EFFECTS OF *GMELINA ARBOREA* ROXB LEAVES ON HAEMATOLOGICAL INDICES OF *COTURNIX COTURNIX JAPONICA* TEMMICK

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ABSTRACT

This study was a thirty six weeks feeding trial to determine the effect of inclusion of varying levels of Gmelina arborea leaf meal (GALM) on haematological indices of two weeks old Japanese quail (Coturnix coturnix japonica). Ninty six birds were allocated to four dietary treatment groups replicated thrice with 8 birds per replicate in a completely randomized experimental design. Treatments A – D were fed A 0 g/kg (control), B 50 g/kg, C 100 g/kg and D 150 g/kg of GALM. Feed and water were provided ad libitum. Haematological indices were assayed using standard protocols and data collected subjected to ANOVA. There were significant difference (p<0.05) in haemogblobin concentration (Hb) and white blood cell (WBC) count, while no significant difference (p>0.05) were observed in pack cell volume (PCV) and red blood cell (RBC) count. The haematological values observed in the study fell within values of Hb (12.30 g/dl), PCV (37 %), and ranges of RBC (2.30 – 3.86 x 10^6 µl), WBC (20 – 40 x 10^6 µl) recommended for normal healthy quails. The birds fed GALM remain clinically normal throughout the period of the experiment and thus the quantity of GALM used in this study can be recommended for inclusion in Japanese quail diets for shorter duration.

Keywords: *Gmelina arborea,* Haematological indices, *Coturnix coturnix japonica,* Japanese quails

INTRODUCTION

Animal protein deficiency, particularly among the rural populace, has remained an issue of concern in developing countries. In order to meet the demand, the production of food must be increased especially those of the basic nutrients, energy and protein. Also factors such as inadequate supply of day chicks, disease, high cost of drugs and the time taken to raise the birds led to search for an alternative source of poultry production and this led to subsequent introduction of quail birds in Nigeria (Adamu *et al.*, 2015). The Japanese quails have the potential to serve as an excellent and cheap source of animal protein for Nigerians (Babangida and Ubosi, 2006; Ani *et al.*, 2009). Distinct characteristics of the Japanese quail which include rapid growth thus enabling the quail to be ready for consumption at 5 – 6 weeks of age, early sexual maturity which results in short generation interval, disease resistance, less capital requirement, high rate of lay and much lower feed and space requirement than domestic fowl (Adeogun and Adeoye, 2004) have further given the bird advantages and attention. Among birds, the meat and eggs of quails have less fat content and has been suggested as good quality meat source for diabetic patients and those with high blood pressure (Agwunobi and Ina-Bor, 2007). Conversely, livestock farmers, especially those

in Nigeria and neighboring countries are faced with the problem of continuous inadequacy of feed ingredients. The high cost of conventional feedstuff like maize, guinea corn and millet, in the developing countries such as Nigeria, caused animal nutritionists to advocate for the use of non-conventional feed ingredients for farm animals feed production (Durunna *et al*., 2006). One of such ingredients is *Gmelina arborea* leaf meal, a product of *Gmelina arborea* tree which is not in competition with man's dietary needs (Ahemen *et al*., 2018).

The potential of leaf meals from tropical trees and shrubs to provide relatively higher levels of crude protein and minerals, and low crude fiber levels than tropical grasses has been studied (Esonu *et al*., 2003; Fasina *et al*., 2004; Okagbare *et al*., 2004; Amata, 2010). Despite the amount of research done on the use of nonconventional feeding stuffs that could have a major impact on livestock production, they continue to be unused, underdeveloped or underutilized. A critical factor in this regard has been the lack of proper understanding of the nutritional principles underlying their utilization (Amata and Lebari, 2011).

Evaluation of the haematological profile of quails provide useful information about their physical condition, making them useful tools in differentiating apparently healthy birds from abnormal or diseased ones (Agina *et al*., 2017). Although, the Japanese quail is fairly resistant to diseases, determination of haematological parameters in quails helps in the clinical management of disorders of the blood and bone marrow. In addition, the relatively short life span of Japanese quail (3 to 4 generations per year) is a useful experimental tool with which to study the effect of age, nutrition, disease and environment on haematological parameters of birds (Ottinger, 2001; Holmes *et al*., 2003). So there is need to look for ingredients to be added to the poultry feed for enhance growth performance of the quail birds. This led to the research on leafy supplement such as *Gmelina arborea* to ascertain its effect on quail bird production.

The deciduous plant, *Gmelina arborea* is widely distributed in the tropics of Africa and warm temperature regions. *Gmelina arborea* is a novel leafy feed stuffs and not much study has been done on the utilization of *Gmelina arborea* in monogastric animal nutrition (Annongu and Folorunso, 2003; Annongu and Fasanya, 2004). Based on the above this research work was conducted to evaluate the effects of inclusion of varying levels of *Gmelina arborea* meal on haematological indices of Japanese quail.

MATERIALS AND METHODS

This study was carried out at the Animal House of the Zoology Department, Nnamdi Azikiwe University, Awka, Awka South Local Government Area, Anambra State, Nigeria. The *Gmelina arborea* leaves were collected from Awka, air dried to a constant weight and milled into fine powder to produce the *Gmelina arborea* leaf meal (GALM).

Phytochemical tests for the presence or absence of alkaloid, saponnin, flavanoid, tannin and steroid were carried out on the GALM used in feeding the quails using the procedure outlined by Harborne (1998).

All the animals used in this experiment were handled in accordance with the guidelines and ethical conduct for the used of non-human animals in research (APA, 2012). A total of 96 Japanese quail were used for the study that lasted for six weeks. The experimental cage was the colony closed cage housing system. Each of the four tiers had three partitions representing four treatments replicated thrice. The animals were allocated into the four treatment groups with three replicates of eight birds each with the ratio of 1 male to 3 females (Momoh *et al*., 2014) based on live weights.

The treatment groups were labeled A to D and correspond with the inclusion of GALM in the poultry diets. The inclusion levels were; Group A 0 g/kg (control), Group B 50 g/kg, Group C 100 g/kg and Group D 150 g/kg.

The feeds used during the study were formulated feeds with *Gmelina arborea* leaf meal as presented in Table 1. Feeds and water were provided *ad libitum.* The feed with different levels of inclusion of GALM was analyzed for proximate composition (AOAC, 2005).

Table 1: Proximate and nutrient composition of the feed formulated with varying levels of *Gmelina arborea* leaf meal (GALM) fed to quail birds

Ingredients	Group A (0 g/kg GALM) Control	Group B (50 g/kg GALM)	Group C (100 g/kg GALM)	Group D (150 g/kg GALM)
GALM	0.00	5.00	10.00	15.00
Maize	45.02	45.02	45.02	45.02
Wheat offal	10.00	10.00	10.00	10.00
Soya Beans	11.76	11.76	11.76	11.76
Groundnut Cake	23.52	23.52	23.52	23.52
Bone Meal	3.00	3.00	3.00	3.00
Limestone	6.00	6.00	6.00	6.00
Salt	0.20	0.20	0.20	0.20
Methionine	0.15	0.15	0.15	0.15
Lysine	0.10	0.10	0.10	0.10
Premix	0.25	0.25	0.25	0.25
Nutrient composition				
Moisture	3.70	4.20	4.70	4.90
Crude Fat	4.80	5.60	5.80	6.10
Ash	10.50	12.80	13.40	13.70
Protein	17.30	17.80	18.40	18.90
Crude Fibre	7.70	6.90	6.10	5.40
Carbohydrate	55.90	52.70	51.60	50.90
Ca/P	1.68	1.88	1.86	1.79
Na/P	0.04	0.05	0.06	0.06

Quails are comparatively resistant to diseases than chicken and other poultry birds (Abao *et al.*, 2015; Nasar *et al.*, 2016; Arya *et al.*, 2018), thus no vaccine was given to quails (Abao *et al.*, 2015; Redoy *et al.*, 2017). Biosecurity measures adopted were that no visitors and new birds were allowed into the experimental area. The cages, environment, feeding and drinking troughs were thoroughly cleaned daily. Artificial lighting was used to provide the birds with 16 hours during the experimental period (Lima *et al.*, 2016).

Data Collection: The following haematological parameters were measured using the methods of Mitruka and Rawnsley (1977); packed cell volume (PCV), haemoglobin concentration (Hb), red blood cells (RBC) count and white blood cells (WBC) count.

Statistical Analysis: The data of haematological indices of the quails were subjected to Analysis of Variance (ANOVA) using SPSS (2007). The least significant difference (LSD) was used to separate means with significant differences at 5 % significant level.

RESULTS AND DISCUSSION

The feed with 150 g/kg of GALM had the highest ash value (13.70 %), crude fat (6.10 %) and crude protein (18.90 %) (Table 1). The crude protein values are was line with the crude protein recommended by Murakami *et al.* (1993) who recommended 18 % crude protein, but lower than 20 % recommended by NRC (1994) and Babangida and Ubosi (2006) for production period of quail birds. The crude protein value in this study was higher than 17 % recommended by Bawa *et al.* (2011). The increased in crude protein content of feed in this study with increasing inclusion GALM was not surprising as Okagbare *et al.* (2004), Amata and Lebari (2011) and Aye (2016) reported high protein content in *Gmelina arborea* leaf meal. Thus, *Gmelina arborea* leaf meal may be an important source of dietary protein for both human and livestock judging from its proximate composition (Aye, 2016).

The calcium: phosphorous ratio of feeds used in this study had similar values with those of Nieman *et al.* (1992) who reported that in animals Ca/P ratio above 2.0 helps to increase the absorption of calcium in the small intestine.

Food is considered "good", if the ratio Ca/P > 1 and "poor" if Ca/P < 0.05 (Nieman *et al.*, 1992). The Ca/P values for all the treatments were all greater than 1 (Ca/P > 1). The sodium: phosphorous ratio of feeds used in this study were equal and less than 0.06 (Na/P < 0.06) (Table 1).

Phytochemical in *Gmelina arborea:* Phytochemical analyses of the *Gmelina arborea* showed the presence of flavonoid, saponins, tannin, alkaloid, phenol and steroid. Tannin, phenol and saponins were strongly present, and flavonoid, steroid and alkaloid were moderately present (Table 2).

Table 2: Phytochemical composition of *Gmelina arborea* leaf meal

Phytochemical parameter	Qualitative results
Flavonoid	+
Tannin	++
Steroid	+
Saponin	++
Phenol	++
Alkaloid	+

Key: + = present; ++ = strongly present

These findings was in agreement with the findings of Osakwe (2003) and Aye (2016) who reported the presence of high concentration of of tannin and other anti-nutritional substances in their biomass which affected their optimal utilization by animals. Furthermore, the phytochemical content of feed in this study was in agreement with Okpara *et al*. (2016) who reported the presence of tannin, alkaloid, saponin, flavonoid and steroid as depicting potential toxicity of the feed resources. They further opine that air-drying of the leaf meal did not reduce the nutritive values but reduced effect of the anti-nutritional factors (ANF's). Similarly, Iswarya *et al.* (2017) reported that GALM is rich in phenol, saponin, alkaloid, tannins, flavonoid and steroid. The presence of these phytochemicals in *Gmelina arborea* makes it a potential plant for pharmaceutical and medical uses. All these contributed to the healthy status of the birds throughout the study though it was observed that four birds died from the control diet. This mortality was not associated to the dietary inclusion of GALM since the control diet had zero inclusion. The zero mortality recorded among the quails fed air-dried GALM confirmed that air-drying was effective in detoxifying anti-nutritional constituents to a safe level.

Haematological Performance of Quails

Pack cell volume (PCV) (Haematocrit): The result obtained for PCV at the beginning of the study showed no significant difference ($p>0.05$) ($p = 0.98$) among the dietary treatments (Table 3). The PCV value obtained from each treatment was lower than 35 % which suggests that the birds were susceptible to anaemia (Ihedioha, 2004; Thrall, 2006). The result obtained at the end of the study showed no significant difference ($p>0.05$) ($p = 0.052$) among the dietary treatments (Table 4). The PCV values obtained from all treatments were higher than 35 % which suggest that the birds were no longer susceptible to anaemia (Ihedioha, 2004; Thrall, 2006).

The result was in line with the findings Babangida and Ubosi (2006), Usman *et al.* (2008), Tuleun *et al.* (2011) and Anggraeni *et al*. (2016) who reported no significant difference ($p>0.05$) in PCV values of quails across the dietary treatment. Haematocrit value is the percentage (by volume) of the blood that consists of red blood cells after being centrifuged and indicates the total number of erythrocytes in the blood. Changes in PCV could be caused by a number of natural factors that include sex, age, geographical elevation, parasitism, energy expenditure, nutrition and genetics (Fair *et al.,* 2007). Haematocrit also increased with age from hatching, due to increased erythropoiesis, so that adult birds generally have greater PCV values than nestlings or juveniles (Fair *et al*., 2007). The result of this study was not in line with the findings of Fatokun *et al*. (2013) who reported significant ($p<0.05$) difference in PCV of quails across the dietary treatment but agreed with finding that decrease in PCV may be due to increased crude fibre across dietary treatments (Fatokun *et al*., 2013).

Table 3: Haematological indices of quails fed with different inclusions of *Gmelina arborea* leaf meal at the beginning of the experiment

Parameters	Group A (0 g/kg GALM) Control	Group B (50 g/kg GALM)	Group C (100 g/kg GALM)	Group D (150 g/kg GALM)
PCV (%)	33.33 ± 1.50	34.67 ± 3.00	34.00 ± 3.50	34.33± 4.50
Hb (g/dl)	8.20 ± 0.25	8.17 ± 0.35	8.47 ± 0.64	8.30 ± 0.35
WBC (X 10^3 µl)	9.35 ± 0.45	8.93 ± 0.63	9.32 ± 0.21	9.31± 0.43
RBC (X 10^6 µl)	2.41 ± 0.03	2.43 ± 0.04	2.42 ± 0.05	2.39 ± 0.05

PCV = pack cell volume, Hb = haemoglobin, WBC = white blood cell, RBC = red blood cell, no significant difference occurred in all treatment means (p>0.05)

Table 4: Haematological indices of quails fed with different inclusions of *Gmelina arborea* leaf meal at the end of the experiment

Parameters	Group A (0 g/kg GALM) Control	Group B (50 g/kg GALM)	Group C (100 g/kg GALM)	Group D (150 g/kg GALM)
PCV (%)	49.67 ± 3.00	43.67 ± 4.00	50.33 ± 4.00	54.33 ± 5.50
Hb (g/dl)	12.93 ±0.20^c	11.47 ± 0.30^b	10.90 ± 0.25^a	10.57 ± 0.15^a
WBC (X 10^3 µl)	19.15 ± 0.55^a	21.78 ± 0.67^b	23.68 ± 0.40^c	24.26 ± 0.38^d
RBC (X 10^6 µl)	3.11 ± 0.12	3.13 ± 0.21	3.09 ± 0.18	3.01 ± 0.70

PCV = pack cell volume, Hb = haemoglobin, WBC = white blood cell, RBC = red blood cell, different alphabet superscript on the same row = significant difference (p<0.05) among the means

Haemoglobin (Hb) concentration: The result obtained at the beginning showed no significant difference (p>0.05) (p = 0.96) among the dietary treatments (Table 3). The result obtained at the end showed significant difference (p<0.05) (p = 0.00) among the dietary treatments (Table 4). This was not in line with the findings of Usman *et al*. (2008), Tuleun *et al.* (2011), Fatokun *et al*. (2013) and Anggraeni *et al*. (2016) who reported no significant (p>0.05) difference in haemoglobin concentration of quails across the dietary treatment and also decrease in Hb may be due to increased crude fibre (Fatokun *et al.,* 2013). But the numerical reductions in Hb contents of the blood of the birds were indications that the oxygen carrying capacity of the animals' blood may be reduced as opined (Fatokun *et al*., 2013). Haemoglobin is a component of the erythrocytes. Haemoglobin is a simple protein, red in colour poster on the erythrocytes which function in the binding of oxygen (Anggraeni *et al*., 2016).

Red blood cell (RBC) count: The RBC result obtained at the beginning of the study showed no significant difference (p>0.05) (p = 0.18) among the dietary treatments (Table 3). The result obtained at the end of the study showed no significant difference (p>0.05) (p = 0.83) among the dietary treatments (Table 4). This is was in line with the findings of Usman *et al*. (2008), Tuleun *et al.* (2011) and Anggraeni *et al*. (2016) who reported no significant difference (p>0.05) in RBC of quails across the dietary treatment. Erythrocytes are blood cells that have a nucleus and play a role in carrying haemoglobin to bind oxygen throughout the body (Anggraeni *et al*., 2016). The result for RBC in this study was not in line with the findings of Fatokun *et al*. (2013) who reported significant difference (p<0.05) of RBC count in quails across the dietary treatments, and that decrease in RBC may be due to increased crude fibre.

White blood cell (WBC) count: The WBC result obtained at the beginning of the study showed no significant difference (p>0.05) (p = 0.75) among the dietary treatments (Table 3). The result obtained at the end of the study showed significant difference (p<0.05) (p = 0.00) among the dietary treatments (Table 4). This was in line with the findings of Fatokun *et al*. (2013) who reported significant difference (p<0.05) in WBC count of quails across the dietary treatment and that the increase in WBC counts of the birds on the *G. arborea* based diets implied that the ingestion of GALM, maybe responsible for the increased production of the

blood components. Besides the lower WBC count in birds fed the control diet than those obtained in birds fed GALM may be explained by an increased need to challenge the foreign body in form of anti-nutritional factors in the diets formulated with different levels of inclusion of GALM. The increasing concentration of anti-nutritional factors with increasing GALM may have elicited increased production of WBC to attack the foreign bodies (Fatokun *et al*., 2013). WBC plays a vital role in antibody formation to protect animals against diseases (Iwuji and Herbert, 2012). Leucocytes are active cells that provide fast and powerful defence against foreign bodies and pathogens that cause infection.

Conclusion: The data obtained from the present study showed that *Gmelina arborea* is a tree with significant number and amounts of phytochemicals. There was no observable negative effect on the health status of quails fed with the varying levels of GALM indicating healthy effect of the *Gmelina arborea* on the birds since no medication was used throughout the period of study. Conclusively, the haematological values observed in the study fell within the values of Hb (12.30 g/dl), PCV (37 %), and ranges of RBC (2.30 – 3.86 X 10^6 µl), WBC (20 – 40 X 10^6 µl) recommended by Sturkie and Grimmger (1976) for normal healthy quails.

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