PERFORMANCE, EGG QUALITY, BLOOD PROFILES AND CARCASS INDICES OF LAYING HENS FED WITH ALUM-WATER PROCESSED *ICACINIA MANNI* BASED DIET

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ABSTRACT

This study was carried out to examine the effect of Icacinia manni meal processed in alum water on performance, carcass, organs, egg quality characteristic, hematological and serum indices of laying hens. Freshly harvested I. manni tubers were washed, chopped into pieces, sun-dried and milled to produce sun-dried I. manni meal. The I. manni meal was soaked in alum-treated water, allowed to ferment for 72 hours. Thereafter, the meal was cooked for 1 hour, sun-dried, milled and sieved to produce I. manni alum-water fermented meal (IAFM). Three diets were formulated, diet I contained maize as the source of energy, diet 2 and 3 contained IAFM at 10 and 20 % levels respectively, replacing maize in the diets. 180 laying hens were used, each diet was fed to a group of 60 laying hens in a completely randomized design. Each group was subdivided into 3 replicates of 20 laying hens. The experiment lasted 12 weeks, data obtained were analyzed. There were no significant (p>0.05) differences in feed intake, body weight changes, feed conversion ratio, egg production and egg weight values. Egg quality indices were not significantly (p>0.05) affected by the treatment. All investigated blood profiles were not significantly (p>0.05) influence by the dietary treatment except the enzymes (ALP and AST). Dressed weight, live weight, dressing percentage, organs weights and abdominal fats were not significantly (p>0.05) affected by the treatments. It was concluded that IAFM can be included in layer's diet up to 20 % with no deleterious effects on the animal.

Keywords: Laying hens, Performance, Egg quality, Blood profiles, Carcass indices, *Icacinia manni*

INTRODUCTION

The production of eggs and meat is of great economic importance in poultry industry. The success of producing enough of these poultry products depend mainly on the availability of feeding ingredients. Feed alone account for about 70 % of the total cost of poultry production (Olorede *et al.,* 1996). The energy components of feed constitute the largest proportion of poultry diet. Maize is a conventional energy source in Nigeria and it constitutes the major feedstuff in poultry feed production, thus the supply of maize therefore determines the availability of compounded poultry feeds.

The demand for maize has always exceeded it supply due to the stiff competition that exist in it usage by humans, animals as well as the confectionary and milling industries. This has resulted in the scarcity and high cost of the grain thus, making it uneconomical to be used as a major source of energy in poultry diets (Udedibie and Asoluka, 2008). Therefore, there is need to replace maize with non-conventional, cheaper, non-competitive and readily available alternative feed source.

Icacinia manni (Earth ball) seems to have potentials as a source of dietary energy for

poultry in Nigeria. *I. manni* is a shrub with modified tuber which is mainly carbohydrate. *I. manni* is one out of the thirteen known species of *Icacinia* plant. The tuber weights up to 20kg vary in shape and colour depending on the soil type and stage of maturity.

It is a common wild field crop; it grows in the forest, fallow or wasted land and is locally abundant in Nigeria especially in the humid climate of Akwa Ibom State (Akobundu and Agyakwa, 1998).

Several researches have been carried on the value of *I. manni* as animal feed. Umoren *et al.* (2007) reported a satisfactory growth response of fermented *I. manni* at 15 % level of inclusion in broilers diet. Asuquo and Udedibie (2012) reported a depressed feed intake of laying hens fed *I. manni* meal and Effiong and Akpan (2017) reported that *I. manni* fermented in rumen digesta filtrate enhances the growth performance of finisher broilers at 20 % level of inclusion. Essien and Sam (2018) observed a positive growth response of broiler finisher chickens fed 20 % *I. manni* processed in saline. Effiong and Jimmy (2019) reported that the dressing percentage, relative cut-parts and internal organs of broiler chickens were not significantly affected when fed diet containing fermented and toasted *I. manni* meal. Scott *et al*. (1947) and Ekpo and Udedibie (2012) reported that *I. manni* contains gummy substances suspected to be galactomannan.

Galactomannans are group of polysaccharide with rigid hydrophilic backbone (poly mannose or mannan) and grated galactose units. They exhibit surface activity and are able to stabilize emulsions. Galactomannan with higher galactose content show little tendency to form gel due to the steric hindrance posed by galactose residues and generally exhibit lower viscosity. The variability in galactose composition and distribution along the mannan main chains is responsible for variation in their solubility. Also, specific hydroxyl groups of mannose and galactose are responsible for extensive inter and intra chain hydrogen bond formation resulting in differences in solubility, thermal stability and viscosity (Srichamroen *et al.,* 2008).

Galactomannan molecules are resistant to human digestive secretions in small intestine an hence function as dietary fibre. Srichamroen *et al*. (2008) reported that galactomannan derivative quar gum reduces or lowers glucose uptake from the small intestine when fed to rat due to it gel forming action.

Different methods of feed processing such as toasting, sun-drying and moist-heat treatment used by various researchers (Owokere, 2010; Asuquo and Udedibie 2012; Ekpo and Udedibie 2012) failed to improve the nutritive value of *I. manni* meal.

However, Umoren *et al.* (2007) reported a satisfactory growth response by broilers fed fermented *I. manni* with cassava. Fermentation is one of the local ways of detoxification of tuberous crops. During fermentation, cells of the fermented products are broken by enzymes or microorganisms, this facilitate the hydrolysis and washing-off of anti-nutritional substances (NRC, 1992; Okafor *et. al.,* 1999; Frediansyah, 2017).

According to Wikipedia (2021), alum is a crystalline hydrated double sulphate salt of aluminum. Alzomor *et al.* (2014) opined that alum sulphate may be used as an astringent which is a substance that causes contractions or shrinkage of tissues and that dry up secretion. Therefore, it may have the ability to loosen sticky substances as well as remove the gummy substance found in *I. manni* meal. The efficacy of alum as an astringent needed to be assessed in the processing of *I. manni* meal. Thus, this study was designed to determine the effect of *I. manni alum*-water fermented meal (IAFM) on the laying performance, egg quality characteristics, organs, haematology and serum biochemical indices of laying hens.

MATERIALS AND METHODS

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm of Akwa Ibom State University, Obio-Akpa Campus. Obio-Akpa is located between latitude 5^0 17′ N and 5^0 27′N and between 7^0 27′N and 7^0 58′E with an annual rainfall ranging from 3500 – 5000 mm and monthly temperature

range of 24 – 26^{0}C and relative humidity between 60 – 90 % (Wikipedia, 2016).

Source of *Icacinia manni* and Processing Method: Fresh *I. manni* were harvested from fallow land within the University Community. The tubers were washed, chopped into pieces and sun-dried for four days. The chips were milled to produce *I. manni* meal. The meal was later soaked in alum-treated water prepared by dissolving alum in water at the rate of 1 kg alum in 50 litres of water and allowed to ferment for 72 hours. Thereafter, the fermented *I. manni* meal was bagged and the fermented water squeezed out. The meal was boiled with fresh water for one hour and later sundried. The sundried meal was run through a hammer mill using a 2 mm sieve to homogenize it and produced the *I. manni* alum-water fermented meal (IAFM).

Toxicity, Anti-nutritive (Phytochemical) and Proximate Composition of *I. manni* Alum-Water Fermented Meal: From an earlier study alum-water processed *I. manni* meal had rich phytochemicals such as hydrogen cyanide, oxalate, saponins, flavonoid alkaloid, phytate and tannin (12.10, 26.16, 3.00, 4.15, 3.05, 0.00 and 0.00 mg/1000 g) levels respectively (Essien, 2015). Similarly, from an earlier study, the proximate composition of alum-water processed *I. manni* meal were crude protein, crude fibre, ether extract, ash and nitrogen free extracts (3.81, 2.74, 1.26, 4.12 and 67.23 % DM) respectively (Essien, 2015). The report also showed that fermentation in alum-treated water was able to eliminate phytate and tannin completely in *I. manni* (Essien, 2015).

Experimental Diets: Three experimental diets were formulated for the laying hens. The diet labeled T_1 (Control), T_2 and T_3 contained 0, 10 and 20 % levels of inclusion of *I. manni* meal process in alum treated water. The ingredients and nutrient compositions of the experimental diets are represented in Table 1.

Birds and Experimental Design: One hundred and eighty Isa brown at six weeks of laying life were used for the trial. The birds were weighed and allotted to three dietary treatments with nine replicate of 20 birds each in a completely randomized design (CRD) the laying hens were housed in a deep litter pen, feed and water were supplied *ad libitum*. Litter materials were changed by weekly. Birds were routinely vaccinated against Marek disease, New castle disease, Infectious bursal disease, and fowl pox between day 1 and 18 weeks of age (Stewart-Brown, 2015). A two week adaptation period to the experimental diet was observed before data collection.

Data Collection: The birds were weighted in grams at the beginning of the experiment and at the end of the feeding trials to determine changes in their body weights. Feed intake was determined by subtracting the weight of the left over feed from the weight of the feed fed the previous day. Feed conversion ratio was determined by dividing the weight of feed intake by egg weight (feed (g)/egg (g) (Clark *et al.,* 2019; Olajide, 2021).

Hen-day production was determined by dividing total egg production by the total number of layers. Data on laying performance of birds were collected weekly while those on egg quality were obtained five weeks to the end of the study. Eggs were collected twice daily and the trial lasted 12 weeks.

External Egg Quality Evaluation

Egg weight: Three eggs were randomly collected weekly from each replicate, labeled and weighed using electronic weighing balance to 0.01 g.

Egg length: The length of each was measured from the pointed end to the broad end with the aid of a vernier caliper with accuracy of 0.1 mm.

Egg width: Egg width was measured to the nearest 0.1 mm with vernier caliper. The egg width was taken as the diameter of widest cross-sectional region.

Table 1: Ingredient and nutrient composition of laying hens fed with alum-water processed *Icacinia manni* based diet

Ingredients and proximate composition	T1 (Control 0 % IAFM)	T2 (10 % IAFM)	T3 (20 % IAFM)
Yellow maize	50.00	40.00	30.00
***Icacinia manni* meal**	0.00	10.00	20.00
Soya bean meal	2.00	2.00	2.00
Blood meal	2.00	2.00	2.00
Fish meal	2.00	2.00	2.00
Palm kernel cake	5.00	5.00	5.00
Wheat offal	8.00	8.00	8.00
Bone meal	10.00	10.00	10:00
Common salt	0.25	0.25	0.25
Mineral /Vitamin Premix*	0.25	0.25	0.25
L-lysine	0.25	0.25	0.25
L-methionine	0.25	0.25	0.25
Total	100	100	100
Calculated chemical composition (%DM)			
Crude protein	18.46	18.44	18.42
Ether extract	3.93	4.03	4.07
Crude fibre	2.14	3.17	3.20
Ash	3.14	3.41	3.40
NFE	72.33	70.95	70.91
ME (Mcal/kg)	2.57	2.56	2.53

*IAFM: Icacinia manni tuber meal processed in Alum-treated water. *To provide the following per kg of feed: Vitamin A, 10,000 iu; vitamin D3, 200 iu, vitamin E, 12 mg, vitamin k, 2 mg, vitamin B1 , 1.5 mg; vitamin B2, 4 mg; vitamin B6 1.5 mg; vitamin B12, 12 mg; Niacin, 5 mg; panthothenic acid, 5 mg; Folic acid, 5 mg; Biotin, 2 mg; chorine chloride, 100 mg; manganese, 75 mg; zinc, 5 mg; iron, 2 mg; copper, 5 mg; iodine, 1.0 mg; selenium, 2.0 mg; cobalt, 5 mg; Antioxidant, 125 mg*

Egg shape index: Egg shape index was calculated as the percentage of the egg width to the length. Egg shape index = Egg width (mm)/Egg length (mm) x 100.

Egg shell thickness: Eggs were broken in a small petri-dish. The shell thickness of each broken egg was measured with a micrometer screw gauge to the nearest 0.01 mm. The measurement was taken from the pointed end, the middle and broad end of the egg and mean was obtained.

Eggshell weight: The broken egg shell was sundried for two days and the weight taken using sensitive top loading electric weighing balance to the nearest 0.01 g. The shell weight was expressed as a percentage of the egg weight and recorded as percent shell (% Shell) for individual egg.

Egg breaking strength: Egg breaking strength was calculated using the formula suggested by Arad and Marder (1982) and presented as EBS = 50.86 x [EW] 0.915, where EW = egg weight (gram).

Internal Egg Quality Evaluation

Three eggs per replicate were randomly selected from the total eggs collected per week for measurement of internal quality of eggs.

Albumen index: Albumen index was determined as the ratio of the albumen height to the diameter. Albumen index (A1) = H/0.5D, where H = height of thick albumen at the boundary with the yolk and D = Average of long and short diameter of albumen measured on the smooth surface.

Albumen weight: The albumen of broken fresh eggs were carefully separated from the yolk and weighed. The albumen weight was expressed as a percentage of the egg weight and recorded as percent albumen (% albumen) for individual egg sample.

Albumen height: Albumen height was determined by using spherometer. The measurement was taken at albumen widest expanse and midway between the yolk edge and the external edge of the thick albumen.

Yolk weight: The yolk was carefully separated from the albumen using a plastic separator and weighed individually with an electric sensitive weighing balance to the nearest 0.01 g.

Yolk width: The yolk width was measured around the widest horizontal circumference using vernier caliper measured to the nearest 0.1 mm.

Yolk index: This was taken as the ratio of the yolk height to the width without the removal of the yolk from the albumen.

Haugh unit: Haugh unit was determined by the formula of Haugh (1937) thus: Hu= 100log ($H + 7.57 - 1.7w^{0.75}$), where H = height of albumen in mm and W = weight of eggs in gram

Serological and Hematological Indices Determination: At the end of the feeding trial 10 ml of blood samples were drawn from three birds per replicate through the jugular vein. 5 ml of the blood samples were transferred into labeled and sterilized dry test tubes or bottles containing ethylene diaminetetracetic acid (EDTA) anticoagulant for haematological assay. Packed cell volume (PCV) and was determined using the capillary haematocrit centrifuge and haematocrit reader as described by Coles (1986). Haemoglobin (Hb) concentration, red blood cells (RBC) and white blood cell (WBC) counts were determined by cyanomethaemoglobin method as described by Coles (1986). Platelets and white blood cell differential counts were determined using 22 and 60 – part differential haematological analyzer (Mythic 22 and Mythic 60, Orphée, Switzerland). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated thus: MCV (%) = PCV x 10/RBC, MCH (%) = Hb x 10/ RBC, MCHC (%) = Hb x 100/PCV (Coles, 1986).

Another 5 ml of the blood sample were transferred into anticoagulant free bottles and was used to determine blood biochemical component such as total protein (g/dl), urea (mg/dl), creatinine (mg/l), albumin (g/dl), globulin (g/dl), calcium (mg/dl), phosphorus (mg/dl), glucose (g/dl), sodium (mmol/l), potassium (mmol/l) and chloride (mmol/l), aspartate amino transaminase (AST/µ/l) and alkaline phosphatase (ALP/µ/l). Total protein was determined by biuret method (Reinhold, 1953), albumin was determined by bromocresol green method (Henry, 1974) and creatinine by Basques-Lustosa's method (Lamb *et al*., 2006). Serum globulin was estimated based on the difference between the concentrations of total protein and albumin. Calcium and phosphorus was determined by the method of Gitelman (1967). Glucose was determined by the method of Trinder (1969). Sodium and potassium were determined by Tietz's method (Tietz, 1976). Chloride was determined by the methods of Schoenfeld and Lewellan (1964). Urea was determined by WHO (1980). AST and ALP was determined using Cobas Mira Automatic Analyzer (Roche Diagnostic system, Basel, Switzerland) at 37^{o}C with the aid of commercial kits (Lab test Diagnostica Lagoa Santa, MG, Brazil). Samples reading were performed using spectrophotometer (Lasany Single Beam Visible Spectrophotometer (Li-720), Lasany International, Panchkula) with light wave length adequate for each test.

Carcass and Organ Weight Evaluation: At the end of the experimental period (12 weeks) three birds were randomly selected from each replicate starved overnight of feed, weighed, slaughtered, defeathered and eviscerated. The live weights and dressed weights were obtained. The weights of the internal organs (heart, liver, kidney and gizzard) as well as abdominal fat were recorded.

Statistical Analysis: The various parameters measured were subjected to analysis of variance (ANOVA) using SPSS version 20.0 (IBM Corporation, Armonk, USA). Significant means were separated using Duncan's New multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

The results of the proximate and phytochemical composition of *I. manni* are presented in Table 2.

Table 2: Proximate and photochemical composition of raw and processed *Icacinia manni* tuber meal

Component (%DM)	RIMM	IAFM
Moisture	11.33	20.84
Crude protein	3.43	3.81
Crude fibre	2.53	2.74
Ash %	3.81	4.12
Ether Extract	2.56	1.26
Nitrogen free extract	76.34	67.23
Gross energy (KCAL/g)	3.88	3.79
Anti-nutrients (mg/100g):		
Phytate	24.03	0.00
Cyanogenic glycosides	20.20	12.10
Oxalate	56.12	26.16
Tannin	31.00	0.00
Saponin	5.32	3.00
Flavonoid	6.00	4.15
Alkaloid	5.60	3.05

RIMM – Raw Icacinia manni meal, IAFM = Icacinia manni alum-water fermented meal

The proximate composition of *I. manni* alum-water fermented meal (IAFM) showed low levels of crude protein, ash, crude fibre and ether extract with a high nitrogen free extract value which makes it a potential energy source feed stuff.

The result of the phytochemical or anti-nutritional substances in IAFM indicated low level of cyanogenic glycoside, saponins, flavonoid and alkaloid. There was no trace of tannin and phytate in IAFM. These results were in agreement with the observation of Sandberg (1991) where fermentation reduces the phytate level in plant foods. The loss of phytate during fermentation is believed to be due to enzyme phytase naturally present in the tuber or secreted by fermentative micro-organisms.

The results of the performance of laying hens fed diets containing IAFM is presented in Table 3. IAFM based diets had no significant ($p>0.05$) effects on feed intake of the laying hens. The results, disagreed with the report of Asuquo and Udedibie (2012) where fed intake of laying hens fed toasted *I. manni* meal decreased significantly.

No influence of the dietary treatments was observed among the treatment on body weight changes except for the control that recorded a numerically higher body weight value than the rest of the groups. It is important to note that body weight has been reported to be the single most important factor controlling egg weight for young pullets (Leeson and Summer, 2010). The IAFM treatment diets had no significant effects ($p>0.05$) on the feed conversion ratio of the laying hens. Laying hens in the control group had the least and the best feed conversion ratio. These results were in agreement with the findings of Asuquo and Udedibie (2012). These authors observed a high feed conversion ratio in laying hens fed toasted *I. manni* meal.

No influence of the IAFM base diets was observed on hen day egg production and egg weight. But the groups fed 20 % IAFM recorded numerically higher hen-day egg production percentage and also laid heavier and bigger eggs than the rest of the group, while the control recorded the least egg weight. This result was contrary to the findings of Asuquo and Udedibie (2012) where hen day egg production and egg weight of laying hens showed a significant ($p<0.05$) reduction in values when fed toasted *I. manni* meal.

No significant difference ($p>0.05$) was observed on egg quality characteristics (Table 4) of the laying hens. Egg width, egg length and egg shape index were statistically similar ($p>0.05$) in their values across the treatments. The values for egg shape index obtained in this study were higher than 0.75 which is regarded as the most satisfactory when eggs are to be package in specialized containers for transportation (Smith, 1990). Belyavin and Boorman (1981) observed that elongated (low index) and heavier eggs were

more prone to cracking. Good egg shape enhances marketing and profitability as round eggs do not show appearances and long eggs are much likely to break during packaging. The values of egg shape index obtained in this study showed that the laying birds produced eggs of good shape. This was in agreement with the study of Esiegwu (2012) who reported that good eggs shape index was gotten when laying birds were fed bitter kola meal.

Albumen height, albumen weight, albumen diameter and albumen index did not record significant differences ($p>0.05$) in their values across treatments. Egg containing large proportion of thick albumen is regarded as being of high quality (Harms and Hussein, 1993). They also reported that albumen weight was more closely associated with egg weight than yolk weight. Egg quality is the most important price contributing factor in table eggs. The values obtained for albumen parameters in this study showed that IAFM is capable of producing egg of high quality without producing any deleterious effect.

Table 3: Performance of experimental laying hens fed with alum-water processed *Icacinia manni* based diet

Parameter	Experimental Diets		
	T1 (Control 0 % IAFM)	T2 (10 % IAFM)	T3 (20 % IAFM)
Initial body weight (kg)	1.68 ± 0.04	1.71 ± 0.05	1.74 ± 0.05
Final body weight (kg)	1.77 ± 0.03	1.81 ± 0.04	1.74 ± 0.02
Average body weight changes (kg)	0.09 ± 0.01	0.10 ± 0.01	0.05 ± 0.01
Average feed intake (g/day)	113.43 ± 2.89	115.99 ± 3.11	119.93 ± 3.29
Average egg weight (g)	50.50 ± 0.55	52.68 ± 0.95	53.30 ± 1.00
Average hen-day egg production (%)	68.45 ± 1.50	70.24 ± 1.85	73.67 ± 2.10
Feed conversion ratio [feed (g)/eggs (g)]	2.25 ± 0.02	2.23 ± 0.04	2.21 ± 0.03

IAFM = Icacinia manni alum-water fermented meal

Table 4: Egg quality indices of laying hens fed with alum-water processed *Icacinia manni* based diet

Parameter	Experimental Diets		
	T1 (Control 0 % IAFM)	T2 (10 % IAFM)	T3 (20 % IAFM)
Haugh unit	95.12 ± 1.00	98.02 ± 0.81	94.99 ± 0.99
Egg shell thickness (mm)	0.53 ± 0.01	0.56 ± 0.02	0.55 ± 0.01
Egg shell weight (g)	7.07 ± 0.02	7.03 ± 0.03	5.97 ± 0.01
Egg shape index	0.79 ± 0.00	0.77 ± 0.01	0.76 ± 0.01
Egg circumference (cm)	4.48 ± 0.01	4.44 ± 0.01	4.29 ± 0.01
Egg length (cm)	5.68 ± 0.01	5.88 ± 0.01	5.78 ± 0.02
Egg breaking strength	1885.80 ± 11.07	1958.20 ± 12.02	2015.10 ± 13.01
Egg specific gravity	1.15 ± 0.01	1.14 ± 0.01	1.14 ± 0.01
Albumen weight (g)	25.80 ± 0.21	26.00 ± 0.23	25.73 ± 0.20
Albumen Diameter (cm)	7.55 ± 0.06	6.96 ± 0.05	6.83 ± 0.04
Album index	1.36 ± 0.01	1.26 ± 0.01	1.19 ± 0.01
Albumen height (cm)	8.03 ± 0.02	8.75 ± 0.04	8.13 ± 0.03
Yolk diameter (cm)	3.24 ± 0.05	3.89 ± 0.08	3.76 ± 0.09
Yolk height (cm)	3.98 ± 0.04	4.07 ± 0.05	3.82 ± 0.04
Yolk index	1.25 ± 0.01	1.05 ± 0.01	1.01 ± 0.01
Yolk weight (g)	25.8 ± 0.07	26.00 ± 0.12	25.73 ± 0.10
Relative yolk wt. (g)	31.55 ± 0.01	31.57 ± 0.01	31.62 ± 0.02

IAFM = Icacinia manni alum-water fermented meal

Yolk diameter, yolk height, yolk weight and yolk index were not significantly ($p>0.05$) affected by the treatment diets. The yolk index could indicate the ability of the egg to spread easily when broken which is undesirable quality to the consumer. This can also happen when eggs are stored for longer period.

The treatment diets did not have any significant ($p>0.05$) effect on egg shell thickness and shell weights. Breakage or cracking of egg shell in market channel is a serious concern to commercial egg producers. Shell thickness and weight affect the egg economic value whether as hatching egg or food product. Ketta and Tůmová (2016) grouped factors influencing egg shell quality into internal and external factors.

Among the external factors was diet's nutrition composition for laying hens. The non-significant difference ($p>0.05$) in shell weight and thickness observed in this study showed that the diet nutrition composition was adequate in calcium and phosphorous and also the IAFM based diet did not negatively interfere with calcium metabolism. This agreed with the report of Reichmann and Connor (1977) who asserted that the level of calcium in the diets of the laying birds had its greatest impact on shell quality as measured by shell thickness, that shell quality improved significantly with the increase in calcium level. Also, Reddy *et al.* (1968) noticed that when calcium level increased in the diet of the laying birds, there was a gradual increase in egg production.

The haugh unit of the laying hens was not significantly ($p>0.05$) affected by IAFM diets. Haugh unit is the measure of albumen quality. The values obtained for haugh unit in this study were higher than 72. Haugh unit of 72 and above is regarded as an indicator of freshness in eggs and a grade AA quality egg (North, 1978). Enyenihi *et al.* (2009) observed enhanced haugh unit of eggs laid by layers fed wetted and un-wetted sun-dried cassava tuber meal.

Egg specific gravity and egg breaking strengths were not affected by the diets. No significant ($p>0.05$) difference was recorded across the treatments. Wells (1967) reported that egg specific gravity is a reliable assessment of shell strength. He added that egg specific gravity is highly correlated to egg shell thickness. He further suggested that the measurement of shell deformation is of comparable reliability. Thus the result obtained in this study showed that the treatment did not negatively influence egg specific gravity and egg breaking strength of the experimental birds.

Haematological and Serum biochemical Indices: Haematological and serum biochemical results are shown on Tables 5 and 6. Significant differences ($p>0.05$) were not recorded in all the hematological parameters determined in this study. The diets did not show negative effect on the RBC counts, PCVs and Hb concentrations of the birds. Aletor and Egberongbe (1992) indicated that blood variables that are most consistently affected by dietary types include the RBC, PCV and plasma protein. Adejumo (2004) reported that PCV and Hb concentrations were correlated with the quality of the diet and the nutritional status of the animal. PCV is a blood toxicity reduction index and its abnormal level point to the presence of a toxic factor in the diet which has effect on blood formation (Oyawoye and Ogunkunle, 1998).

The Hb concentration and RBC values obtained in this study suggest that the IAFM diets were free of anti-nutritive factor and toxins.

The WBC counts, lymphocytes and neutrophils were not significantly affected ($p>0.05$) by the diets. But the results of the study showed a numerical increase in these values for laying birds fed 10 and 20 % IAFM diets over the control diet. This result was in agreement with the report of Essien and Sam (2018) where broilers fed *Icacinia manni* meal processed in saline had no significant difference ($p<0.05$) in their WBC counts, lymphocytes and neutrophils values. WBC plays a major role in defending the body against disease causing micro-organisms such as bacteria viruses and fungi (Britannica, 2020). A deficiency in WBC counts may result in an increased susceptibility to infections. The higher but non-significant values of WBC obtained in this study for laying birds fed 10 and 20 % IAFM diets is suggestive of enhanced immune system.

Table 5: Hematological indices of laying hens fed with alum-water processed *Icacinia manni* based diet

Parameter	Experimental Diets		
	T1 (Control 0 % IAFM)	T2 (10 % IAFM)	T3 (20 % IAFM)
WBC ($x10^5$/U1)	$6.55x10^5 \pm 2.01x10^3$	$6.67x10^5 \pm 2.13x10^3$	$7.01x10^5 \pm 2.19\ 1x10^3$
RBC ($x10^6$/ul)	$2.18x10^6 \pm 1.01x10^5$	$2.24x10^6 \pm 1.21x10^5$	$2.01x10^6 \pm 1.05x10^3$
Hb (g/dl)	9.40 ± 0.22	9.33 ± 0.21	8.97 ± 0.18
PCV (%)	29.77 ± 0.51	30.73 ± 0.64	28.13 ± 0.43
Platelets ($x10^4$/ul)	$2.30x10^4 \pm 1.01x10^3$	$2.41x10^4 \pm 1.02x10^3$	$2.43x10^4 \pm 1.11x10^3$
MCV (%)	136.07 ± 2.71	137.40 ± 2.23	137.00 ± 2.80
MCHC (g/dl)	29.13 ± 1.23	30.37 ± 1.32	30.10 ± 1.27
MCH (pg)	42.43 ± 1.32	41.70 ± 1.30	41.23 ± 1.31
Lymphocyte (%)	96.97 ± 1.53	96.33 ± 1.21	97.10 ± 1.11
Neutrophils (%)	12.07 ± 1.02	12.50 ± 1.13	12.10 ± 1.21
Eosinophils (%)	10.50 ± 0.09	8.83 ± 0.11	8.80 ± 0.13

IAFM = Icacinia manni alum-water fermented meal

Table 6: Serum biochemical indices of laying hens fed with alum-water processed *Icacinia manni* based diet

Parameter	Experimental Diets		
	T1 (Control 0 % IAFM)	T2 (10 % IAFM)	T3 (20 % IAFM)
Total protein (g/dl)	4.56 ± 0.32	7.41 ± 0.53	7.01 ± 0.63
Urea (mg/dl)	3.77 ± 0.31	5.37 ± 0.45	4.60 ± 0.32
Creatinine (mg/1)	53.51 ± 0.84	54.02 ± 0.98	53.41 ± 0.89
Albumen (g/dl)	3.15 ± 0.32	3.61 ± 0.39	3.76 ± 0.42
Globulin (g/dl)	1.41 ± 0.21	3.80 ± 0.23	3.25 ± 0.32
Calcium (mg/dl)	6.60 ± 0.46	2.07 ± 0.22	2.09 ± 0.23
Phosphorus (mg/dl)	2.08 ± 0.01	6.61 ± 0.08	6.58 ± 0.07
Glucose (g/dl)	2.08 ± 0.26	2.68 ± 0.25	3.57 ± 0.27
Sodium (mmol/L)	9.87 ± 0.33	9.60 ± 0.34	8.50 ± 0.31
Potassium (mmol/l)	3.00 ± 0.12	2.93 ± 0.23	2.60 ± 0.24
Chloride (mmol/l)	177.08 ± 2.32	166.25 ± 2.12	168.41 ± 2.14
Alkaline Phosphatase (u/l)	137.75 ± 2.01^a	143.21 ± 2.11^b	157.61 ± 2.17^c
Aspartate Amino Transferase (u/l)	208.25 ± 2.21^a	219.31 ± 2.27^b	225.42 ± 2.32^c

IAFM = Icacinia manni alum-water fermented meal. a, b and c = means in the same row with different superscript are significantly different ($p<0.05$)

This is consonance with the report of Watson *et al.* (2021) that white blood cells act as immunity cells which flow through the blood stream to fight viruses, bacteria, and other foreign invaders that threatens health. Also, Graczyk *et al.* (2003) reported that white blood cells and its differential leucocytes were used as indicators of stress response and sensitive biomarkers crucial to immune functions.

Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin count (MCHC) of the laying hens were not influenced by dietary treatments. The importance of MCV, MCH and MCHC lies in their use in the diagnosis of anemia and an index of capacity of bone marrow to produce RBC (Aletor and Egberongbe, 1992). All the haematological parameters values obtained in this study were within the normal range recommended for chickens (Mitruka and Rawnsley, 1977).

Among the serum biochemical parameters determined, the enzymes, alkaline phosphatase (ALP) and aspartate amino

transferase (AST) were significantly different ($p<0.05$) across treatments. The values for ALP and AST were significantly higher ($p<0.05$) in laying hens fed IAFM based diets than in hens fed the control diet. Serum enzymes help in maintaining the integrity of the internal organs especially liver and kidney. They are considered to be the markers of liver function (Alter, 2008). Thus, the significant increases obtained in this study suggest that the anti-nutritional substances present in *I. manni* were not completely eliminated.

Serum protein showed a numerical increase in laying hens fed 10 and 20 % IAFM based diets over the laying birds fed the control diet. Alagawany *et al*. (2016) reported that high serum protein is an indicator of sufficient protein intake in laying birds. Protein plays an important role in the diet formulation of poultry to maintain high immunity, improve growth and feed utilization (Alagawany *et al*., 2016). The values observed in this study showed that the test ingredient did not inhibit protein metabolism and that the protein found in *I. manni* was of a good quality.

Urea and creatinine levels in laying birds were not affected by the dietary treatments. This suggested that the protein of the three diets were well metabolized. Ross *et al.* (1978) reported that serum urea comes from the animal diet and the breakdown of tissue proteins. Ugwu *et al.* (2020) reported that creatinine has been found to be a fairly reliable indicator of kidney function. He further added that the stability in the serum creatinine and urea level suggests that the diets did not affect the kidney of the animals.

Albumen, globulin, total bilirubin and conjugated bilirubin levels in laying birds followed the same trend. Obikaonu *et al.* (2020) reported that albumen, globulin of broiler birds were not affected by dietary treatments. Ukpanukpong *et al.* (2018) reported that there was no significant difference ($p>0.05$) in conjugated and total bilirubin values when Bambara seed meal was fed to broiler chickens at 5% dietary level. Calcium and phosphorous were not significantly affected ($p>0.05$) by the dietary treatments.

Calcium and phosphorous are constituents of the skeletal structure of birds and are essential in egg formation (Pelicia *et al.,* 2009). The result of this study showed that the diets did not interfere negatively with calcium metabolism. This result tended to agree with the result of the phytochemical analysis which indicated non-presence of phytate in *I. manni* processed in alum-water.

Glucose, Sodium potassium and chlorine were not significantly affected ($p>0.05$) by the dietary treatments. All the values for biochemical indices obtained in this study were within the normal range for chickens (Mitruka and Rawnsley, 1977).

Carcass and Organ Weight Evaluation: Results of the carcass and organ weight of the laying hens fed IAFM based diets are shown in Table 7. The live weight, dressed weight and dressing percentage of the laying hens were not significantly affected ($p>0.05$) by the dietary treatments. Furthermore, the liver, heart and kidney weights were not significantly influence ($p>0.05$) by the dietary treatments. But there were numerical increase in the weight value of liver of laying hens fed 10 and 20 % IAFM. The liver weight increased with increased level of *I. manni*. There was no observed abnormality on the organs. Liver and kidney are involved in the elimination of toxins and metabolic wastes from animal's body. Also enlargement of this organ is always linked with the presence of anti-nutritional factors in the diet. These results tended to agree with Asuquo and Udedibie (2012) and where liver weight of laying hens and broiler birds fed moist heat treated and toasted *I. manni* did not differ significantly ($p>0.05$).

The gizzard and abdominal fat of the laying hens were not affected by the diet. But the abdominal fat weight of the laying hens fed 10 and 20 % IAFM based diet were relatively higher than that of laying hens fed the control diet. These results agree with the findings by Enyenihi *et al.* (2013) where laying hens developed more abdominal fat when fed diet containing 10 % peeled, fermented and gelatinized cassava.

Table 7: Internal organ weights / dressed weight of laying hens fed with alum-water processed *Icacinia manni* based diet

Parameter	Experimental Diets		
	T1 (Control 0 % IAFM)	T2 (10 % IAFM)	T3 (20 % IAFM)
Live weight (kg)	1.82 ± 0.07	1.78 ± 0.05	1.68 ± 0.03
Dressed weight (kg)	1.07 ± 0.02	1.06 ± 0.02	1.02 ± 0.01
Dressing (%)	58.79 ± 1.42	59.60 ± 1.47	59.50 ± 1.34
Liver (% of BW)	1.54 ± 0.08	1.57 ± 0.12	1.61 ± 0.16
Gizzard (% of BW)	1.43 ± 0.07	1.57 ± 0.08	1.65 ± 0.11
Heart (% of BW)	0.37 ± 0.01	0.45 ± 0.02	0.42 ± 0.01
Kidney (% of BW)	0.26 ± 0.01	0.22 ± 0.01	0.27 ± 0.02
Abdominal fat (% of BW)	2.32 ± 0.11	2.77 ± 0.15	2.98 ± 0.17

IAFM = Icacinia manni alum-water fermented meal

Conclusion: The study showed that detoxification of *I. manni* by fermenting in alum water greatly reduced the levels of anti-nutritional substances found in it that limits it usage as feed ingredients by animals; especially non-ruminant. The removal of these anti-nutritional substances, particularly the gum, has improved the nutritive value of the meal thereby rendering it acceptable to laying hens. *I. manni* processed in alum-treated water did not have any adverse effect on feed intake, body weight changes, feed conversion ratio, carcass/organ weights, egg quality indices and haematological profile of laying hens. *I. manni* process in alum water enhanced hen day egg production and egg weight at 20 % dietary level. Thus, the finding of the study suggests that laying hens can be fed with ration containing 20 % *I. manni* meal processed in alum water partly replacing maize without any detrimental health challenges.

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REFERENCES

ADEJUMO, D. O. (2004). Haematology, growth and performance of broiler finishers fed rations supplemented with Indian almond (*Terminalia catappa*) husk and kernel meal. *Ibadan Journal of Agricultural Research*, 1(1): 1 – 6.

AKOBUNDU, I. O. and AGYAKWA, C. W. (1998). *A Hand Book of West African Weeds*. 2nd Revised Edition, International Institute of Tropical Agriculture, Ibadan, INTEC Printers, Ibadan, Nigeria.

ALAGAWANY, M., EL-HACK, M. E. A., FARAG, M. R., RUCHI, T., SWATI, S., KUMARAGURUBARAN, K. and KULDEEP, D. (2016). Positive and negative impacts of dietary protein levels in laying hens. *Asian Journal of Animal Sciences*, 10(2): 165 – 174.

ALETOR, V. A. and EGBERONGBE, O. (1992). Feeding differently processed soya bean. Part 2. An assessment of haematological responses in the chicken. *Die Nahrung*, 36(4): 364 – 369.

ALTER, D. (2008). *Liver-Function Testing*. Medical Laboratory Observer, Sarasota, Florida, USA. https://www.mlo-online.com/home/article/13003063/liverfunction-testing Accessed March 20, 2021.

ALZOMOR, A. K., MOHARRAM, A. S. and AL ABSI, N. M. (2014). Formulation and evaluation of potash alum as deodorant lotion and after shaving astringent as cream and gel. *International Current Pharmaceutical Journal*, 3(2): 228 – 233.

ARAD, Z. and MARDER, J. (1982). Effect of gradual acclimation to high ambient temperatures on egg-shell quality of the

Sinai Bedouin fowl, the commercial white leghorn and their crossbreds. *British Poultry Science*, 23(2): 113 – 119.

ASUQUO, L. E. and UDEDIBIE, A. B. I. (2012). Effect of toasted *Icacinia manni* meal on the performance of layer hens. *International Journal of Agriculture and Rural Development,* 15(3): 1162 – 1168.

BELYAVIN, G. G. and BOORMAN, K. N. (1981). Physical characteristics of intact and cracked eggs. *British Poultry Science*, 22(1): 9 – 15.

BRITANNICA (2020). *White Blood Cells*. Encyclopedia Britannica. https://www.britannica.com/science/white-bloodcells Accessed March 20, 2020.

CLARK, C. E., AKTER, Y., HUNGERFORD, A., THOMSON, P., ISLAM, M. R., GROVES, P. J. and O'SHEA, C. J. (2019). The intake pattern and feed preference of layer hens selected for high or low feed conversion ratio. *PloS One*, 14(9): e0222304. https://doi.org/10.1371/journal.pone.0222304

COLES, E. H. (1986). *Veterinary Clinical Pathology*. 4th Edition, W.B. Saunders Company, Philadelphia, USA.

DUNCAN, D. B. (1955). Multiple range and multiple F tests. *Biometrics*, 11(1): 1 – 42.

EFFIONG, O. O. and AKPAN, V. E. (2017). Performance of finisher broiler chickens fed diets containing graded levels of rumen digesta filtrate fermented Earth ball (*Icacinia manni*) meal. *Journal of Agriculture and Ecology Research International*, 13(2): 1 – 8.

EFFIONG, O. O. and JIMMY, E. (2019). Chemical composition of toasted and fermented Earth ball (*Icacinia manni*) meal. Determination of their feeding value using broiler chickens. *Global Journal of Agricultural Sciences,* 18(1): 1 – 7.

EKPO, K. O. and UDEDIBIE, A. B. I. (2012). Moist heat treatment as a method of improving the nutritive value of *Icacinia manni* (Earth ball) for broilers. *International Journal of Agriculture and Rural Development,* 15(3): 1154 – 1161.

ENYENIHI, G. E., ESIEGWU, A. C., ESONU, B. O., UCHEGBU, M. C. and UDEDIBIE, A. B. I. (2013). Gelatinization of fermented cassava tuber meal and its nutritive value for laying hens. *Journal of Agricultural Technology*, 9(5): 1137 – 1149.

ENYENIHI, G. E., UDEDIBIE, A. B. I., AKPAN, M. J., OBASI, O. L. and SOLOMON, I. P. (2009). Effects of 5 hour wetting of sun-dried cassava tuber meal on the hydrocyanide content and dietary value of the meal for laying hens. *Asian Journal of Animal and Veterinary Advances*, 4(6): 326 – 331.

ESIEGWU, A. C. (2012). *The Value of Garcinia kola (Bitter Kola) as Feed Ingredient and Anti-Microbial Agent for Layers and Rabbits.* Ph.D. Thesis, Federal University of Technology, Owerri, Nigeria.

ESSIEN, C. A. (2015). *Phytochemical and Nutritional Evaluation of Icacinia manni (Earth Ball) Tuber Meal as Source of Dietary Energy in Poultry Production.* Ph.D. Thesis, Federal University of Technology, Owerri, Nigeria.

ESSIEN, C. A. and SAM, I. M. (2018). Performance and haematological response of finisher broiler chickens fed *Icacinia manni* (Earth ball). *European Journal of Physical and Agricultural Sciences,* 6(1): 1 – 7.

FREDIANSYAH, A. (2017). Microbial fermentation as means of improving cassava production in Indonesia. Pages 123 – 137. *In*: WAISUNDARA, V. Y. (Ed.). *Cassava*. InTechOpen, London. https://doi.org/10.5772/intechopen.71966

GITELMAN, H. J. (1967). An improved automated procedure for the determination of calcium in biological specimens. *Analytical Biochemistry*, 18(3): 521 – 531.

GRACZYK, S., PLISZCZAK-KRÓL, A., KOTOŃSKI, B., WILCZEK, J. and CHMIELAK, Z. (2003). Examinations of hematological and metabolic changes mechanisms of acute stress in turkeys. *Electronic*

Journal of Polish Agricultural Universities (EJPAU): Veterinary Medicine, 6(1): 05. http://www.ejpau.media.pl/volume6/issue1/veterinary/art-05.html

HARMS, R. H. and HUSSEIN, S. M. (1993). Variations in yolk: albumen ratio in hen eggs from commercial flocks. *Journal of Applied Poultry Research*, 2(2): 166 – 170.

HAUGH, R. R. (1937). A new method for determining the quality of an egg. *US Egg and Poultry Management,* 39: 27 – 49.

HENRY, R. J. (1974). *Clinical Chemistry Principles and Techniques*. 2nd Edition, Harper and Row, New York.

KETTA, M. and TŮMOVÁ, E. (2016). Eggshell structure, measurements, and quality-affecting factors in laying hens: a review. *Czech Journal of Animal Science*, 61(7): 299 – 309.

LAMB, E., NEWMAN, D. J. and PRICE, C. P. (2006). Kidney function tests. Pages 797 – 835. *In*: BURTIS, C. A., ASHWOOD, E. R. and BRUNS, D. E. (Eds.). *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics.* 4th Edition, Elsevier Saunders, St. Louis, Missouri, USA.

LEESON, S. and SUMMER, J. D. (2010). *Broiler Breeder Production*. Nottingham University Press, United Kingdom.

MITRUKA, B. M. and RAWNSLEY, H. M. (1977). *Clinical Biochemical and Hematological Reference Values in Normal Experimental Animal*. Mason Publishing Company, New York, USA.

NORTH, M. O. (1978). Standard relationship between week of egg production and egg size of commercial leghorns. Pages 420 – 421. *In:* NORTH, M. O. (Ed.). *Commercial Chicken Production Manual*. 2nd Edition, AVI Publishing Company Incorporated, West Port, Connecticut, USA.

NRC (1992). *Applications of Biotechnology to Fermented Foods: Report of an Ad Hoc Panel of the Board on Science and Technology for International Development.* National Academies Press, Washington DC, USA. https://doi.org/10.17226/1939

OBIKAONU, H. O. OBIORA, S. N. and OBIAGWU, P. (2020). Evaluation of different materials on hematological and serum biochemical indices of intensively managed finisher broiler chickens. *Nigeria Journal of Animal Production*, 47(3): 59 – 66.

OKAFOR, N., AZUBIKE, C. and IBENEGBU, C. (1999). Carriers for starter cultures for the production of garri, a fermented food derived from cassava. *World Journal of Microbiology and Biotechnology*, 15(2): 231 – 234.

OLAJIDE, R. (2021). Pride of Barbados (*Delonix regia*) seed meal improved the egg quality parameters of black Harco laying hens. *Nigeria Journal of Animal Production*, 48(2): 79 – 89.

OLOREDE, B. R., ONIFADE, A. A., OKPARA, A. O. and BABATUNDE, G. M. (1996). Growth, nutrient retention, haematology and serum chemistry of broiler chickens fed sheabutter cake or palm kernel cake in the humid tropics. *Journal of Applied Animal Research*, 10(2): 173 – 180.

OWOKERE, O. E. (2010). *The Potentials of Icacinia manni as Feed Ingredient in Broiler Diets*. M.Sc. Dissertation, University of Uyo, Uyo, Nigeria.

OYAWOYE, E. O. and OGUNKUNLE, M. (1998). Chemical analysis and biochemical effects of raw Jack beans on broiler. *Proceeding of the Nigerian Society of Animal Production,* 23: 141 – 142.

PELICIA, K., GARCIA, E. A., FAITARONE, A. B. G., SILVA, A. P., BERTO, D. A., MOLINO, A. B. and VERCESE, F. (2009). Calcium and available phosphorus levels for laying hens in second production cycle. *Brazilian Journal of Poultry Science*, 11(1): 39 – 49.

REDDY, C. V., SANFORD, P. E. and CLEGG, R. E. (1968). Influence of calcium in laying rations on shell quality and interior quality of eggs. *Poultry Science*, 47(4): 1077 – 1083.

REICHMANN, K. G. and CONNOR, J. K. (1977). Influence of dietary calcium and phosphorus on metabolism and

production in laying hens. *British Poultry Science*, 18(6): 633 – 640.

REINHOLD, J. C. (1953). Manual determination of serum total protein, albumin and globulin fraction by Burette method. *In:* REINER, M. (Ed.). *Standard Method of Clinical Chemistry.* Academics Press, New York.

ROSS, J. G., CHRISTIE, G., HALLIDAY, W. G. and JONES, R. M. (1978). Haematological and blood chemistry - comparison values for clinical pathology in poultry. *Veterinary Record*, 102(2): 29 – 31.

SANDBERG, A. S. (1991). The effect of food processing on phytate hydrolysis and availability of iron and zinc. *Advances in Experimental Medicine and Biology,* 289: 499 – 508.

SCHOENFELD, R. G. and LEWELLAN, C. J. (1964). A colorimetric method for determination of serum chloride. *Clinical Chemistry*, 10(6): 533 – 539

SCOTT, H. M., MATTERSON, L. D. and SINGSEN, E. P. (1947). Nutritional factors influencing growth and efficiency of feed utilization. 1. The effect of the source of carbohydrate. *Poultry Science,* 26(5): 554 – 554.

SMITH, A. J. (1990). *Poultry*. The Tropical Agriculturist, CTA / Macmillan Press Limited, London.

SRICHAMROEN, A., FIELD, C. J., THOMSON, A. B. and BASU, T. K. (2008).The modifying effects of galactomannan from Canadian-grown fenugreek (*Trigonella foenum graecum* L.) on the glycemic and lipidemic status in rats. *Journal of Clinical Biochemistry and Nutrition*, 43(3): 167 – 174.

STEWART-BROWN, B. (2015). *Vaccination Programs in Poultry.* MSD Manual, Veterinary Manual, Merck and Company Incorporated, Kenilworth, New Jersey, USA. https://www.msdvetmanual.com/poultry/nutrition-and-management-poultry/vaccination-programs-in-poultry Accessed December 4, 2016.

TIETZ, N. W. (1976). *Fundamental of Clinical Chemistry*. W. B. Saunders and Company, Philadelphia, USA.

TRINDER, P. (1969). Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. *Journal of Clinical Pathology*, 22(2): 158 – 161.

UDEDIBIE, A. B. I. and ASOLUKA, O. C. (2008). Effects of 5-hour wetting of sun-dried cassava tuber meal on the HCN content and dietary value of the meal for young broiler chicks. *Nigerian Journal of Animal Production*, 35(1): 25 – 31.

UGWU, D. O., JIWUBA, P. C., UBOGU, V. R. and AKAZUE, R. C. (2020). Phytochemical properties of *Hibiscus sabdariffa* calyx and the effects of its aqueous extract supplementation on haematological and serum biochemical indices of broiler birds. *Nigerian Journal of Animal Science*, 22(3): 165 – 172.

UKPANUKPONG, R. U., BASSEY, S. O., OGUNBODE, S. M. BASIRU, D. I., OMANG, W. A. and ETENG, M. U. (2018). Effect of processed *Vigna subterranean* seeds on alkaline phosphatase, conjugated and total bilirubin levels in broiler chickens finishing phase. *International Journal of Current Research in Biosciences and Plant Biology,* 5(8): 15 – 23.

UMOREN, U. E., ISIKA, M. A., ASANGA, E. P. and EZEIGWE, O. N. (2007). Effect of replacement of maize with earth ball (*Icacinia manni*) meal on the performance of broiler chickens. *Pakistan Journal of Biological Sciences*, 10(14): 2368 – 2373.

WATSON, L. R., TURLEY, R. and GERSTEN, T. (2021). *Health Encyclopedia*. University of Rochester Medical Centre, Rochester, New York.

WELLS, R. G. (1967). Egg shell strength, the relationship between egg specific gravity and egg shell deformation and their reliability as indicators of shell strength. *British Poultry Science,* 8(3): 193 – 199.

WHO (1980). *Manual of Basic Techniques for a Health Laboratory.* World Health Organization, Geneva, Switzerland. https://www.who.int/medical_devices/p

ublications/manual_healthlab_tech/en/ Accessed December 4, 2016.

WIKIPEDIA (2016). *Akwa Ibom State*. Wikipedia. https://en.wikipedia.org/wiki/Akwa_Ibom_State Accessed December 4, 2016.

WIKIPEDIA (2021). *Alum*. Wikipedia. https://en.wikipedia.org/wiki/Alum Accessed March 20, 2021.