumen index, Haugh unit and chemical composition (Stadelman, 1977; Song et.al. 2000). Chicken egg has been very well studied for its composition, however such information are not so abundantly documented in other poultry species. Hence, this study examines the proximate composition of Quail eggs fed varying levels of edible frog.

Materials and Method

The study was carried out at the aviary unit of the Department of Wildlife and Ecotourism Management, University of Ibadan. The University of Ibadan is located in the northern limit of lowland rainforest zone between latitude 7°26"N and longitude 3°54"E. It lies in a transitional zone between the rainforest and derived savanna zone. The humidity is relatively dry with mean annual rainfall of about 1220mm of double peak during June and August which last for almost 8months(April to October) and dry season between November and March (Adesina *et al.*, 2014).

A total number of 64 day old Japanese quail at the beginning of the trial was distributed into 4 treatments with replicates (16 birds per treatment and 8 birds per replicate i.e each treatment was replicated twice). The experimental diets include T_1 (control diet) contained 6% fishmeal + 0% frog meal, T_2 contained 4% fishmeal + 5% frog meal, T_3 contained 2% fishmeal + 10% frog meal and T_4 contained 0% fishmeal + 15% frog meal (Table 2). According to Prabakaran, (2003) protein and energy constitute about 90% of the total cost of the diet and any attempts at economizing on the diet means reducing the level of the protein and energy hence one of the most important factors in the formulation of quail rations is that the energy-protein ratio must be balanced in such a way as to get the maximum production with the minimum wastage. At 10 weeks old, 3 quail egg samples (3 replicates) were randomly selected from each treatment for proximate analysis of eggs of the quails fed varying levels of edible frog meal.

Table 2: Ingredient inclusion (%) of layer ration fed to quails

	Experimental diet (treatment)			
Ingredients	**1**	**2**	**3**	**4**
Maize	68.60	70.70	73.74	74.40
Soybean meal	15.20	10.10	4.06	0.40
Wheat offal	2	2	2	2
Groundnut cake	2	2	2	2
Palm kernel cake	2	2	2	2
Fish meal	6	4	2	0
Frog meal	0	5	10	15
Lime stone	1.5	1.5	1.5	1.5

Bone meal	2	2	2	2
Salt	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25
Methionine	0.1	0.1	0.1	0.1
Lysine	0.1	0.1	0.1	0.1
Total	100	100	100	100
Calculated CP	19.16	19.33	19.20	19.83

Method of Nutrient Analysis

The proximate composition of the quail eggs for dry matter, crude protein, crude fiber, ash, moisture content and ether extract were determined according to AOAC (1990). Calcium was determined by atomic absorption spectrophotometer and phosphorus analyzed by Technicon II Auto analyzer, which is based on calorimetric method.

Moisture Content: The determination of moisture content is one of the most important and widely used measurements in samples that absorb and retain water. Chemical analyses are normally made on dry matter basis. Moisture content determination look very simple in concept but in practice the accurate determination is complicated by number of factors which vary considerably from one sample to another.

Among the factors are the relative amount of water available and the ease with which the moisture can be removed. Methods that are based upon the removal of water from the sample and its measurement by loss of weight or the amount of water separated.

Air or vacuum oven drying at 70 -80°C are considered to be reliable methods provide that there is no chemical decomposition of the sample and water is the only volatile constituent removed. Sample should be dried to a constant weight.

Procedure

1. A clean and well labelled dish that has been oven dried was weighed (W1)
2. Enough sample was added into the dish and weighed (W2)
3. The dish and content was transferred into the thermo setting oven at about 105°c for about 24 hours
4. The dish was transferred from oven to desiccators, cooled for about one hour and weighted (W3)
5. % Moisture content was calculated.
6. Experiment was performed in replicates.

$$\% \text{ Moisture} = \frac{\text{Loss in weight}}{\text{Wt. of sample before drying}} \times 100$$

$$\% \text{ Dry matter} = \frac{W3-W1}{W2-W1} \times 100$$

Ash: The ash of a biological material is an analytical term for the inorganic residue that remains after the organic matter present in the original material since there may be losses due to the volatilization or chemical interaction between constituents. The importance of the ash content is that it gives an idea of the amount of mineral elements present and the content of organic matter in the sample. The organic matter accounts for quantitative constituents of proteins, lipid or fat, carbohydrate plus nucleic acid. Sample rich in organic matter can be preheated on flame or hot plate.

Procedure

1. Silica dish (crucible) was placed into muffle furnace for about 15 minutes at 350°C.

2. The dish was removed, cooled in a desiccator for about one hour and weighed (W1).
3. Enough sample was added into the dish (0.5 -2g) and the content weighed (W2).
4. The sample was pre dried.
5. The dish was places inside the muffle furnace and the temperature slowly increased from 200°C - 450°C to avoid incomplete ashing. Ash sample until it becomes whitish in colour. If ashing is in complete (evidence of black particles) within a reasonable period remove crucible, cool, moisten with few drops of distilled water, dry on water bath and return to the furnace.
6. The dish was removed from furnace to desicator and allowed to cool at room temperature.
7. The dish and content was reweighed (W3).

Calculation:

$$\% \text{ Ash} = \frac{W3\text{-}W1}{W2\text{-}W1} \times 100$$

% Organic matter = 100 - % Ash.

Ether Extract

Lipid (Fat) Extraction – Soxhlet Extraction Method: Fats are mixtures of various glyceride of fatty acids which are soluble in certain organic solvents. Extraction is carried out with soxhlet apparatus with ether or petroleum ether, the usual procedure is to continuously extract the fat content with 40/60°C petroleum ether in a convenient extractor (Soxhlet extractor). The ether extraction method is based on the principle that non-polar components of the sample are easily extracted into organic solvent. Direct extraction gives the proportion of free fat but gives no clue to the particular fatty acids. The soxhlet extractor is mostly suitable for dried samples.

Procedure

1. Crucible previously dried was weighed (W1).
2. Enough sample was added into the thimble and weighed again (W2).
3. 500ml round bottom flask was weighed W3.
4. The flask was filled with petroleum ether up to 2/3 of the 500ml flask.
5. The soxhlet extractor was filled up with a reflux condenser. The heat source was adjusted so that the solvent boils gently, left to siphon over several hours (5-6 hours).
6. The condenser was detached and the crucible removed, petroleum ether distilled from the flask.
7. The flask containing the fat residue was dried in an air oven at 100°C for 5 minutes. Cooled in a desiccator and weighed (W4).
8. The crucible is placed in the beaker in an oven at 50°C and dry constant weight with sample. Cool in desiccator and weigh (W5).

$$\% \text{ Ether Extract} = \frac{W1\text{-}W3}{W2} \times 100$$

Crude Fibre: Crude fibre is that portion of the plant material which is not ash or dissolves in boiling solution of 1.25% H_2SO_4 or 1.25% NaOH. Crude fibre was originally thought to be indigestible portion of any main food. It is known however that fibre consists of cellulose which can be digested to a considerable extent by both ruminant and non ruminant. The interest in fibre in food and feed has increased based on the noticed number of serious illnesses associated with diet low on fibre. Fibre swells and form gelatinous mass with high water retention capacity with the digestive system. Finding show that fibre product can absorb cholesterol, toxic agents and raise the excretion of bile acids and sterols.

Procedure

1. About 3.5 – 5g sample was transferred into 500ml conical flask.
2. 200ml of boiling 1.25% H2SO4 was added and allowed to boil gently for 30 minutes exactly using cooling finger to maintain constant volume.
3. Filtered through poplin cloth or filter paper by suction using Buchner funnel, rinsed well with hot distilled water, separated material back into the flask with spatula.
4. Added 200ml of boiling 1.25% NaOH and few drops of antifoaming agent and boiled gently for 30 minutes using cooling finger (KOH can be used in the place of NAOH) and vegetable oil as antifoaming agent.
5. Filtered through poplin cloth and wash with hot distilled water. Rinsed four times with hot distilled water, and once with 10% Hcl, four times again with hot water, twice with methylated spirit and three times with petroleum ether (where methylated spirit is not available). Ethanol could be used as a substitute for methylated spirit.
6. Servaged the residue into crucible after drain, dried in the oven at 105^{0}C, cooled in desiccator and weigh W2.
7. Placed in muffle furnace at about 300^{0}C for about 30 minutes.
8. Removed into desicator and allowed to cool to room temperature weigh again W3.

$$\% \text{ Crude Fibre} = \frac{W2\text{-}W3}{W1} \times 100$$

Crude Protein Using Kjedahl Method: The accepted standard method for the determination of nitrogen in samples involve the complete digestion of sample in hot concentrated acid, and in the presence of an appropriate metal ion catalyst is to convert all nitrogen in the nitrogenous materials in the sample into ammonium ion. Upon the addition of alkali to the digest, ammonia is released which may then either be distilled out of the sample and determined by simple acid-base titration, or the ammonia can be reacted with an appropriate reagent such as phenol and sodium hypochlorite, to give a coloured derivative which can be measured with colourimeter or spectrophotometer. The Kjeldahl digestion is usually performed by heating the sample with H_2SO_4 – containing substances which promote oxidation of organic matter by increasing the boiling point of the acid (K_2SO_4 or Na_2SO_4) and Se or Cu which increase the state of oxidation or organic matter. These reagents here referred to as digestion catalyst. It is necessary to digest the sample for certain period until you obtain a clear solution to ensure accurate results.

Method of Data Analysis

Data were analyzed using Analysis of Variance and T-test.

Result and Discussion

The result in table 3 shows the proximate composition of the quail eggs revealing that the percentage crude protein content of eggs increased with corresponding percentage increase in frog meal in Quail diet. However, Dudusola (2010) reported moisture content of 74.26%, crude protein 11.98%, crude fat 11.91% and crude ash of 1.04% in quail eggs reared in farm conditions while Tunsaringkarn *et al.*, (2013) reported contents of moisture, protein, fat and ash were 72.25%, 12.7%, 9.89% and 1.06% respectively from quail eggs collected from local markets. Ether extract % was higher in this study when compared to that of Thomas *et al.*, (2016) where he reported 11.99%. Table 4 presents result of T-test of significant difference between chemical composition and treatment of varying levels of edible frog in Quail meals. The results show that there is a significant difference that exist in crude protein chemical composition (t= 30.28, P<0.05), Crude fibre (t= 30.23, P<0.05), ash (t=7.00, P<0.05), Ether extract (t=66.82, P<0.05), Moisture content (t= 164.8, P<0.05) and Dry Matter (t= 89.07, P<0.05). From the result, it is deduced that all the chemical composition i.e. Crude protein, Crude fibre, ash, Ether extract, moisture content and dry matter are good feed for Quail eggs. Hence, Quail eggs from the third treatment (T_3) have the highest percentage (33.95) of crude protein.

Table 3: Proximate Composition of Eggs of Quail Fed Edible Frog Meal

ID	% Crude Protein	% Crude Fibre	% Ash	% Ether Extract	% Moisture Content	% Dry Matter
T1	31.68	0.01	2.00	31.00	65.68	34.32
T2	32.55	0.01	2.00	31.00	63.96	36.04
T3	33.95	0.01	2.00	32.00	65.43	34.57
T4	28.96	0.01	2.00	33.00	64.60	35.40

Table 4: Result of t-test of significant between chemical composition and treatment of varying levels of frog meal.

Chemical composition	Numbers of Treatments	df	Mean Difference	STD Error of Mean	T	P-value	Decision
Crude protein	4	3	31.78	2.102	30.23	0.000	Significant
Crude fibre	4	3	31.78	2.102	30.23	0.000	Significant
Ash	4	3	1.750	0.500	7.00	0.006	Significant
Ether extract	4	3	31.75	0.957	66.32	0.000	Significant
Moisture content	4	3	64.91	0.787	164.8	0.000	Significant
Dry matter	4	3	35.08	0.787	89.07	0.000	Significant

NB: df = Degree of freedom (df = n-1)
P-value is significant at 0.05 level of significant

Conclusion

This study revealed that percentage increase of frog meal in quail diet gives a corresponding increase in percentage protein content of its egg. Therefore, adoption of frog meal into Quail diet will improve the protein quality of their eggs. Further studies will seek to establish the economic feasibility and profitability of edible frog meal in Quail diet compared to other protein meal sources.

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