ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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International Journal of Poultry Science 8 (5): 475-479, 2009
ISSN 1682-8356

Effect of Dietary Graded Levels of *Leucaena leucocephala* Seeds on Layers Performance, Egg Quality and Blood Parameters

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Abstract: The layers experiment was conducted to evaluate the effect of different levels of leucaena seed (0, 8, 16 and 24%) on 27 weeks of old laying hens. Parameters studied were egg production, external and internal quality characteristics of eggs, haematological indices and plasma constituents. Feed intake, hen-day egg production, FCR (kg feed/kg egg) and FCR (kg feed/dozens of egg) were depressed with the inclusion of leucaena seeds. Thereafter, birds were placed on control diet for two weeks, which revealed improvement of the performance except for FCR (kg feed/dozens of egg) for birds fed 24% leucaena seeds. Higher egg weight, maximum length and egg shell surface were recorded for birds fed 16% leucaena seeds compared to the others. Maximum width, shell weight and shell% were reduced for birds fed 24% leucaena seeds. Internal quality characteristics of eggs were not significantly affected by the dietary treatments except albumin weight, albumin%, yolk index and yolk color score. Yolk color score consistently increased with the inclusion of leucaena seeds.

Key words: Leucaena, lsayers, performance, egg characteristics

INTRODUCTION

Many of the conventional ingredients used in poultry diets are anticipated to be in short supply. Meanwhile there will be an increase in the global demand for protein sources, which are expected to be met largely by legumes and other non-conventional sources. The incorporation of grain legumes in poultry diets, however, is limited because of uncertainty about their nutritional quality. Although the analyzed total amino acid and energy contents of grain legumes are quite similar, their protein qualities and metabolizable energy values are quite variable and possibly influenced by the content of a number of Antinutritive Factors (ANF) such as protease inhibitors, lectins, tannins and Non-starch Polysaccharides (NSP) (Wiryawan, 1997). Babiker (1997) used leucaena seeds at 0, 5, 10, 15 and 20% in layer chickens diet. He reported that non of the dietary treatments had a significant effect on feed intake, egg production and feed efficiency. On the other hand, leucaena can be used to improve yolk colour of poultry eggs, due to its high content of vitamin A precursor carotenoids (NFTA, 1990). Therefore, the objective of this experiment was to investigate the effect of dietary leucaena seeds on layers.

MATERIALS AND METHODS

The experiment was conducted in an open- sided poultry house at the poultry unit in the college of Agricultural studies, Sudan University of Science and Technology. One hundred 24 weeks of age, Hisex laying hens were purchased from local market. The birds were previously vaccinated at hatch against Marek's disease. They were also vaccinated against Infectious Bronchitis (IB) at five days of age, infectious bursal disease (Gumboro) at two weeks of age, Newcastle disease at 3 weeks of age and at 11 weeks against fowl pox. The birds were beak-trimmed at 10 days of age. The birds were placed single in batteries and had been fed the control diet. At the beginning of the trial period, when the birds were 27 weeks of age, they were divided into four equal treatment groups of 25 birds in each and these were further subdivided into five replicate groups of five birds each. Birds of each replicate were assigned randomly in cages on 3 tiers wire blocks (50x42x40 cm) of laying battery.

Four isocaloric and isonitrogenous experimental diets were formulated according to nutrient specifications recommended by National Research Council (NRC, 1994), as follows: rations (A) 0% *Leucaena leucocephala* seed, (B) 8% leucaena seeds, (C) 16% leucaena seeds and (D) 24% leucaena seeds. The composition of these diets appears in Table 1. Feed samples were analyzed for proximate composition according to the methods outlined in the AOAC methods of analysis (1990). The birds in each cage had continuous access to two drinker nipples and the experimental diets were fed *ad libitum*. A day-length of 15 hours was provided throughout the laying period by a combination of natural daylight and artificial light.

Feed intake and body weight were determined weekly. Eggs were collected twice a day and the records of daily

Table 1: Composition of experimental layer diets containing graded levels of *Leucaena leucocephala*

	Levels of *Leucaena leucocephala* seeds, %			
Ingredients, %	0 (A)	8 (B)	16 (C)	24 (D)
Sorghum	64.50	63.47	63.27	61.9
Ground nut meal	6.79	4.00	1.00	0.00
Sesame meal	6.00	5.00	2.50	0.00
Wheat bran	9.66	6.50	4.16	1.00
Super concentrates[1]	5.00	5.00	5.00	5.00
Dicalcium phosphate	0.60	0.63	0.67	0.70
Oyster shell	7.00	7.00	7.00	7.00
Nacl	0.40	0.40	0.40	0.40
Lysine	0.05	0.00	0.00	0.00
Calculated analysis				
ME (kcal/kg)	2852.03	2858.56	2861.71	2863.93
CP%	17.61	17.80	17.52	17.82
Crude fiber%	4.15	4.42	4.67	4.98
Ca%	3.43	3.42	3.39	3.36
Available Phosphorous%	0.31	0.31	0.31	0.31
Lysine%	0.69	0.69	0.72	0.78
Methionine%	0.36	0.36	0.34	0.32
Methionine + Cystine%	0.56	0.55	0.51	0.49
Determined analysis				
CP%	19.61	19.15	19.77	19.96
Crude fiber%	4.88	4.95	5.11	5.20
EE%	3.10	4.05	3.45	2.75
Ash%	15.7	13.5	13.75	13.2
NFE%	50.01	53.45	51.12	51.58
Ca%	3.57	3.60	3.51	3.50
Total phosphorous%	0.85	0.91	0.90	0.86

[1]p 40%, ME 2000 kcal/kg, C.fiber 3%, EE 8%, Ash 34%, Ca 10%, Av. P 1.74%, Lysine 6%, Methionine 2.8%, Methionine+Cystine 3.1%. Vitamin A 240000 IU/Kg, Vitamin D3 60000 IU/Kg, Vitamin E 200 Mg/Kg, Vitamin K3 40 Mg/Kg, Vitamin B1/ Thiamin 30 Mg/Kg, Vitamin B2/ Riboflavin 100 Mg/Kg, Niacin Vitamin PP 700 Mg/Kg, Pantothenic acid/ Vitamin B3 147 Mg/Kg, Vitamin B6/ Pyridoxine 50 Mg/Kg, Vitamin B12 400 Mcg/Kg, Choline 8000 Mg/Kg, Folic Acid 10 Mg/Kg, Iron 1000 Mg/Kg, Manganese 1860 Mg/Kg, Copper 156 Mg/Kg, Zinc 1080 Mg/Kg, Iodine 20 Mg/Kg, Cobalt 20 Mg/Kg, Selenium 5 Mg/Kg.

egg production and eggs weight were kept throughout the experimental period. Average hen-day production percentages were calculated from the total number of eggs actually collected, expressed as percentages of the expected number of eggs for each group per week over the six-week period. Both weekly external and internal quality characteristics were measured from eggs. Each week, 2 fresh eggs from each replicate group were weighed and considered for measurement of maximum length, cm and maximum width, cm. shell samples from top, middle and bottom of the eggs were measured for thickness using 0.2 mm Vernier caliper (Mitutoyo-Japan) and the mean was calculated before statistical analysis. Shell weight, g and egg shape index (maximum width/ maximum length) also measured. Shell percentages were calculated as the proportion of shell weight/egg weight. Egg shell surface was calculated using the formula developed by Carter (1975) i.e. Area= $3.9782W^{0.7056}$, W= weight of fresh egg. Sample eggs from each replicate were broken with a blunt knife, contents poured on a piece of flat glass positioned on a flat surface and different components were separated and weighed. The albumen height mm was taken by 0.2 mm Vernier caliper (Mitutoyo-Japan) on the middle of thick albumen in both sides opposite to the chalaza as an average of two sides. The yolk height, mm, albumen and yolk diameters, cm were measured using 0.2 mm Vernier caliper (Mitutoyo-Japan). The albumin and yolk indices were calculated as the proportion of the height of each to the diameter. Albumin yolk index was calculated as the proportion of albumin weight/yolk weight. Albumin and yolk percentages were calculated as the proportion of weight of each/egg weight. Haugh unit was calculated by using the following formula (Haugh, 1937, cited by Alleoni and Antunes, 2001).

$$\text{Haughunits} = 100 \log\left[H - \frac{\sqrt{G(30W^{0.37} - 100)}}{100} + 1.9\right]$$

Where H= Albumen height (millimeters), G = is a constant (32) related to the constant of gravitation and W= Weight of egg (g).

Yolk colour scores were recorded by a Roche Yolk Colour Fan graded from 1 to 15 (F. Hoffman-La Roche Ltd., Switzerland). At termination of the experiment, two to three milliliters of blood samples were taken from two birds per pen from brachial vein using sterilized syringes and received in 10 ml test tube that contained ETDA. At the end of feeding trial, chickens adjusted once again on the control diet for 2 weeks. Likewise, daily and weekly performance and external quality characteristics of eggs were recorded. The experiment was arranged in a Completely Randomized Design (CRD). Data were subjected to analysis of variance and treatment means compared using the Duncan's multiple range tests (Steel and Torrie, 1980) in SAS (1985) version 6.12. Regression analysis was used to determine the possible effect of leucaena levels on feed intake, egg production, body weight change, FCR, PER and yolk color.

RESULTS AND DISCUSSION

Results of the effect of dietary treatments on overall performance (28^{th}-31^{st} week of age) of layer chickens are given in Table 2. There was no significant ($p \geq 0.05$) effect of dietary treatments on change in body weight and egg weight. However, the other parameters were significantly ($p \leq 0.01$) influenced by the dietary treatments. Feed intake was consistently depressed as the level of leucaena seeds increased. Regression analysis (Table 3.) indicated that feed intake is negatively correlated ($p \leq 0.05$) with inclusion of leucaena seeds. Hen-day egg production%, FCR (kg feed/kg egg) and FCR (kg feed/dozens of egg) of birds fed control and 8% leucaena seeds were better than those fed 16 and 24% leucaena seeds. These results agreed with D'Mello (1991) who noticed an adverse effect of inclusion of leucaena leaf meal on feed intake and egg production.

Table 2: Effect of dietary graded levels of *Leucaena leucocephala* seeds on layers performance (28th - 31st week of age)

	Levels of *Leucaena leucocephala* seeds, %				
Parameters	0 (A)	8 (B)	16 (C)	24 (D)	±SEM
Feed intake, (g/bird/day)	83.77^{a}±2.90	73.07^{b}±7.37	68.48^{b}±10.70	51.08^{c}±7.01	3.36
Egg production, hen-day %	72.50^{a}±9.58	62.00±^{a}16.86	38.57^{b}±9.07	30.36^{b}±7.47	5.07
Egg wt, (g/egg)	45.47±0.91	46.89±1.62	47.55±3.00	45.67±0.84	0.81
FCR (kg feed/kg egg)	2.56^{b}±0.29	2.85^{b}±0.58	4.30^{a}±0.66	4.45^{a}±0.40	0.23
FCR (kg feed/dozens of egg)	1.40^{b}±0.17	1.60^{b}±0.31	2.45^{a}±0.32	2.42^{a}±0.23	0.12
Change in body weight, (g)	-9.90±21.05	-11.60±5.90	-17.20±8.84	-21.60±23.94	8.43

Values are means (±SD)of 5 replicates per treatment. ^{abc}Means with different superscripts in the same row were significantly different ($p \leq 0.05$). SEM: Standard error of the means from ANOVA d.f 16.

Table 3: Linear regression equations relating levels of inclusion of *Leucaena leucocephala* seeds to performance parameters of laying hens

Parameter	Equation	R^2
Feed intake	–128.31X+84.499	0.9476*
Egg production	–187.31X+73.335	0.9648*
Egg weight	1.575X+46.206	0.0268NS
Feed conversion ratio (Kg feed/Kg egg)	8.9X+2.472	0.8918NS
Feed conversion ratio (Kg feed/dozen of egg)	4.8875X+1.381	0.8544NS
Body weight change	–50.875X–8.97	0.9637*
Egg yolk color	12.813X+1.325	0.9046*

*$p \leq 0.05$, NS = Not Significant

Table 4: External quality characteristics of eggs from layers fed dietary graded levels of *Leucaena leucocephala* seeds (28th -31st week of age)

	Levels of *Leucaena leucocephala* seeds, %				
Parameters	0 (A)	8 (B)	16 (C)	24 (D)	±SEM
Egg weight, (g)	45.31±^{b}±1.99	45.45^{b}±1.99	48.02^{a}±1.33	43.86^{b}±0.35	0.70
Max. length, (cm)	5.31^{bc}±0.11	5.40^{b}±0.09	5.45^{a}±0.09	5.28^{c}±0.03	0.04
Max. width, (cm)	4.26 ^{ab}±0.04	4.25^{abc}±0.07	4.29^{a}±0.03	4.18^{c}±0.02	0.02
Shell weight, (g)	5.54^{a}±0.14	5.64^{a}±0.20	5.62^{a}±0.21	5.06^{b}±0.07	0.07
Shell thickness, (mm)	0.316±0.004	0.325±0.014	0.320±0.010	0.314±0.009	0.004
Egg Shape Index	0.803±0.008	0.788±0.021	0.787±0.019	0.793±0.006	0.007
Egg Shell surface	58.62^{b}±1.81	58.75^{b}±1.81	61.07^{a}±1.24	57.31^{b}±0.31	0.64
%Shell	12.27^{ab}±0.31	12.47^{a}±0.74	11.73^{bc}±0.16	11.55^{c}±0.21	0.19

Values are means of 5 replicates per treatment. ^{abc}Means with different superscripts in the same row were significantly different ($p \leq 0.05$). SEM: Standard error of the means from ANOVA d.f 16.

The consistent reduction in feed intake associated with the inclusion of leucaena seeds in layer chickens diets may due to some factors such as mimosine, which may induced poor appetite (Sethi and Kulkarni, 1995). Poor layer performance with the inclusion of leucaena seeds may be due to insufficient intake of digestible nutrients (D'Mello *et al*., 1987) and negative effect of ANFs such as mimosine, protease inhibitors, haemagglutinins, tannin and phytate. On the other hand, amino acid availability due to reduction in feed intake (Boorman, 1979) may be also responsible for this poor performance. Mimosine brought its effect through inhibition of protein biosynthesis in living body or by suppressing the action of metal chelating enzymes. In contrary Babiker (1997) reported that the inclusion of leucaena seeds up to 20% had no negative effect on layer chickens performance. In the present study, the results of deleterious effect of high levels of leucaena seeds on layers performance was ascertained by the better performance of layers through recovery period at which feed intake increased by 23.79, 14.94 and 70.20% for birds that previously fed 8, 16 and 24% respectively. Moreover, supplementation of birds previously fed 8, 16 and 24% leucaena seeds with control diet prominently improved egg production by 31.34, 38.53 and 45.88%, respectively.

Mean values of external quality characteristics of eggs from 28th -31st week of age layers fed dietary graded levels of leucaena seeds (Table 4.) depicted the non effect of dietary treatments on shell thickness and egg shape index. Egg sample weight, maximum length and egg shell surface were higher for birds fed 16% leucaena seeds compared to the other dietary treatments. Maximum width, shell weight and shell% of birds fed 24% leucaena seeds were lower compared to the other dietary treatments. Poor shell weight may be due to Ca and P chelation by phytate and/or mimosine (Sethi and Kulkarni, 1995 and Sell *et al*., 2000).

Table 5: Internal quality characteristics of eggs from layers fed dietary graded levels of *Leucaena leucocephala* seeds (28th- 31st week of age)

	Levels of *Leucaena leucocephala* seeds, %				
Parameters	0 (A)	8 (B)	16 (C)	24 (D)	±SEM
Albumen height, (mm)	8.22±0.17	8.20±0.20	8.04±0.26	8.31±0.07	0.08
Albumen diameter, (cm)	7.18±0.16	7.12±0.09	7.15±0.17	7.19±0.08	0.06
Albumin weight, (g)	27.93^{b}±1.76	28.58^{b}±2.13	31.09^{a}±1.02	27.86^{b}±0.53	0.67
Albumin Index	0.115±0.004	0.115±0.004	0.113±0.006	0.116±0.002	0.002
Albumin %	61.60^{b}±1.60	62.75^{ab}2.35	64.59^{a}±0.83	63.47^{ab}±0.70	0.68
Yolk height, (mm)	10.37±0.24	10.75±0.69	10.30±0.32	10.42±0.12	0.18
Yolk diameter, (cm)	3.36±0.10	3.29±0.05	3.36±0.06	3.31±0.04	0.03
Yolk weight, (g)	11.77±0.83	11.23±0.64	11.49±0.43	11.01±0.04	0.25
Yolk index	0.309^{b}±0.005	0.327^{a}±0.019	0.307^{b}±0.010	0.315^{ab}±0.002	0.005
Yolk %	25.98±1.86	24.78±1.69	24.04±1.21	25.12±0.27	0.63
Albumin Yolk Index	2.42±0.26	2.57±0.28	2.70±0.17	2.53±0.05	0.09
Yolk color score	1.00^{c}±0.00	2.60^{b}±0.29	3.85^{a}±0.14	4.00^{a}±0.18	0.08
Haugh unit	99.22±0.80	99.18±0.92	98.31±1.14	99.73±0.31	0.38

Values are means of 5 replicates per treatment. ^{abc}Means with different superscripts in the same row were significantly different ($p \leq 0.05$). SEM: Standard error of the means from ANOVA d.f 16.

Table 6: Effect of dietary graded levels of *Leucaena leucocephala* seeds on some haematological parameters and plasma constituents of layers (28th-31st week of age)

	Levels of *Leucaena leucocephala* seeds, %				
Parameters	0 (A)	8 (B)	16(C)	24 (D)	±SEM
PCV%	37.80^{b}±3.90	32.40^{b}±4.04	37.40^{b}±4.56	45.20^{a}±4.21	1.87
Hb g/dL	22.47±4.53	20.73±2.12	22.23±1.35	24.13±1.74	1.22
Neutrophils%	35.80±3.11	36.00±3.94	32.00±2.74	31.40±3.13	1.46
Lymphocytes%	43.00±3.08	44.00±6.52	45.00±0.00	48.20±1.30	1.64
Monocytes%	5.60±1.34	5.60±0.55	6.60±0.55	5.40±1.34	0.46
Eosinophils%	11.80±1.10	11.40±1.82	12.20±0.84	11.60±0.89	0.55
Basophils%	3.80±1.92	3.00±1.00	4.20±1.10	3.40±0.55	0.56
GOT U/L	40.20±1.79	36.80±2.86	36.80±1.79	38.60±2.97	1.08
ALP U/L	86.40±1.14	85.40±1.67	86.60±1.14	84.20±1.48	0.62
Ca mg/dL	7.76±0.11	7.92±0.13	7.90±0.16	7.78±0.16	0.06
Na mEq/L	169.60±2.97	176.40±2.70	174.20±6.42	169.40±6.23	2.19
K mEq/L	4.66±0.17	4.64±0.18	4.66±0.21	4.66±0.15	0.08
Pi mg/100ml	3.86±0.19	4.06±0.18	4.14±0.34	3.86±0.17	0.10
Total protein g/dL	4.14±0.60	5.08±1.27	5.16±0.21	5.03±0.46	0.33
Albumin g/dL	1.82 ^{b}±0.24	2.04^{ab}±0.30	2.26^{a}±0.16	2.33^{a}±0.12	0.10
Globulin g/dL	2.32±0.42	3.04±1.01	2.90±0.19	2.70±0.42	0.26
Cholesterol mg/dL	192.40±5.73	188.80±8.20	186.20±6.72	182.40±8.88	3.35
Uric acid mg/dL	2.82±0.34	2.76±0.21	2.56±0.09	2.50±0.19	0.10
Glucose mg/dL	193.55±9.74	195.70±9.62	189.25±10.09	193.12±17.10	5.39

Values are means of 5 replicates per treatment. ^{ab}Means with different superscripts in the same row were significantly different ($p \leq 0.05$). SEM: Standard error of the means from ANOVA d.f 16.

The effect of graded levels of leucaena seeds on internal quality characteristics of eggs of 28th-31st week of age layers (Table 5) revealed that albumen height, albumen diameter, albumin Index, yolk height, yolk diameter, yolk weight, yolk %, albumin yolk index and Haugh unit did not appear to be affected by the dietary treatments. Similar findings of Haugh unit were reported by (Odunsi *et al*., 2002). In the current study, albumin weight of birds fed 16% leucaena seeds was higher than other dietary treatments, while albumin% of birds fed 16% leucaena seeds was higher than the control. Yolk index of the birds fed 8% leucaena seeds was higher than the other groups. The improvement in egg yolk may be due to the presence of carotenes. A similar observation was reported by NFTA (1990).

The effect of graded levels leucaena seeds on haematological parameters and plasma constituents of 28th-31st week of age layers (Table 6.) showed that PCV% of birds fed 24% leucaena seeds was higher than the other dietary treatments, while Hb, neutrophils%, lymphocytes%, monocytes%, eosinophils% and basophils% were not influenced by

the treatments. This indicated that the dietary treatments had no negative effect on the health of the chickens. Dietary treatments did not affect plasma GOT and ALP, this indicated that no signs of liver damage appeared. Similarly Adam (1974) and Benno (1963) attributed the release of plasma GOT and ALP to liver cell necrosis. Plasma Ca, Na, K, P, total protein, globulin, cholesterol, uric acid and glucose were not affected by the treatments. Plasma albumin of birds fed leucaena seed diets was higher than control.

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