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**Vegetable (*Telfairia occidentalis*) Leaf Meal as Dietary Protein
Supplement in Laying Hens: Egg Laying Performance,
Egg Quality and Hematological Implications**

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Abstract: The objective of this study was to investigate by evaluating the bio-nutritional potency of leaf meal prepared from *Telfairia occidentalis* as a protein supplement in laying hens. An experiment was carried out with Hy-line Variety Brown layer birds beginning at the first phase of egg lay spanning a period of 3 months. Four isonitrogenous and isocaloric diets were formulated in which the major difference was the percentage inclusion levels of the *Telfairia occidentalis* leaf meal (TOLM) at 0, 10, 15, 20% in diets 1, 2, 3 and 4, respectively. There were no statistically significant differences ($p>0.05$) in the body weight changes, egg weight, albumen height and shell thickness. The mean egg weight values ranged between 63.20 ± 1.85 and 66.55 ± 3.14 g while albumen height ranged between 4.97 ± 0.26 and 5.30 ± 1.07 mm. The shell thickness also ranged from 0.47 ± 0.09 to 0.52 ± 0.08 mm. However, the yolk colour scores showed significant differences ($p<0.05$) among eggs collected from the different diets with yolk colouration of eggs from diets 3 and 4 having the deepest yellowish colouration. The Haugh unit (Hu) values calculated for all experimental hens on all diets were statistically similar ($p>0.05$). The hen day production of hens on diet 3 (15% TOLM inclusion) was consistently higher than others at an average of $63.54\pm7.38\%$. Hematological investigation did not reveal any deleterious effects on the performance and no physical health hazards were observed in all experimental hens. It is evident that TOLM if well prepared could be a potential source of plant protein particularly at 15% inclusion level in poultry layer diets.

Key words: Hematological studies, egg internal and external qualities

INTRODUCTION

The shortage of animal protein particularly in developing countries in Africa (Nigeria inclusive) has necessitated investigations of several novel sources of protein. This acute shortage of protein had been attributed to the phenomenal rise in the price of animal feeds which accounts for about 75-85% of the recurrent production cost in intensive monogastric animal production in Africa (Fetuga, 1977). This has escalated prices of animal products and by-products thus making animal protein generally beyond the reach of the average citizens in the developing countries. With the teaming population, there is an attendant rise in the demand for animal products in recent years but a careful survey of some relevant literatures and policy statements on the supply and consumption of animal protein reveals a growing concern with the widening gap between estimated protein requirement and actual protein consumption in many tropical developing countries including Nigeria (Fasuyi, 2007).

The shortage of animal protein is worsened by the state of economy in most developing countries which makes domestic production of animal protein to decline rapidly giving room for the importation

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of several frozen meats especially poultry meat. The net effect of the deficit is manifested in the prevalence of various diseases such as kwashiorkor, marasmus (Onwudike and Oke, 1986) and mental deficiencies (NRC, 1993). The ban on importation of agricultural raw materials has led to an acute shortage of caloric sources, deprivation of foreign investors and as a result the feed mill industry is currently witnessing a down turn in output, efficiency and capacity utilization. It is obvious that all these factors have contributed to the rising prices of animal feeds.

From the foregoing, it is therefore pertinent that some efforts must be geared towards improving the per capital animal protein consumption of the developing countries through the incorporation of relatively less expensive ingredients as feedstuffs. The major objective of this research work was to study by evaluating the bio-nutritional potency of leaf meal prepared from *Telfairia occidentalis* (TOLM) as a protein supplement in laying hens and to expose any deleterious effects associated with the consumption of this novel protein source through haematological investigations.

MATERIALS AND METHODS

Collection and Preparation of TOLM

Freshly harvested fluted pumpkin (*Telfairia occidentalis*) leaves were purchased from local farmers in different farming locations within the university community. The fresh leaves were immediately subjected to sun drying in an open cleaned concrete floor space until moisture content became constant at about 13%. The sun dried leaves were later milled using a locally fabricated milling machine. The milled *Telfairia occidentalis* leaf meal (TOLM) were collected in jute bags and tightly tied to forestall the attack of insects and other pests. They were also kept in a clean, well ventilated room prior to the feed formulation to ensure that the quality was maintained. Samples were taken from the bagged TOLM for proximate analyses, amino acid and mineral determination. Thereafter, the TOLM was used to formulate diets along with other ingredients purchased locally (Table 1-3).

Table 1: Proximate composition (g/100 g dry matter), gross energy (kcal g⁻¹) and amino acid profile (g/16 g N) of *Telfairia occidentalis* leaf meal (TOLM) (means, n = 2)

Composition (g/100 g)	TOLM
Dry matter	90.96
Crude protein	35.14
Ether extracts	9.61
Crude fibre	12.68
Ash	10.87
Nitrogen free extract	31.72
Gross energy (kcal g ⁻¹)	3.21
Amino acids*	
Alanine	6.51
Aspartic acid	6.21
Arginine	5.02
Glycine	6.10
Glutamic acid	11.01
Histidine	1.38
Isoleucine	5.10
Lysine	2.10
Methionine	2.48
Cystine	1.08
Meth. + Cys.	4.56
Leucine	7.58
Serine	3.91
Threonine	3.81
Phenylalanine	4.85
Valine	6.20
Tyrosine	5.62
Tryptophan	3.12

*Source: Fasuyi (2006a)

Table 2: Mineral composition of *Telfairia occidentalis* leaf meal (TOLM) (means, n = 2)

TOLM	Ca	P	K	Na	Mg	Fe	Mn	Cu	Zn
	(g/100 g)								
	(ppm)								
	2.6	1.2	6.2	5.1	4.7	5875	182	136	1036

Source: Fasuyi (2007)

Table 3: Phytic acid, phytin-P, oxalate, tannic acid and cyanide contents of *Telfairia occidentalis* leaf meal (TOLM) (means, n = 2)

TOLM	Phytic acid (mg/100 g)	Phytin-P (mg/100 g)	Phytin-P As % of total P	Oxalate (mg/100 g)	Tannin (mg/100 g)	HCN (mg/100 g)
	189.2	120.1	8.05	80.7	43.0	61.2

Source: Aletor and Adeogun (1995) and Fasuyi (2007)

Table 4: Composition of experimental diet (g/100 g)

Ingredients	Diets			
	1	2	3	4
	Inclusion levels of TOLM (%)			
	0	10	15	20
Maize (9% CP)	48.0	47.5	47.0	47.0
Wheat offals (15.3% CP)	16.0	17.0	16.0	15.0
Brewers dried grains (25.3% CP)	10.0	5.0	4.0	3.0
Fish meal (68.0% CP)	2.0	2.0	2.0	2.0
Soyabean meal (45.0%)	14.0	8.0	5.0	2.0
Palm oil	0.0	0.5	1.0	1.0
TOLM*	0.0	10.0	15.0	20.0
Bone meal	2.0	2.0	2.0	2.0
Oyster shell	7.0	7.0	7.0	7.0
L-Lysine	0.2	0.2	0.2	0.2
DL-Methionine	0.1	0.1	0.1	0.1
NaCl	0.2	0.2	0.2	0.2
Premix**	0.5	0.5	0.5	0.5
Total	100.0	100.0	100.0	100.0
Calculated				
Crude protein (%)	16.5	16.5	16.5	16.5
ME (kcal kg ⁻¹)	2496.1	2443.5	2448.0	2428.7
Crude fibre (%)	5.3	5.4	5.3	5.5
Ether extract (%)	4.1	4.4	4.2	4.3
Analysed				
Crude protein (%)	16.1	16.2	16.0	16.1
Crude fibre (%)	6.1	6.3	6.2	6.3
Ether extract (%)	5.2	5.3	5.3	5.4

*: TOLM, *Telfairia occidentalis* leaf meal, **: Contained Vitamins A 800 I.U.; D3 (1,4731.C.U); Riboflavin 4.20 mg; Pantothenic acid 5.0 mg; Nicotinic acid 20.0 mg; Folic acid 0.5 mg; Choline 300 mg; Vitamin K, 2.0 mg; Vitamin B₁₂, 0.01 mg; Vitamin E, 2.5I.U; Manganese, 56.0 mg; Iodine, 1.0 mg; Iron 20.0 mg; Copper 10.0 mg; Zinc 50.0 mg and Cobalt 1.25 mg

Proximate and Amino Acids Composition

Proximate composition of the *Telfairia occidentalis* leaf meal (TOLM) was determined (AOAC, 1995) while the amino acids were determined using Biochrom 20 Auto Loader Version, Model 80-2107-07 amino acid analyzer. The gross and digestible energy values were computed by method described by Ng and Wee (1989).

Experimental Diets

The TOLM was processed as earlier discussed in the Teaching and Research Farm of the University of Ado-Ekiti, Nigeria. The results of the proximate compositions earlier determined were used in the formulation of the different diets. Four isonitrogenous and isocaloric layers diets were formulated with the feed ingredients shown in Table 4. Diet 1 was the control diet and it served as the

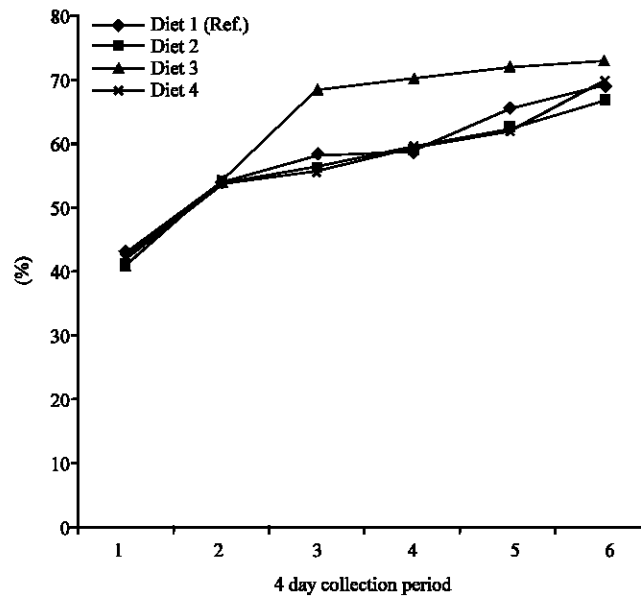


Fig. 1: Hen day production (%) for laying period

reference diet with which other diets were compared. Diets 2, 3 and 4 were formulated such that TOLM was introduced into the diets at graduated levels of 10.0, 15.0 and 20.0%, respectively. All diets were also supplemented with feed-grade methionine, lysine and vitamin/mineral premix.

Bird Husbandry and Experimental Design

Two hundred and forty layer birds that were previously kept on commercial layers diet were randomly selected from a stock of Hy-line Variety Brown hens at the first phase of the hen year. The experimental design adopted for this study was the completely randomized type. The laying birds were randomly allotted into individual cages with consideration for a uniform average weight, replicated thrice under four dietary treatments. Twenty layer birds were used per replicate making sixty birds per treatment. The birds were placed on the experimental diets immediately but the collection of eggs commenced after a 3 day stabilization (acclimatization) period. The birds were given feed and water *ad libitum* throughout the entire experimental period of 90 days.

Data Collection

Feed intake was determined on a daily basis by subtracting the weight (g) of the left over feed from the weight (g) of the feed initially offered. Feed efficiency was computed by calculating the amount of feed consumed per dozen of eggs laid (Fig. 1). The percentage hen-day production was computed as the percentage of the total number of eggs over the total number of days by number of hen i.e.

$$\% \text{ hen day production} = \frac{\text{Total No. of eggs}}{\text{Total No. of days} \times \text{No. of hens}} \times \frac{100}{1}$$

Weekly egg production was determined by collecting eggs laid per replicate every day and pooling together for counting the total collection for seven days. All eggs were sampled for weight by collecting

the fresh eggs per replicate per day and then weighing. The various weights were recorded against each replicate. The average egg weight for each replicate was later determined at the end of the experimental period. Determination of internal egg quality was carried out after the determination of egg weight. The fresh eggs were broken out on a flat non- absorbent surface (smooth-surface transparent glass slab) and the weight of the thick albumen was measured with a tripod micrometer as described by Haugh (1937). The yolk colour was scored with the aid of Roche Yolk colour fan. The two egg membranes were pulled off the shells immediately after being broken and the shells so peeled were air-dried for a day after which the egg shell thickness was determined with a micrometer screw gauge. The values so obtained were used to compute the average shell thickness per replicate. The Haugh's unit was calculated per replicate from the values obtained from albumen height and egg weight by employing the formula:

$$\text{Haugh's unit} = 100 \log (H + 7.57 - 1.7W^{0.37})$$

Where:

H = Observed albumen height in millimeters

W = Observed egg weight in grams

Mortality was calculated on a percentage basis i.e.,

$$\% \text{ mortality} = \frac{\text{No. of birds that died}}{\text{No. of birds housed}} \times \frac{100}{1}$$

Blood Collection for Analysis

This was carried out in the last week of the trial. Ten birds were selected per treatment and blood samples collected by way of venipuncture of the external jugular vein. Blood was delivered into two sets of clean labeled bijour bottles. One set with and another set without EDTA. The blood in the EDTA containing bijour bottles was processed for haematology while those in bottles without EDTA were processed for serum. The serum was kept deep frozen prior to analysis.

Hematological and Serum Analyses

The Packed Cell Volume (PCV) was estimated by spinning about 75 μ L of each blood sample in heparinized capillary tubes in a hematocrit micro centrifuge for 5 min while the total Red Blood Cell (RBC) was determined using normal saline as the diluting fluid. The Hemoglobin Concentration (HbC) was estimated using cyanomethaemoglobin method while the Mean Corpuscular Haemoglobin (MCH) and the Mean Corpuscular Volume (MCV) were calculated by the method described by Lamb (1981). The Erythrocyte Sedimentation Rate (ESR) of the blood as well as the total protein, albumin and globulin of serum were also determined by the method described by Lamb (1981).

Feed Cost Analysis

The cost of each experimental diet, average cost of actual feed consumed by experimental laying hens on each dietary treatment and the feed cost per kg egg produced by laying hens on each dietary treatment were calculated making use of the unit cost of each ingredient used in the diet formulation, average feed consumption values obtained during the experiment and the weight in kg of eggs pooled for each dietary treatment. All costing were done in Nigerian currency (Nigeria Naira is equivalent to 125USD as at the time of this study).

Statistical Analysis

Data collected on the performance indices, hematological indices and other egg quality parameters were subjected to analysis of variance (Steel and Torrie, 1960). Where significant differences were found, the means were compared using the Duncan's Multiple Range Test (DMRT) (Duncan, 1955).

RESULTS AND DISCUSSION

Average Egg Production Data for Laying Period

There was no significant weight change ($p>0.05$) in the experimental hens before and after the experimental period (Table 5). However, there were slight inconsequential increases/decreases in the weights of some experimental hens. The feed conversion was calculated on the feed consumed per dozen eggs laid. The feed conversion values obtained for all experimental hens were similar ($p>0.05$) with a range between 2752.00 ± 674.28 in diet 3 and 2869.33 ± 217.82 in diet 4 (Table 5). The hen day production (%) values in Table 5 were similar ($p>0.05$) for hens on diets 2, 4 and the control diet. Hens on diet 3 also had similar ($p>0.05$) hen day production (%) value with hens on the control diet.

Feed intake was also constant and similar ($p>0.05$) for all the experimental pullets since there was uniformity in the several factors affecting feed consumption. Such factors include nutrient content (particularly caloric content), house temperature, production rate, egg size and body weight (Anonymous, 2004). The suggested feed consumption rate for the Brown layer strain of layers under normal field conditions using an energy adequate diet was also met. The daily energy values based on the energy prediction equation with modifications based on actual performance experience for the Hy-line Brown egg layer is given (Anonymous, 2004) as:

$$\text{Daily energy values (kcal bird}^{-1} \text{ day}^{-1}) = W(140-2T) + 2E + 5\Delta W$$

Where:

- W = Current body weight in kilograms
- T = Average ambient temperature in degree Celsius
- ΔW = Body weight gain in $\text{g bird}^{-1} \text{ day}^{-1}$
- E = Daily egg mass in $\text{g bird}^{-1} \text{ day}^{-1}$

E can be calculated as:

$$\frac{\% \text{ production} \times \text{Egg weight in grams}}{100}$$

The feed consumed per dozen of eggs is the yardstick used for the measurement of feed efficiency (Oluyemi and Roberts, 2000). The determination of feed efficiency done by considering the total egg production and total feed intake is perhaps the major index in egg production measurement in laying birds. These values were similar for all experimental laying birds indicating a uniform feed efficiency index. These feed efficiency values were in agreement with standard values obtained and reported in literature (Hamilton, 1978; Onwudike and Oke, 1986). These values were also in consonance with values obtained in experiments where cassava (*Manihot esculenta*, Crantz) leaf protein concentrates (CLPC) and siam weed (*Chromolaena odorata*) leaf meal were used as protein supplements in layers' diets (Fasuyi *et al.*, 2005; Fasuyi, 2006b).

The percentage hen day production varied slightly but significantly ($p<0.05$) among the experimental laying hens with the highest value of $63.54\pm7.38\%$ recorded for pullets on diet 3 (15% TOLM dietary inclusion). Interestingly, the percentage hen day production of laying hens on diet 4 (20% TOLM inclusion) was similar to the value obtained for hens on the control diet 1 without

Table 5: Performance of laying hens and egg quality parameters for laying period

	Diets			
	1	2	3	4
	Inclusion levels of TOLM (%)			
Egg laying indices	0	10	15	20
Body weight (g)	1685.83	1776.50	1614.50	1643.00
Weight change (Δ WG)	0.43 ^a	2.76 ^a	-1.53 ^a	-1.51 ^a
Expected feed consumption day ⁻¹ (g)*	100.30	100.30	100.30	100.30
Average feed consumption day ⁻¹ (g)	100.90	101.20	101.20	101.40
Feed conversion (feed consumed/dozen egg produced, g)	2864.81 ^a	2880.00 ^a	2752.00 ^a	2869.33 ^a
Hen day production (%)	57.64 ^a	52.09 ^b	63.54 ^a	54.16 ^{ab}
Egg weight (g)	64.95 ^a	66.02 ^a	63.20 ^a	66.55 ^a
Albumen height (mm)	4.97 ^a	5.17 ^a	5.01 ^a	5.30 ^a
Yolk colour	4.81 ^a	8.58 ^{ab}	9.46 ^b	11.20 ^c
Shell thickness (mm)	0.47 ^a	0.49 ^a	0.52 ^a	0.51 ^a
Haugh unit	65.46 ^a	65.99 ^a	66.59 ^a	66.96 ^a
Liveability (%)	100.00	100.00	100.00	100.00
Cost of feed (Nigeria Naira kg ⁻¹)	35.36	33.27	29.01	27.37
Cost of feed consumed (Nigeria Naira)	321.10	303.02	264.22	249.78
Feed cost per kg egg (Nigeria Naira kg ⁻¹)	71.69 ^a	69.47 ^b	58.45 ^c	56.67 ^c

*: Commercial Management Guide, Hyline Brown Variety, 2002-2004, Means with same superscripts in the same horizontal row are not significantly different ($p>0.05$), Nigeria Naira is equivalent of 125USD as at the time of this study

TOLM. It is extrapolative that at certain dietary inclusion levels of TOLM, there was the possibility of an increased blood flow to the ovaries thereby leading to more ovarian follicle formation which ultimately increased egg production. This theory was corroborated by the study carried out on *Mansonina altissima* on laying birds (Ogbangba and Wekhe, 2005).

Apart from the nutritional qualities of TOLM as a potential protein source in poultry feed depicted by its chemical and proximate compositions and its equally favourable amino acids composition (Aletor and Adeogun, 1995; Fasuyi, 2007), many researchers have confirmed the presence of substances that contain antibiotics, growth promoting agents and stimulants in the green plants especially leaves (Uku, 2002; Ogbangba and Wekhe, 2005).

Egg Quality Characteristics

Egg quality characteristics are shown in Table 5. The egg weight mean values were similar ($p>0.05$) for all hens on all diets. This was also true for albumen height and shell thickness. The mean egg weight values had a range of 63.20 $\pm$ 1.85 g in eggs collected for hens on diet 3 to 66.55 $\pm$ 3.14 g in eggs collected for hens on diet 4. The albumen height had a range of 4.97 $\pm$ 0.26 mm in the eggs collected from hens on the control diet to 5.30 $\pm$ 1.07 mm in eggs collected from hens in diet 4. The shell thickness also ranged from 0.47 $\pm$ 0.09 mm in eggs collected from hens on the control diet to 0.52 $\pm$ 0.08 mm in eggs collected from hens on diet 3. The yolk colour scores were similar ($p>0.05$) for eggs collected from hens on diets 2 and 3 on one hand, diets 3 and 4 on another hand while the yolk colour score for eggs collected from hens on the control diet was statistically different ($p<0.05$) from all other scores. The Haugh unit (Hu) values calculated for all experimental hens on all diets were statistically similar ($p>0.05$).

The similarity in egg weight values for all eggs laid by experimental birds indicated that the dietary inclusion of TOLM in layers' diets even up to 20% (diet 4) did not affect the weight of the eggs. The average weight of the eggs also conformed and compared favourably (even higher in some cases) with values reported for layers in available literatures (Alkali and Filkry, 1972; Ernst *et al.*, 1975; Oluyemi and Roberts, 2000; Fasuyi, 2006b).

The albumen height and Haugh unit have correlative relationship (Oluyemi and Roberts, 2000). The height of the albumen determines the Haugh unit of the egg. When the albumen height is high, the

value of the Haugh unit is greatly enhanced and the better the quality of the egg (Oluyemi and Roberts, 2000). The albumen height values obtained for the experimental birds were similar ($p>0.05$). The Haugh unit values were also similar ($p>0.05$) for the eggs laid by the experimental laying birds. The average values of these two egg quality parameters conformed to values reported for standard commercial egg production guides and other available literatures (Ranvindrion and Blair, 1992; Anonymous, 2004). The average values of the Haugh unit and albumen height in this study were also in conformity with values obtained when siam weed (*Chromolaena odorata*) leaf meals and cassava (*Manihot esculenta*, Crantz) leaf protein concentrates (CLPC) were used in different experiments as protein supplements in layers' diet (Fasuyi *et al.*, 2005; Fasuyi, 2006b).

There was a deepening in the yellowish colouration of the yolk across the diets. The increase in the dietary inclusion levels of TOLM from diet 2 (10% inclusion) to diet 4 (20% inclusion) led to a corresponding increase in yolk colouration. Significant statistical differences ($p<0.05$) occurred in the yolk colour scores. Physical observation during the experiment also revealed a corresponding yellow pigmentation of the skin, beaks, ear lobes and shanks of the layer birds fed the TOLM-based diets. It is conceivable that TOLM had a high level of β -carotene which is a precursor of vitamin A. The carotenoid content of TOLM is easily convertible into vitamin A through its metabolism in the guts of the birds (Kuzmicky *et al.*, 1968; Onwudike and Oke, 1986).

The average shell thickness measured for eggs collected from the experimental laying hens had similar values ($p>0.05$) indicating a similar relative density for the eggs. The egg shell thickness is an important indicator of the specific gravity (relative density) of eggs since both are positively correlated (Oluyemi and Roberts, 2000). It is therefore conceivable that TOLM had no depressing effects on the specific gravity of eggs laid by the experimental laying birds.

The livability of the laying hens stood at one hundred percent since no mortality was recorded throughout the experimental period. The livability of the laying hens showed that the inclusion of TOLM did not constitute any physically visible health hazards obliterating the fear of the presence of some antinutritional factors in TOLM particularly phytates and oxalates (Leung *et al.*, 1968; Aletor and Adeogun, 1995) by the processing effects of shredding and sun drying the freshly harvested fluted pumpkin leaves may have contributed to the reduction of such ANFs (Fasuyi and Aletor, 2005). A combination of processing effects particularly sun drying have been reported to be effective in reducing the levels of antinutrients in cassava leaves (Fasuyi, 2005).

Haematological Indices Measured in Birds on TOLM-Based Diets

The haematological indices of experimental birds are shown in Table 6. Apart from the Erythrocyte Sedimentation Rate (ESR) values that showed significant differences ($p<0.05$), all other hematological parameters investigated showed no significant differences for all the birds on the

Table 6: Hematological indices of laying hens fed varying levels of dietary TOLM

Parameters	Diets			
	1	2	3	4
	Inclusion levels of TOLM (%)			
	0	10	15	20
PCV (%)	25.6	25.2	25.7	26.1
RBC count ($\times 10^6 \text{ mm}^{-3}$)	2.1	2.2	2.3	2.1
HbC (g/100 mL)	2.2	2.1	2.1	2.1
MCHC	7.2	7.0	7.2	7.1
MCH (pg)	9.4	9.4	9.2	9.4
MCV	128.1	127.9	128.0	127.9
ESR (mm)	4.1	4.2	4.2	4.1

TOLM: *Telfairia occidentalis* Leaf Meal, PCV: Packed Cell Volume, RBC: Red Blood Cell, WBC: White Blood Cell, HbC: Hemoglobin Concentration, MCHC: Mean Cell Hemoglobin Concentration, MCH: Mean Cell Hemoglobin, MCV: Mean Cell Volume, ESR: Erythrocyte Sedimentation Rate, All means in the same horizontal row are not significantly different ($p<0.05$)

Table 7: Serum metabolites of laying hens fed varying dietary levels of TOLM

Parameters	Diets			
	1	2	3	4
	Inclusion levels of TOLM (%)			
	0	10	15	20
Total serum protein (g/100 g)	9.7	10.1	9.9	9.8
Albumin (g/100 g)	0.7	0.6	0.6	0.7
Globulin (g/100 g)	9.1	9.2	9.0	9.1
Albumin/Globulin ratio	0.1	0.1	0.1	0.1

All means in the same horizontal row are not significantly different ($p < 0.05$). TOLM: *Telfairia occidentalis* leaf meal

4 treatment diets ($p > 0.05$). The ESR values for birds on diets 1 (control diet), 2 and 3 showed no significant differences ($p > 0.05$). The serum metabolites of laying birds fed the varying levels of TOLM as protein supplement are shown in Table 7. The total serum protein, albumin, globulin and albumin/globulin ratio showed no significant differences ($p > 0.05$).

Haematological examination is an assessment of the clinical and nutritional health status of animals in feeding trials. The most commonly examined hematological parameters in nutritional studies include PCV, RBC, HBC, MCHC, MCV and clotting time (Aletor and Egberongbe, 1992; Olorode and Longe, 2000; Adeyemi *et al.*, 2000). The haematological examination of the present study shows no adverse condition on the health status of the experimental birds as a result of the dietary inclusion of TOLM. The PCV and RBC experimental values were similar to those reported for chicks fed winged bean and full fat jatropha seeds (Igene, 1999; Adeyemi *et al.*, 2000). The threshold of tolerance of hemoglobin concentration was surpassed in all the experimental birds. All values were in the neighbourhood of those given in the control treatment during the hematological study and thrombocyte ultrastructure of the adult fowl fed dietary rapeseed meal (Maxwell, 1982). The blood physical properties indicated ESR as the only parameter that was significantly influenced by the dietary treatments. It is widely believed that the frictional resistance of the surrounding plasma, which holds the cells in suspension and the gravitational pull on the erythrocyte, mostly determines the ESR. The high value of ESR may precipitate acute general infections and malignant tumors (Frandsen, 1986). However, the ESR values obtained in this study were relatively low and may not be considered as having any significant effect on the health status of the test animals.

Total serum protein, albumin and globulin syntheses were generally similar for all the dietary treatments in the present study. The values obtained for the serum metabolites were generally similar with no significant differences ($p > 0.05$) and are comparable with many other reports (Igene, 1999; Adeyemi *et al.*, 2000).

Feed Cost Analysis

Expectedly, the cost of feed reduced from diet 1 to diet 4 apparently as a result of the increasing inclusion rate of TOLM across the diets. TOLM even though a vegetable used in human diets is relatively cheap to produce as a result of the agronomic ease of production (Leung *et al.* 1968). The cost of actual feed consumed by the laying hens on the diets also decreased across the diets from diet 1 to diet 4. The feed cost per Kg egg produced also followed the same pattern with significance differences ($p < 0.05$) among the treatment mean values. Only diets 3 and 4 (15 and 20% TOLM inclusions, respectively) had similar feed cost/kg egg values ($p > 0.05$).

CONCLUSIONS

Since the major preoccupation of poultry farmers is to produce poultry products at the lowest possible cost, it is pertinent that feed ingredients should be reasonably cheap and nutritionally potent

to support the production of poultry particularly in developing countries like Nigeria. The inclusion of TOLM in layers diets portends a positive economic implication since TOLM is a lot cheaper and more readily available than most conventional protein sources in monogastric animal feeds. Even though, fluted pumpkin leaves are equally consumed by man, the agronomy of production of this crop makes it a lot cheaper than most other plant protein sources used in poultry feed.

The inclusion levels of TOLM between 10 and 20% in laying hens had no deleterious effects on hen day production and other important egg production indices. There was also no apparent physical health hazards on the layer birds fed these diets. In fact, increased egg production was noticed in hens fed diets with 15% TOLM dietary inclusion.

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