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# Effect of Different Levels of the Processed Lablab purpureus Seeds on Laying Performance, Egg Quality and Serum Parameters

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Abstract: *L. purpureus* is considered to substitute some of conventional plant protein sources in poultry diets. In 12 weeks feeding trial the processed *L. purpureus* seeds were investigated for its impacts on laying performance, egg quality and serum constituents of Hisex White hens. Diets contain 0, 5, 10, 15 and 20% *L. purpureus* were allotted randomly following completely randomized design to 25 units of three birds each. Hen day egg production, egg weight, egg mass, FCR and weight gains were significantly (p<0.01) high up to 10% *L. purpureus*. Shell percentage and thickness were significantly (p<0.05) high in control and 20% *L. purpureus* groups. 15 and 20% *L. purpureus* dietary groups were of high egg albumin index and percentage. The utmost (p<0.05) yolk index and percentage were shown in 15 and 10% *L. purpureus* groups, respectively. Comparable high Haugh units were shown in different treatment groups. 15% *L. purpureus* groups, recorded significant (p<0.01) high serum total protein and globulin. All *L. purpureus* treatment groups were of significant (p<0.05) low serum albumin. Serum glucose was significantly (p<0.01) lowered in *L. purpureus* groups, while serum cholesterol was insignificantly (p>0.05) reduced due to *L. purpureus* inclusion. The 20 and 10% *L. purpureus* groups when compared to control were found of low serum P and Ca, respectively. In conclusion, up to 10% dietary inclusion of *L. purpureus* is appropriate to provide similar laying performance as the standard layers diet.

Key words: Egg quality, Lablab purpureus, laying performance, serum constituents

## INTRODUCTION

The development of poultry industry depends mainly on providing cheap and cost effective feeds (Akinmutimi and Okwu, 2006). Most of the conventional plant protein sources as peanut, sesame cakes and soybean meal might sometimes be limited in poultry feeding. That is due to their costly prices (Onu and Okongwu, 2006). This situation inevitably guides to look for unconventional substitutes as legumes, especially those of downgraded varieties and of no human preference (Ojewola and Ewam, 2005). This is the case for the legume of our study L. purpureus formerly known as Dolichos lablab (Mortuza and Tzen, 2009). The utilization of legumes seeds as protein sources for poultry is limited for the uncertainty of their nutritional quality (Elamin and Abdelati, 2008). Legumes are, to some extent, cheap and rich in most nutrients. L. purpureus is considered as suitable source of functional protein, due to its good balance of high bio-available amino acids (Arinathan et al., 2003; Ragab et al., 2010). Moreover, it is well suited to wide range of environments and soil types and is drought resistant and cold tolerant (Venkatachalam and

Sathe, 2007). It is lesser-known and hasn't received attention by nutritionists (Ramakrishna et al., 2006), L. purpureus as other grain legumes was reported to contain variety of antinutritional factors as trypsin, chymotrypsin, amylase inhibitors, phytic acid, flatulence factors, etc. (Vijayakumaris et al., 1995; Devaraj and Manjunath, 1995; Ramakrishna, 2008). processing as aqueous and dry heating, toasting, germination and boiling could be undergone to L. seeds to minimize their purpureus negative physiological effects (Osman, 2007; Ramakrishna et al., 2006).

The objective of this work is to investigate the influence of different levels of the processed *L. purpureus* seeds on layers performance traits, egg quality measurements and serum constituents.

# **MATERIALS AND METHODS**

**Housing, experimental birds:** The experiment was carried out in an open sided house, in the poultry unit, University of Khartoum. It lasted for 12 weeks period. 75 Highsex layers of 26 weeks' age were selected then

adapted for three weeks on standard layer ration. At 29th weeks' age hens were on the average body weight of 1450 g and maximum laying rate of 81%. Groups of three birds with nearly identical weights were replicated five times under five experimental diets. Water and feed were provided *ad-libitum*. Birds were brought in a wooden shave deep litter system. Each cage was supplied with single local nest.

Seeds processing and chemical analysis: *L. purpureus* seeds variety are the Rongai of brown color. Seeds were brought from the market, then were cleaned, mixed, submerged in water for 48 h and boiled for 30 min. Water change was every 24 h and before the boiling process. Thereafter, seeds were sun dried for 72 h. After the milling of seeds to particle size of I mm seeds were analyzed for proximate composition according to the standardized method VDLUFA 4th Ed. (Naumann *et al.*, 2004).

**Experimental diets:** Five experimental diets contained 0, 5, 10, 15 and 20% *L. purpureus* seeds of the whole diets were formulated to meet the outlined recommendation

Table 1: Proximate composition of raw and processed *L. purpureus* seeds (% DM)

Composition	Raw seeds	Soaked and boiled seeds		
Dry matter(1) (2)	93.30	88.90		
Crude protein(1)	23.40	25.70		
Ash <sup>(1)</sup>	3.73	2.20		
Ether extract(3)	0.92	2.12		
Crude fiber(3)	10.20	11.50		
NFE <sup>(4)</sup>	61.80	58.50		

<sup>(1)</sup> Values are averages of three determinations

of Hisex Breeder Management Guide. Proximate composition of *L. purpureus* seeds are shown in Table 1. Feed ingredients, calculated and determined analysis of experimental diets according to AOAC (1990) methods are shown in Table 2.

Data collection: Feed intake and body weight were determined as averages for each replicate on weekly basis. Fresh laid eggs were weighted daily and pooled for every week. Feed Conversion Ratio (FCR) was

Table 2: Composition of treatment diets containing L. purpureus seeds

	Levels of Lablab purpureus seeds (%)							
Ingredients	0 LP	5 LP	10 LP	15 LP	20 LP			
Sorghum	63.40	59.30	54.80	50.90	46.50			
Groundnut cake	13.70	12.50	11.50	13.60	13.02			
Sesame cake	5.59	5.97	6.20	3.25	3.00			
Lablab purpureus	0.00	5.00	10.00	15.00	20.00			
Wheat bran	2.74	2.66	2.95	2.60	2.85			
Super concentrate(1)	5.00	5.00	5.00	5.00	5.00			
Di-calcium	0.72	0.72	0.72	0.76	0.77			
Lime stone	7.98	7.98	7.99	8.09	8.11			
Salt	0.40	0.40	0.40	0.40	0.40			
Lysine	0.22	0.17	0.13	0.07	0.02			
Methionine	0.00	0.00	0.00	0.03	0.03			
Vit. + min <sup>(2)</sup>	0.30	0.30	0.30	0.30	0.30			
Calculated analysis								
ME (kcal/kg)	2867.00	2870.00	2869.00	2870.00	2868.00			
CP (%)	18.90	18.90	18.90	18.90	18.90			
Crude fiber (%)	3.91	3.96	4.05	4.11	4.19			
Ca (%)	3.85	3.85	3.85	3.85	3.85			
Av. Phosphorus (%)	0.42	0.41	0.41	0.41	0.42			
Lysine (%)	0.88	0.88	0.88	0.88	0.88			
Methionine (%)	0.46	0.46	0.46	0.46	0.46			
Meth.+ Cystine (%)	0.67	0.67	0.67	0.67	0.66			
Determined analysis								
CP (%)	18.50	18.80	18.70	19.10	18.10			
Crude fiber (%)	4.20	4.03	4.03	4.00	3.91			
EE (%)	3.40	2.65	2.95	3.25	2.65			
Ash (%)	6.15	6.25	5.57	6.50	6.90			
NFE (%)	63.70	62.30	63.12	61.20	64.50			

Supplied the following per kg = 35% CP, 2150 kcal ME, 4% C.F, 5% EE, 10% Ca, 4.5% P, 5.7% lysine and 4.5% methionine.

<sup>&</sup>lt;sup>(2)</sup>DM on fresh basis

<sup>&</sup>lt;sup>(3)</sup>Values are averages of two determinations

<sup>(4)</sup> Values are calculated by difference as following: (NFE) = 100 - (Ash + CP + EE + CF)

 $<sup>^2</sup>$ Supplied the following per kg of the diet: Vitamin A 500.000IU, Vitamin D<sub>3</sub> 100.000IU, Vitamin E 750 mg, Vitamin K<sub>3</sub> 100 mg, Vitamin B<sub>1</sub> 75 mg, Vitamin B<sub>2</sub> 200 mg, Pantothenic acid 450 mg, Vitamin B<sub>6</sub> 100 mg, Vitamin B<sub>12</sub> 1.25 mg, Nicotinic acid 1.250, Folic acid 37.5 mg, Choline Chloride 25.00 mg, Iron 3.000 mg, Copper 300 mg, Manganese 3.000 mg, Zinc 2.500 mg, Iodine 25 mg, Selenium 10 mg, Phosphorus 12.9%, Lysine11.30%, Methionine 4.9%

calculated either as weekly feed consumption (kg) per weekly produced (kg) eggs weight, or weekly feed consumption (g) per weekly dozens of laid eggs. Henday egg production was calculated from the number of laid eggs expressed as a percentage of the number of hens per pen on weekly basis. Egg masses were computed by multiplying hen day egg production by eggs weights.

Egg quality measurements: In two weeks intervals, ten eggs per treatment were selected for eggs characteristic study. After weighing of freshly laid eggs, they were broken in a wide flat Petri dish. Egg shells were weighted then peeled of any membranes. Shell samples from top, middle and bottom, were measured for thickness using 0.01 mm micrometer screw gauge model.

Yolk was carefully removed from albumin then weighed. Albumen weight was calculated by difference. Shell, Yolk and Albumin percentages were calculated as rates of the whole eggs weights. Height and diameter of yolk and Albumin for indices calculation were measured using 0.02 mm Vernier.

Haugh's unit was computed by using the formula:

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

H = Albumen height in millimeters.

W = Egg weight in grams.

Blood collection and serum analysis: Blood samples were taken in the trial last week from 10 birds per

treatment, by method of venipuncture of the brachial vein by syringe. Serum samples after collection were kept deep frozen prior to analysis.

**Statistical analysis:** The experiment was conducted following the completely randomized design. Data were subjected to analysis of variance according to Steel and Torrie (1960). Treatment means were compared using Duncan multiple range test (1989).

#### **RESULTS**

Seeds' processing practiced in this work was of slight impact upon nutrients. Meanwhile, it faintly fortified CP, EE and CF; it decreased ash contents and NFE for about 41% and 5.4%, respectively.

Laying performance (30th-41st) week: Regarding feed consumption, none considerable (p>0.05) variation was noticed between treatment groups for the overall and daily feed intake. Total number of laid eggs, hen day egg production and egg mass were shown comparable and significantly (p<0.01) high for birds fed on 0 LP, 5 LP and 10 LP diets. Whereas, the lowest values for these parameters were observed in 15 LP and 20 LP dietary groups. Regarding overall weight of laid eggs, birds on 0 LP and 10 LP diets laid eggs of utmost (p<0.01) overall weight. The least overall eggs' weight was for birds on 15 and 20 LP diets. In both two ways of measuring feed conversion ratio FCR, the poorest and poorer FCR, were proven to 20 LP and 15 LP treatment groups, respectively. Concerning average egg weight, all L. purpureus dietary groups, laid eggs of comparable (p>0.05) weights. A part from others, only the groups on 5 LP and 10 LP diets laid eggs of analogous (p>0.05) weight to control group. Regarding body weight change, the groups on 15 LP and 20 LP diets lost comparable weight, while the others on 0 LP, 5 LP and 10 LP diets both attained equivalents (p>0.05) weights.

Egg quality: High shell percentage and thickness were similarly recorded in favor of 0 LP and 20 LP groups. Comparable high albumin index and percentage were apparent in the 15 LP and 20 LP treatment groups. High yolk index was evident in 15LP dietary group, however, the remaining groups showed similar yolk index. The superior yolk percentage was encountered in the 10LP treatment group. All treatment groups laid eggs of comparable Haugh units.

**Serum parameters**: As illustrated in Table 5, significant (p<0.01) high serum total protein was detected in15 LP

Table 3: Layers hens' performance fed graded levels of processed L. purpureus seeds (30th-41st) weeks of age

	Dietary levels of <i>L. purpureus</i> seeds (%)					
Parameters	0 LP	5 LP	10 LP	 15 LP	20 LP	SEM
Feed intake, g/hen/12week	8907.00	84767.00	8943.00	8570.00	8847.00	398.00
Feed intake, g/hen/day	106.00	101.00	106.00	102.00	105.00	4.74
Total laid eggs, eggs/hen/12week	79.00°	72.00°	78.00°	55.00⁵	47.00b	2.65
Hen-day egg production, %	94.60°	90.50°	92.30ª	65.50 <sup>b</sup>	58.20b	2.58
Total eggs weight, g/hen/12week	4411.00°	3938.00b	4217.00 <sup>ab</sup>	2863.00°	2507.00€	142.00
Egg weight, g/egg	55.60°	54.70ab	54.40ab	52.20b	52.80b	0.78
Egg mass, g/hen/day	52.50°	49.50°	50.20°	34.10 <sup>b</sup>	30.70b	1.30
FCR, g feed/g eggs	2.02°	2.15°	2.12°	3.01 <sup>b</sup>	3.55⁵	0.11
FCR, kg feed/dozens of egg	1.35°	1.41ª	1.39ª	1.89⁵	2.25⁵	0.08
Body weight change, g	61.10 <sup>b</sup>	66.80⁵	40.10⁵	-65.90°	-38.60°	23.50

abMeans in the same raw with different superscripts were significantly different

Table 4: Egg quality of layers fed dietary levels of processed L. purpureus seeds

Parameters	Levels of L. purpureus seeds (%)					
	0 LP	5 LP	10 LP	 15 LP	20 LP	SEM
Shell (%)	11.200°	10.700b	10.700⁵	10.600⁵	11.100°	0.120
Shell thickness (mm)	0.370°	0.350bc	0.360b	0.340°	0.370°	0.004
Albumin index	0.130 <sup>b</sup>	0.129 <sup>b</sup>	0.129 <sup>b</sup>	0.137°	0.131 ab	0.002
Albumin (%)	62.700 <sup>bc</sup>	63.300ab	62.600€	63.900ª	63.700°	0.270
Yolk index	0.410 <sup>b</sup>	0.420b	0.420₺	0.440°	0.420₺	0.010
Yolk (%)	25.900 <sup>b</sup>	26.100b	26.600°	25.600 <sup>bc</sup>	25.200°	0.230
Haugh units	90.600	89.900	90.500	91.200	89.700	0.590

<sup>&</sup>lt;sup>ab</sup>Means in the same raw with different superscripts were significantly different

Table 5: Serum constituents of layers fed dietary levels of processed L. purpureus seeds

Parameters	Levels of L. purpureus seeds (%)					
	0 LP	5 LP	10 LP	15 LP	20 LP	SEM
Total protein (g/dL)	5.45°	6.39 <sup>ab</sup>	5.84 <sup>bc</sup>	6.82°	5.52°	0.23
Albumin (g/dL)	2.75°	2.39b	2.29₺	2.22b	2.14⁵	0.10
Globulin (g/dL)	2.70€	4.00 <sup>ab</sup>	3.55 <sup>b</sup>	4.60°	3.38bc	0.25
Uric acid (mg/dL)	5.83	6.21	6.33	6.28	6.34	0.39
Glucose (mg/dL)	204.00°	193.00°b	169.00⁰	153.00⁰	175.00₺፡	7.71
Cholesterol (mg/dL)	130.00b	124.00b	115.00b	129.00b	154.00°	7.65
P (mg/dL)	7.63°	7.58°	7.09 <sup>ab</sup>	6.76ab	6.36b	0.29
Ca (mg/dL)	14.10 <sup>a</sup>	13.60°	12.90b	12.70 <sup>ab</sup>	12.70 <sup>ab</sup>	0.52

abMeans in the same raw with different superscripts were significantly different. Values are means of 10 blood samples

group; it is in consonance (p>0.05) only with the 5 LP group. As compared to control, comparable (p>0.05) lower serum albumin was detected in all *L. purpureus* treatment groups. Regarding serum globulin, the highest (p<0.01) value was noticed in the 15LP group. With exception of 20 LP group, the control for serum globulin, significantly (p<0.01) ranked below *L. purpureus* groups. Serum uric acid wasn't affected by treatment diets, however, it was slightly (p>0.05) raised in *L. purpureus* dietary groups.

Serum glucose was significantly (p<0.01) lowered in L. purpureus treatments groups. For the cholesterol, an insignificant (p>0.05) reduction was detected in L. purpureus dietary groups, conversely, the group on 20LP diet showed the highest (p<0.05) cholesterol level. Regarding phosphorus P, as compared to control, only the significant (p<0.05) low P was detected in 20 LP group. Serum calcium Ca was slightly (p>0.05) lowered in most L. purpureus treatment groups. However the group on 10LP diet showed significant (p<0.05) lower serum calcium compared to control and 5 LP treatment groups.

### **DISCUSSION**

The study investigated the effect of levels of processed *L. purpureus* seeds in laying performance, egg characteristics and serum constituents of High sex white hens.

Concerning laying performance, the similarity in overall feed intake across treatment groups could be attributed to the equality of dietary treatments in energy and nitrogen components. However, all experimental diets

were formulated on this basis. Moreover, it might be due to well adaptability of layers to differently composed treatment diets. Amaefule *et al.* (2006) either before reported non-significant difference in feed intake of pullets fed differently processed levels of *C. cajan* based diets.

The high production rate (76 number of laid eggs out of 84 housed hens) up to 10 LP level within 12 weeks was remarkable. This reflects the tolerance birds to *L. purpureus* seeds up to this level. Additionally, data collection was within the laying age between 30th and 41st weeks, which is the age range when High sex hens reach peak production. The drop in egg production and the negatively influenced average egg weight at high *L. purpureus* levels were in harmony with that of Abeke *et al.* (2008). In tandem, this was applied as well with the overall weight of laid eggs.

The negative influence on egg weight might be due to some toxic residues left in seeds after processing. It is the fact previously investigated by Akanji and Ologhobo (2007). Tannins and phytic acid are of the residues in the processed L. purpureus seeds (Osman, 2007; Ramakrishna et al., 2006), they as shown by Bawa et al. (2003) are resistant to heat processing and their left over negative effects beyond processing could be exaggerated by increasing the seeds level. They can form complexes with proteins and polypeptides preventing their digestion and assimilation, hence reducing the sufficient protein for egg production (Card and Nesheim, 1975). Where egg mass is computed basing on the egg production rate, so the declined egg mass at high L. purpureus levels was in parallel with egg production declined rate.

Despite the similarity of 15 LP and 20LP dietary groups in feed consumption with 0 LP, 5 LP and 10 LP groups; they oppositely exerted remarkable reduction in the overall weight and total number of laid eggs. It is evidence of low quality of these two diets, which was eventually shown as poor FCR in these treatment groups.

The body weight losses at high L. purpureus levels are similar to that observed by Robinson and Singh (2001). The authors primarily attributed the losses to trypsin inhibitor activity, which was detected by them as the only ANF in direct significant correlation with body weight change. Robinson and Singh (2001) detected that, the viscosity and TIA of L. purpureus were lower than Jack beans and chick peas; however, oppositely, L. purpureus demonstrated the inferior performance. The authors hence suspected the poor performance with L. purpureus diets to factors other than high viscosity and TIA. The sameness of shell thickness of the 20LP and OLP groups is suspected to be of more Ca affluence in the blood stream of 20 LP groups. This was due to their reduced exploitation for egg shell formation owing to the low laying rate. Additionally, it might be in part for the likely serum calcium of the 0 LP and 20 LP groups. This trend applied in turn to shell percentage.

Egg albumin index attained for different treatment groups fall within the normal albumin index of eggs, 0.078-0.13 (Nair and Elizabeth, 1983; Kondaiah *et al.*, 1983. The reduced yolk percentage at high *L. purpureus* levels is preferred by consumers because laid eggs are then expected to include low cholesterol. Caston *et al.* (1994) reported decreases in yolk percentage when 10 or 20% flaxseed diets were fed from 39 to 43 weeks of age. The hormones responsible for yolk formation as follicle stimulating hormones and progesterone were reported to be negatively interfered by haemagglutinins (Pusztai *et al.*, 1975; Card and Nesheim, 1975; Akanji and Ologhobo, 2007). Haemagglutinins residual effect beyond seeds processing might have brought about the slight decrease in yolk percentage at high Lablab beans' level

As for albumin index, the 15 LP group laid eggs of significant high yolk index. The comparable Haugh units of eggs along *L. purpureus* treatment groups when compared with control refer to high albumin quality and freshness state of eggs when measured for quality.

However, Durunna *et al.* (2005) regarded the 72 Haugh units and more as an indicator of eggs' freshness.

The treatment diets were formulated on CP equality basis and when chemically detected for CP, the 15 LP diet was of slightly high CP. However, this might in turn explains the high serum total protein and globulin in these treatment groups. Crevieu *et al.* (1997) stated that some of the pea's albumin molecules were less

susceptible to hydrolysis and digestion and remained indigestible till the end of the digestive tract. Similarly, this case can help to explain why the serum albumin in case of *L. purpureus* feeding is at minimal level.

There was an evidence of a synchronized reduction relationship between albumin and cholesterol demonstrated before by Viveros *et al.* (2007), which correspondingly shown slightly in this study.

The decrease of serum cholesterol and glucose in L. purpureus groups compared to control can be clarified basing on their reduced carrying over on the blood stream. That is owing to their lessened absorption from the intestine, due to the raised viscosity of intestine ingesta. The viscosity might occurred, to some extent as a result of the NSP left after L. purpureus seeds processing (Gallaher et al., 1993a,b; Magni et al., 2004; Viveros et al., 2007). The elevated serum cholesterol in the 20 LP treatment group might be due to their low laying rate. The amount of 209-211 mg/100 mL cholesterol in egg yolk (Chowdhury et al., 2002, 2005) arrives directly from the blood stream. And whereas egg production is decreased, serum cholesterol is expected to rise for no more egg drain to get hold of serum cholesterol. The decrease in serum phosphorus as compared to control wasn't considerable except at 20 LP treatment group. That may be due to the residual capturing effect of either leftover tannin or phytic acid at this high L. purpureus level, which prohibited phosphorus full absorption, assimilation occurrence in the blood stream.

In conclusion, *L. purpureus* seeds processed in the way shown in this study could safely be included up to 10% level in layers' rations without any problems in laying performance.

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