LAYING PERFORMANCE, HAEMATOLOGY AND SERUM BIOCHEMICAL PROFILE OF HENS FED UNFERMENTED AND FERMENTED AFRICAN LOCUST BEANS

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ABSTRACT
The study was carried out to compare the effects of unfermented and fermented African locust bean on laying performance, haematology and serum biochemical profile of hens in a twelve week feeding trial. The unfermented African locust bean (UALB) contained seeds that were dehulled and boiled in water, without going through fermentation. The fermented African locust bean (FALB) contained seeds that were dehulled, boiled in water and made to undergo natural fermentation for 4 days. One hundred, 40- week old, laying hens (Nera black) were randomly assigned to five dietary treatments in which UALB and FALB replaced soybean meal at 50% and 100% levels. Trypsin inhibitor (TI) activity was higher in the raw African locust beans (RALB), but no trace of the TI was contained in the UALB and FALB. However, higher residual amounts of tannin (38.10 mg/g) and phytate (2.80 mg/g) were obtained from the UALB. Egg weight and hen day production were significantly (p < 0.05) reduced in birds fed 100% UALB. Haemoglobin was similar (p > 0.05) across the groups of hens. The packed cell volume, red blood cells and white blood cells were significantly (p < 0.05) reduced in hens fed 100% UALB. Alanine transaminase, aspartate transaminase and alkaline phosphatase were significantly (p < 0.05) higher in birds fed 50% UALB and 100% UALB respectively. Marginal differences were however obtained in all the response indices between hens fed the control diet, 50% FALB and 100% FALB respectively. It was therefore concluded that fermentation was a better processing method for optimal utilization of Africa locust beans in diets of laying hens.

Key words: African locust beans, hens, performance, haematology, serum profile

INTRODUCTION
The high cost of animal protein has directed interest towards several leguminous seed proteins as potential sources of vegetable protein for human food and livestock feed. Among the plant species, grain legumes are considered as the major source of dietary proteins. They are consumed worldwide, especially in developing and under developed countries where consumption of animal protein may be limited as a result of economic, social, cultural or religious factors (Esenwah and Ikenebomeh, 2008). Prominent among the grain legumes in poultry feeding are the conventional soybean and groundnut, but these commodities are faced with dwindling local production especially in sub Saharan Africa, hence the need for continued exploration for underutilized grain legumes.

African locust bean is among the under-utilized grain legumes (Owoyele et al., 1987). According to Omafuvbe et al. (2004), the crude protein content of African locust beans is similar to those of cowpea, jack bean, lima bean and pigeon pea. The crude protein is a rich source of essential amino acids, minerals and vitamins. African locust bean had been successfully fermented for the production of food condiments in seasoning foods in Nigeria and other West African countries (Odunfa, 1986).

The potential of locust beans seed in the nutrition of humans and animals has been assessed (Eka, 1979; 1980). However, being a grain legume, it contains some antinutritional factors such as trypsin inhibitor, tannin, phytate and saponins. Several studies have been carried out by different investigators on improving the nutritive quality of African locust bean. The efficacy of fermentation at improving the nutritive quality of legumes has been noted (Muller, 1988; Omafuvbe et al., 2004). Improved performance traits, nitrogen retention and feed utilization of broilers fed fermented African locust bean as replacement of groundnut cake have been reported by Kolo (2000), Ayanwale and Ari (2002) and Dawodu (2009). Based on these findings, this study was carried out to compare the effects of fermentation of African locust bean on egg laying performance, haematological and serum biochemical indices of hens.

Laying Performance, Haematology and Serum Biochemical Profile of Hens Fed Unfermented and Fermented African Locust Beans

2

MATERIALS AND METHODS

Raw African locust bean were sourced from local markets in Ibadan, South western Nigeria. The seeds were air- dried and sorted to remove unwanted particles. The procedural methods of fermentation as described by Omafuvbe *et al.* (2004) were used to process the African locust beans in this study. Two equal batches of the raw African locust bean were separately boiled in water for 12 hrs. Thereafter, the seeds were soaked further in the water used for boiling for another 12 hrs overnight. After soaking, excess water was drained off while the seeds were manually dehulled using the feet to press them in a large wooden mortar. Further removal of the seed coat was achieved by rubbing the seeds between the palms of the hand and washing with water. The dehulled seeds were then cooked for another 6 hrs . The first batch was subsequently oven-dried at this stage, bagged and labeled unfermented African locust bean (UALB).

The second batch of the cooked and dehulled seeds was put in a fermentor, and allowed to undergo natural fermentation for 72 hrs. Thereafter, the resulting cotyledons were oven dried, bagged and labeled fermented African locust bean (FALB).

Chemical Analysis

Analytical methods of AOAC (1984) were used to determine the crude protein, fibre, ash, ether extract and nitrogen free extract of the raw, unfermented and fermented African locust beans. Concentrations of trypsin inhibitor, tannin and phytate were determined using the procedural methods as outlined by Liener (1979), Dawra *et al.* (1988) and Maga (1983).

Experimental Diets

Five experimental diets (Table 1) were formulated in which UALB and FALB replaced soy bean meal at 50% and 100% levels. Minor adjustments were made in the other ingredients to make the diets isonitrogenous and isocaloric. Methionine and lysine were added to each diet to ensure that the amino acids were not limiting for growth.

Experimental Birds

A total of 100 laying hens (Nera black) of 40 weeks old were randomly allocated into five dietary treatment groups at 20 birds per group in a completely randomized design experiment. Each group was further subdivided into five birds per replicate. The birds were raised in battery cages and adequately dewormed in the first week of the experiment. Food and water were served *ad libitum*. At the end of the 12th week, the experiment was terminated. Parameters used to measure the daily laying performance were feed intake, egg weight, feed conversion ratio and hen day production. The feed conversion ratio of each replicate was determined by dividing the feed intake by the egg weight.

Analytical Measurements

At 84 days, blood samples were collected from eight live birds per group (2 per replicate group) from the jugular vein. Blood samples for haematological analysis were collected into sterilized bottles containing ethylene diamine tetra acetic acid (EDTA) as anti-coagulant while those used for serum biochemical analysis were collected into tubes without EDTA and centrifuged before analysis. Packed cell volume (PCV) was determined using the microhaematocrit method while the haemoglobin content was determined with the cyanomethaemoglobin method (Apata, 1990). Red blood cell count was determined using the Neubauer haemocytometer, while alanine transaminase, aspartate trasaminase and alkaline phosphatase were determined with a spectrophotometer.

Table 1: Diets of birds fed unfermented and fermented African locust beans

Ingredients	Control	50% UALB	100% UALB	50% FALB	100% FALB
Maize	48.60	49.00	49.50	50.50	50.50
Soybeans	18.00	9.00	-	9.00	-
UALB	-	9.00	18.00	-	-
FALB	-	-	-	9.00	18.00
Wheat offal	12.00	11.60	11.10	10.10	10.10
Palm kernel meal	8.50	8.00	8.00	7.50	7.50
Fish meal	1.50	2.00	2.00	1.70	1.90
Bone meal	3.00	3.00	3.00	3.00	3.00
Oyster shell	7.50	7.50	7.50	7.50	7.50
Salt	0.20	2.00	2.00	2.00	2.00
Vitamins	0.30	3.00	3.00	3.00	3.00
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0.30	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.00
Calculated Crude Protein content (%)	17.11	16.49	16.50	16.56	16.82
Calculated Metabolizable energy(kcal/kg)	2645.46	2644.17	2639.71	2637.34	2636.91

UALB = Unfermented African locust Beans; FALB = Fermented African locust Beans,
0.30 premix supplied, per kilogram of diet: vitamin A, 12,000 IU; vitamin D3, 2,000 IU; vitamin E, 50 IU; vitamin B1, 1 mg; vitamin B2, 3 mg; vitamin B6, 1 mg; vitamin B12, 10 µg; vitamin K, 2 mg; copper (cupric sulphate), 75 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; iron, 200 mg; cobalt, 0.5 mg; manganese, 40mg; zinc, 90 mg, iodine, 1 mg; selenium, 0.2 mg; calcium, 31.25 g; sodium, 10 g

Statistical Analyses

The experiment was carried out using the completely randomized design. The data were analyzed by analysis of variance. When analysis of variance indicated a significant treatment effect, means were separated using the Duncan multiple range test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The analysis for proximate composition showed that raw African locust bean (RALB) had high crude protein (CP) content (Table 2). The CP significantly (p < 0.05) increased in the FALB, but marginally (p > 0.05) reduced in the UALB when compared to RALB. The ash, fat, and crude fibre contents were marginally reduced in both the FALB and UALB. The proximate composition obtained in the RALB is similar to the reports of Akoma *et al.,* (2001), Omafuvbe *et al.* (2004) and Ari and Ayanwale (2012). The CP obtained in the RALB is close to the 30.6% reported by Eka (1979).The increase in the CP in the FALB is similar to the findings of Odebunmi *et al.* (2009) and Tortora *et al.* (2002) who attributed significant (p < 0.05) increase in CP of fermented locust beans to structural proteins that are integral part of the microbial cells.

Results showing the antinutritional factors (ANFs) are presented in Table 3. Higher values of tannin (43.5mg/g), phytate (3.5mg/g) and trypsin inhibitor (6.1 Tiu/g) contents were obtained in the RALB. Trypsin inhibitor activity was destroyed in the UALB and FALB, while higher significant (p < 0.05) reductions in tannin, phytate and oxalate were obtained in the FALB. The tannin, phytate and trpsin inhibitor contents obtained in the RALB in this study are close to the values reported by Adeniran *et al.* (2013). The destruction of the trypsin inhibitor in both the UALB and FALB can be attributed to the cooking method used in this study. Moist heating has been established as a convenient means of eliminating trypsin inbitor and heamagglutinin activities in grain legumes (Emiola *et al.,* 2003; Ologbobo *et al*., 1993; Udedibie and Carlini, 1998). Fermentation method of processing locust beans was able to considerably reduce tannin, oxalate, saponinns and phytate (Omafuvbe *et al.,* 2004; Ari and Ayanwale, 2012) .

The egg laying performance is shown in Table 4. Feed intake was similar (p > 0.05) across the groups of hens. However, the egg weight (EW) and hen day production were significantly (p < 0.05) higher in hens fed 100% FALB, 50% FALB and control based diets, respectively. The lowest EW was obtained in hens fed with 100% UALB based diet. The feed conversion ratio (FCR) was higher (p < 0.05) in hens fed 50% UALB and 100% UALB based diets. The similarity in the feed intake across the groups of hens in this study can be attributed to the destruction of the trypsin inhibitor activity in both the UALB and FALB in the process of boiling the seeds. This observation is consistent with the reports of Ologhobo *et al.* (1993) and Akanji (2002) on similarities in feed intake of broiler chickens fed cooked jack bean-based diets and control diet. Akanji *et al.* (2007) fed raw jack beans and bambara nuts and reported significant negative correlations between feed intake and trypsin inhibitor intake. However, the reduction in the egg weight of the hens fed 100% UALB can be attributed to the likely increase in tannin and phytate intakes. The tendency of phytate and oxalate to make bivalent minerals and dietary protein unavailable for metabolism and utilization in chickens as reported by Israr *et al.* (2013) can be attributed to the lower egg weights obtained in the hens fed UALB based diets. Forbes *et al.* (1984) note that phytic acid chelates and thus makes unabsorbable such important minerals as zinc, iron, calcium and magnesium in monogastric animals.

Table 2: Dry matter proximate composition of raw, unfermented and fermented African locust beans

	RALB	UALB	FALB
Crude protein (%)	31.94 [b]	30.81 [b]	36.34 [a]
Ash (%)	3.04	2.91	2.96
Ether Extract (%)	29.01	28.34	27.48
Crude fibre (%)	2.94	2.61	2.56
Carbohydrate (%)	25.08	25.34	19.77
Dry matter (%)	92.01 [a]	90.01 [b]	89.11 [b]

RALB = Raw African Locust beans, UALB = Unfermented African Locust beans, FALB = Fermented African locust bean

Table 3: Anti-nutritional factors in the raw, unfermented and fermented African locust beans

	RALB	UALB	FALB
Trypsin Inhibitor (Tiu/g)	6.10	-	-
Tannin (mg/g)	43.50[a]	38.10[b]	11.40 [c]
Phytate (mg/g)	3.50 [a]	2.80 [b]	1.10 [c]

RALB = Raw African Locust beans,   UALB = Unfermented African Locust beans, FALB – Fermented African locust beans

Table 4: Egg laying performance of hens fed unfermented and fermented African locust beans

	Control	50%UALB	100% UALB	50%FALB	100% FALB	SEM±
FI (g/day)	109.11	111.34	112.42	112.61	113.83	1.69
EW (g)	61.33 [a]	56.31 [c]	54.24 [c]	60.11 [a]	61.43 [a]	0.97
FCR	1.78 [c]	2.01 [a]	2.13 [a]	1.87 [b]	1.85 [b]	0.08
HDP (%)	78.62 [a]	71.31 [c]	65.33 [d]	75.13 [b]	77.41 [b]	1.18

Means with different letters across the same row are significantly (P < 0.05) different
FI = Feed intake; EW = Egg weight; FCR = Feed conversion ratio, HDP = Hen day production

The FCR and lower hen day production in hens fed the 50% UALB and 100% UALB in this study can be attributed to poor utilization of the diets by the hens. Ebadi *et al.* (2005) reported negative effects of tannin on egg weight and egg production of laying hens fed sorghum. According to Ravindran *et al.* (1995) and Kornegay (1996), low ability of poultry to utilize phytate phosphorus is due to insufficient quantity or lack of intestinal phytase secretion. The inability of poultry to utilize phytate phosphorus results in the excretion of large amounts of phosphorus, posing an environmental concern in areas of intensive animal production. The phytate-bound phosphorus is unavailable to monogastric animals as they do not naturally have the phytase enzyme needed to break it down. Mustafa *et al.* (2012) reported the use of microbial phytase to improve egg weight, hen day production and egg quality of chickens fed diets rich in phytate. The effects of fermentation at reducing phenolic compounds, tannin and phytate contents of sorghum gruels were reported by Elicato (2006). This probably led to better egg weight and hen day production in birds fed FALB in this study.

The haematological indices and serum biochemical values are presented in Tables 5 and 6. The haemoglobin values were similar ($p > 0.05$) across the groups of hens. The packed cell volume (PCV) was significantly ($p < 0.05$) higher in hens fed control diet, and followed by those fed 50% FALB and 100% FALB. The lowest PCV value was obtained in hens fed 100% UALB. The red blood cells (RBC) were least of hens fed 50% UALB and 100% UALB. The white blood cells (WBC) followed a similar pattern to that of the RBC. The highest ($p < 0.05$) mean corpuscular volume (MCV) was obtained in hens fed control diet, while those fed 50% FALB and 100% FALB followed in MCV values. The mean corpuscular heamoglobin (MCH) values were similar ($p > 0.05$) between hens fed control diet, 50% FALB and 100% FALB based diets. The neutrophils were significantly ($p < 0.05$) reduced in hens fed 50%

UALB and 100% UALB. The lymphocyte contents were similar ($p > 0.05$) across the groups of hens.

The alanine transaminase (ALT) was significantly ($p < 0.05$) higher in hens fed 50% UALB and 100% UALB. Similar ($p > 0.05$) values of ALT were however obtained in hens fed control diet, 50% FALB and 100% FALB. The highest ($p < 0.05$) aspartate trasaminase (ASP) was obtained in hens fed 100% UALB. The ASP value of hens fed 50% UALB diet was significant ($p < 0.05$) than those of hens fed 100% FALB, 50% FALB and control diet. Hens fed 50% and 100% FALB had similar ASP rate while those fed the control diet had the least ASP rate. The serum total protein was higher ($p < 0.05$) in hens fed control diet, and followed by those fed 50% FALB and 100% FALB. The alkaline phosphatase was higher ($p < 0.05$) in hens fed 100% UALB and 50% UALB diets. The increases in the RBC, PCV and WBC in hens fed FALB based diets are similar to the findings of Ari and Ayanwale (2012) when broiler chickens were fed with fermented African locust bean. The values of alanine transaminase, aspartate trasaminase, alkaline phosphatase and serum total protein obtained in the birds fed UALB based diets are similar to the values reported by Ologhobo *et al.*(1993),Aletor and Ogunyemi(1988) and Aletor and Fetuga (1984) who linked increased activities of hepatic transaminases in rats fed some raw legumes to high catabolism of amino acids and liver cell damage as a result of the inherent anti-nutritional factors.

CONCLUSION
Results of this study showed that egg laying performance; haematology and serum indices of hens were significantly better when FALB was included in their diets. The FALB had higher crude protein, and lower tannin, oxalate and phytate contents than UALB. The results also showed that fermentation is necessary for optimal utilization of dietary Africa locust beans by laying hens.

Table 5: Hematological profile of hens fed of hens fed unfermented and fermented African locust beans

	Control	50%UALB	100% UALB	50%FALB	100% FALB	SEM ±
Hb (g/dl)	8.9	7.8	7.4	8.4	8.6	0.07
PCV (%)	33.4 [a]	29.1 [c]	26.7 [d]	32.3 [b]	32.8 [b]	0.21
RBC (x10^{12}/l)	3.8 [a]	3.1 [b]	2.8 [c]	3.7 [a]	3.8 [a]	0.03
WBC (x10^{9}/l)	2.8 [a]	2.2 [b]	1.8 [c]	2.7 [a]	2.6 [a]	0.01
MCV (fl)	84.8 [a]	80.1 [d]	81.8 [c]	83.5 [b]	83.9 [b]	1.28
MCH (pg/cell)	28.9 [a]	25.8 [b]	25.1 [b]	27.3 [a]	27.9 [a]	0.18
Nitrophils (%)	58.1 [a]	53.4 [b]	52.8 [c]	56.1 [b]	56.4 [b]	0.98
Lymphocytes (%)	42.8	41.5	41.3	42.1	41.9	0.87

Means with different letters in a row are significantly ($p < 0.05$) different); Hb = Hemoglobin, PCV = packed cell volume, RBC = Red blood cells, WBC = White blood cells, MCV = Mean Corpuscular volume, MCH = Mean Corpuscular Hemoglobin, SEM = Standard error of mean

Table 6: Serum biochemical parameters in hens fed unfermented and fermented

	Control	50%UALB	100% UALB	50%FALB	100% FALB	SEM ±
ALT (iu/l)	30.8 [c]	32.8 [b]	34.8 [a]	30.5 [c]	31.1 [c]	0.35
ASP (iu/l)	212.1 [d]	265.7 [b]	276.8 [a]	222.4 [c]	223.7 [c]	3.89
ALP (iu/l)	575.4 [c]	589.4 [a]	590.3 [a]	579.1 [b]	579.3 [b]	4.32
Total protein (g/dl)	2.91 [a]	2.46 [c]	2.20 [d]	2.83 [b]	2.84 [b]	0.11

Means with different letters across the same row are significantly ($P < 0.05$) different

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