International Journal of Agricultural Science Research Vol. 4(3), pp. 049-056, March 2015
Available online at http://academeresearchjournals.org/journal/ijasr
ISSN 2327-3321 ©2015 Academe Research Journals

Full Length Research Paper

Effects of feeding processed kidney bean meal (*Phaseolus vulgaris*) instead of soybean meal on qualities of eggs of white leghorn hens

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Accepted 11 February, 2015

A study was conducted to evaluate the effects of replacing processed kidney bean meal (PKBM) for soybean meal (SBM) on egg quality parameters of white leghorn (WL) chicken. A total of 360 eggs (24 per replicate and 72 eggs per treatment), which were collected from a total of 225 chicken (165 layers and 30 cocks) with uniform body weight (BW) and age, randomly distributed into 15 pens and assigned to five treatments, were used for analysis of egg quality. Treatments were SBM substitution by PKBM at 0, 25, 50, 75 and 100% levels for T1, T2, T3, T4 and T5, respectively. Replacement of SBM with PKBM in the diet did not affect albumen height and weight, Haugh unit, yolk index, diameter, weight and height, and shell thickness. The shell weight was significantly (p<0.05) lower for T5 than T1 but yolk color was significantly (p<0.05) higher for T5 than T1. Thus, it can be concluded that 100% (at a rate of 100 g/kg concentrate diet) of PKBM as a substitution for SBM in the diet of layers did not affect the qualities of the eggs except for yolk color and shell weight.

Key words: Egg quality, Haugh unit, kidney bean, soybean meal, yolk color.

INTRODUCTION

Egg quality is a general term which refers to several standards which can be discussed under two broad categories namely external and internal quality (Monira et al., 2003). External quality is focused on the size and shape of the eggs as well as the structures, thickness and breaking strength of the shell, shell cleanliness, shell and egg weight. Internal quality refers to egg white (albumen) cleanliness and viscosity, albumen weight and height, size of the air cell, yolk weight, shape, strength, height and color, whipping capacity and the relative size of various internal components, and the integrity of the shell membrane (Bain, 2005; Matta et al., 2009). The same authors reported that a domestic fowl's egg contains approximately 64, 27 and 9% of albumen, yolk and shell, respectively. The chalaza is 0.25% of the total egg weight and usually included with albumen weight. Shell membrane is about 0.75% of the total egg weight and generally included with shell weight. Most egg quality traits change mainly as a result of nutrition, effect of environmental condition, genotype, age of hens and laying rate of hens.

Egg weight is an important trait that influences egg and chick quality as well as grading. Egg size can be determined without breaking the eggs and it is measured by weighing balance (Soniaya and Swan, 2004).

The egg weight partly depends on the feed, age of birds, breed, and the environment, particularly the temperature. Protein and amino acids in the diet are the major nutrient controlling egg size (Waldroup and Hellewing, 1995).

Egg shell quality is the major concern of commercial

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egg producers. Egg shell quality is largely based on its thickness and weight. It is a very important structural component of egg since it serves as embryonic chamber for the developing chick, serve as gas exchange medium, prevent contamination by bacteria, and provide mechanical protection of the content and it is unique package for a valuable food (Shi et al., 2009). Egg shells of commercial breeds of chickens are white or brown. Breeds with white earlobes ordinarily lay white eggs and breeds with red earlobes ordinarily lay brown eggs. Shell color has nothing to do with egg flavor, nutritive value, or cooking characteristics, but it affects marketability since it affects customer's preference (Jacqueline et al., 2008).

Egg albumen is the human's most valuable kind of protein and amino acids composition is regarded as a standard. It is characterized by a unique biological value and its true digestibility (TD) is the highest one (TD=97±3%) among the known protein foods (Lewko and Gornowicz, 2009). Egg albumen shows great quantitative and qualitative diversification and is present in all structures. The same authors reported that the freshly laid egg contains 80, 11, 0.6 and 0.4% water, protein, carbohydrate and minerals, respectively. Thin and thick albumen is a sign of poor and good quality eggs, respectively. When a decayed egg is broken out, the yolk is flattened and often displaced to one side and the surrounding area of albumen also collapsed and flattened to produce a wide arc of liquid (Jacqueline et al., 2008). The main egg albumen quality assessment is the determination of Haugh unit which is the criterion of egg quality being used in international trades. The Haugh unit from 79-100 demonstrates best egg albumen quality (Lewko and Gornowicz, 2009).

Yolk quality depends on color, spherical condition and strength or firmness of the vitelline membrane. In Ethiopia, yellow yolk color of eggs is highly preferable than white yolk color, hence the color of the yolk is important to the commercial farm. Yolk color is measured by using Roche Color Fan, which consists of a series of colored cards ranging from pale yellow (1) to deep orange (15) colored plastics tabs. Yolk color depends on the diet of the hens, which is xanthophylls deposition in the yolk. Hens fed mashes containing yellow corn and alfalfa meal laid eggs with yellow yolk, while those eating white corn, grain sorghum (Milo) and wheat or barley laid eggs with light-color (platinum) yolk. The inclusion of higher than the recommended levels or incorrect ratios of pigments in layer ration can lead to orange-red yolk colors (Silverside et al., 2006). Natural yellow orange substances such as marigold petals may be added to light colored feeds to enhance yolk color (Baiyshya, 2008). Etches (1996) also noted that color of the egg yolk can be extensively modified by diet. The incorporation of oxycarotenoids into the diet can lead to the deposition of up to 1 mg of pigment in the yolk and produce a deep orange color.

MATERIALS AND METHODS

Management of experimental chickens

The experimental house has 15 experimental pens partitioned with a wire mesh, each having a 2.5*2 m dimension. It was cleaned and disinfected (with a cavlon, that is, 10 ml in 1 litter H_2O) very well before the commencement of the experiment. Similarly watering and feeding troughs and laying nests were thoroughly cleaned, disinfected and sprayed against external parasites and the floor of each pen was covered with teff straw of 10 cm depth.

The birds required for the experiment were obtained from Haramaya University poultry farm. The 225 birds used for the experiment were uniform in size, age and free from any defects. The birds were vaccinated against Newcastle disease, salmonelosis and coccidiosis at the farm. The birds were adapted to the experimental diets and experimental procedures for 7 days before the actual data collection was started. They were also given aminovate vitamin (that is, 10 g in 20 L of water) and piperazine citrate (that is, 900 ml in 20 L of water) for ascaris deworming. Feed was measured and provided to the birds in group twice a day at 0800 and 1700 hours *ad libitum*. The feeding and watering troughs were cleaned every morning before feeding. Feed was offered in hanging tubular feeders, which was suspended approximately at a height that birds can reach. Water was available all the time and the experiment lasted for 90 days.

Ingredients and experimental rations

The feed ingredients used in formulation of the different experimental rations of the study were processed kidney bean, maize grain, wheat short, soybean meal, noug seed cake, vitamin premix, limestone and salt as shown in Table 1. The kidney bean, maize grain and noug seed cake were coarsely ground before formulating the treatment rations. The five layer rations were formulated on an iso-caloric and iso-nitrogenous basis in such a way to consist of about 2800-2900 KCal/ME per kg DM and 16-17% CP to meet the requirements of layers. The treatments consisted of 0% (T_1), 25% (T_2), 50% (T_3), 75% (T_4) and 100% (T_5) processed kidney bean as a substitute for soybean meal as protein source.

The kidney bean seed was cleaned from dust and dirt materials soaked in water at a proportion of 5 L H_2O to 1 kg bean for five hours, rinsed and poured into boiled water (100°C) at the same proportion and heated for 1 h.

Table 1. Proportion of ingredients (percentage of total ration) used in formulating the experimental rations.

Ingredients (%)	Treatment diets				
	T_1	T_2	T_3	T_4	T_5
Maize grain	38	38	38	38	38
Wheat short	18	18	18	18	18
Soybean meal	10	7.5	5	2.5	0
Kidney bean	0	2.5	5	7.5	10
Noug seed cake	25	25	25	25	25
Limestone	7.8	7.8	7.8	7.8	7.8
Vitamin premix	0.7	0.7	0.7	0.7	0.7
Salt	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100

PKBM = processed kidney bean meal; T_1 = ration containing 0% PKBM; 100% SBM (control); T_2 = ration containing 25% of SBM substituted by PKBM; T_3 = ration containing 50% of SBM substituted by PKBM; T_4 = ration containing 75% of SBM substituted by PKBM; T_5 = ration containing 100% SBM substituted by PKBM.

The cooked kidney bean was rinsed and sun dried by spreading the grain on a canvas until it was sufficiently dried to the ground (Emiola, 2007).

Experimental design and treatments

The experiment was conducted in a completely randomized design with 5 dietary treatments each with three replications. As clearly indicated in Table 2, a total of 195 white leghorn pullets and 30 cockerels which were both 7 months old were obtained from Haramaya University poultry farm.

Measurements and observation

Egg weight and egg mass

Immediately after collection of the eggs, mean egg weight was determined by dividing the total egg weight with the total number of eggs collected from each pen. The average egg mass per day was computed by multiplying the mean egg weight by percentage hen-day egg production (North, 1984).

Egg quality parameters

The internal egg quality was measured through the break out analysis method. A total of 3 eggs per replicate was randomly selected and their quality was measured once in a week, that is, 8 times during the experimental period. Thus, 24 eggs per replicate and 72 eggs per treatment were used for analysis of internal egg quality. The following egg quality parameters were determined.

Egg shell thickness and shell weight: Eggs were broken on a flat mirror and the egg membrane was carefully removed from the shell. Shell thickness was measured using micro meter gauge. The measurements were taken from three sides: the top (small end), bottom (large end) and the middle parts of the egg, and the average of the three measurements was taken as egg shell thickness (Ajuwon et al., 2002). Egg shell weight was taken using sensitive balance after egg shell membrane was carefully removed.

Albumen weight, height and Haugh unit: Immediately after breaking the eggs, the height of albumen was measured by a tripod micrometer and its weight was taken by sensitive balance after being separated from the yolk. The Haugh unit was used to relate the weight of eggs with the height of thick albumen. The Haugh unit was computed using the following formula by Haugh (1937):

$$\text{Haugh unit (HU)} = 100 \text{ Log } [H- \frac{\sqrt{G}\,(30W^{0.37} - 100)}{100} + 1.9]$$

where, HU = Haugh unit (g); G = Gravitational constant, 32.2; H = Albumin height (mm); W = Weight of egg.

Yolk weight, diameter, height, yolk index and color: After the separation of the yolk and albumen, yolk diameter and height was measured using tripod micrometer and yolk weight was measured using a sensitive balance. Yolk index was computed using the

Table 2. Layout of the experiment.

Treatments*	Replication	No. of layers/replication	No. of cockerels/replication
T_1 100% SBM + 0% PKB	3	13	2
T_2 75% SBM + 25% PKB	3	13	2
T_3 50%SBM + 50% PKM	3	13	2
T_4 25%SBM + 75% PKB	3	13	2
T_5 0% SBM + 100% PKB	3	13	2

*SBM = soybean meal; PKB = processed kidney bean; 100% SBM represent the amount of SBM in 1 Kg of layers ration.

Table 3. Egg weight and egg mass of eggs of white leghorn layers fed rations containing different proportions of processed kidney bean as a replacement for soybean meal.

Parameter	Treatments					
	T1	T2	T3	T4	T5	SEM
Egg weight (g)	50.42	50.16	50.39	49.82	49.43	0.38
EM (g/hen/day)	28.94	28.83	29.68	30.77	30.3	2.13

SEM = standard error of mean; g = gram, EM = egg mass; PKBM = processed kidney bean meal; SBM = soybean meal; T_1 = ration containing 0% PKBM; 100% SBM (control); T_2 = ration containing 25% of SBM substituted by PKBM; T_3 = ration containing 50% of SBM substituted by PKBM; T_4 = ration containing 75% of SBM substituted by PKBM; T_5 = ration containing 100% SBM substituted by PKBM.

following formula:

$$\text{Yolk Index} = \frac{\text{Yolk Height}}{\text{Yolk Diameter}}$$

After removing yolk membrane and taking other necessary information, the yolk was thoroughly mixed, sample was taken on a piece of white paper, and yolk color was determined by comparing with the Roche fan measurement strips which have 1-15 strips ranging from pale to orange yellow (Vuilleumiler, 1969).

Statistical analysis and models

The data collected during the study period were subjected to statistical analysis using SAS computer software version 9.1.3. SAS (2008). During data analysis, egg mass, egg weight, shell weight, albumen weight, yolk weight, yolk height, albumen height, yolk diameter, yolk index, Haugh unit and shell thickness were analyzed following one way analysis of variance procedure. When the analysis of variance indicated the existence of significant difference between treatment means, list significant difference (LSD) method was used to locate the treatment means that were significantly different from the others (Gomez and Gomez, 1984). The model used for statistical analysis was:

$$\mathbf{Y_{ij} = \mu + T_i + e_{ij}}$$

where: Y_{ij} = individual observation, T_i = treatment effect, μ = overall mean, and e_{ij} = error term.

General logistic regression analysis was employed for analysis of data recorded on yolk color (1, 2, 3…). The general logistic regression model used is given as follows:

$$\text{Model:} \quad \ln\left\{\frac{\pi}{1-\pi}\right\} = \beta_0 + \beta_1 * (\mathbf{x})$$

Test H0: No treatment effect (that is, β1 = 0) versus HA: Significant treatment effect (β1 ≠ 0)

where π = probability, β = slope, and x = treatment.

RESULTS AND DISCUSSION

Egg weight and mass

There was no significant (p>0.05) difference in egg weight among treatments (Table 3). As Waldroup and Hellewing (1995) reported, the methionine level of the diet affects egg weight. Therefore, in the present experiment, the methionine level of the diet might have

Table 4. Egg quality parameters of white leghorn chicken fed rations containing different levels of processed kidney bean as a replacement for soybean meal.

Parameter	Treatments					
	T1	T2	T3	T4	T5	SEM
Shell thickness (μm)	0.33	0.33	0.32	0.31	0.31	0.00
Shell weight (g)	6.00[a]	6.02[a]	5.61[ab]	5.39[b]	5.41[b]	0.14
Albumen weight (g)	31.00	30.13	29.27	29.67	29.91	0.52
Albumen height (mm)	7.85	8.16	7.84	8.41	7.86	0.26
Haugh unit	90.42	92.00	91.25	93.5	90.00	1.41
Yolk weight (g)	15.67	15.65	15.55	15.45	15.33	0.25
Yolk height (mm)	16.01	16.08	15.92	15.60	15.80	0.16
Yolk diameter (cm)	3.65	3.68	3.65	3.61	3.62	0.02
Yolk index	0.44	0.43	0.44	0.43	0.44	0.00
Yolk color	3.28[b]	3.33[b]	3.88[ab]	3.81[ab]	4.23[a]	0.23

[a] and [b] = Means within a row with different superscripts are significantly different at (P< 0.05); g = gram; μm = micro meter; mm = mili meter; cm = cent meter; SEM = standard error of mean; PKBM = processed kidney bean meal; SBM = soybean meal; T_1 = ration containing 0% PKBM; 100% SBM (control); T_2 = ration containing 25% of SBM substituted by PKBM; T_3 = ration containing 50% of SBM substituted by PKBM; T_4 = ration containing 75% of SBM substituted by PKBM; T_5 = ration containing 100% SBM substituted by PKBM.

been similar as soybean meal is replaced by processed kidney bean meal. Wu et al. (2005) also reported that early egg weight increased by increasing dietary protein, and similar protein intakes among treatments might have accounted for the similar egg weight observed in this study. Summer and Leeson (1993) reported that egg weight was affected by body weight of laying hens, which was not affected in this study. There was no significant (p>0.05) difference in egg mass among treatments, like that of egg weight. Fakhraei et al. (2010) noted that egg mass followed the same trend as egg production is consistent to the observation in this study.

Egg quality parameters

Egg shell weight and thickness

The mean egg shell weight and shell thickness resulting from feeding of the five treatment rations is shown in Table 4. The result showed no (p>0.05) difference among treatments in egg shell thickness. Similarly, Senkoylu et al. (2005) did not found significant (P>0.05) difference in eggshell thickness among hens fed diets consisting of different levels (0, 10, 16, and 22%) of dietary full fat SBM. But egg shell weight in the present experiment was significantly (p<0.05) different among the treatments and that T4 and T5 had lower shell weight than other treatments. Lower shell weight and thickness at higher level of PKBM may be due to the relatively low Ca and P content of the kidney bean, as compared with SBM, although the experimental diets appeared to have comparable Ca and P contents.

Albumen weight, height and Haugh unit

There was no significant (p>0.05) difference in albumen weight, height and Haugh unit (HU) among treatments (Table 4). As documented by William (1992), albumen quality is not greatly influenced by nutrition, but decline in HU is mostly related to age of the hen and egg storage conditions. Layers used in the present experiment are in their first year of production, and the eggs used for quality analysis were fresh. Thus, a difference in HU as a result of layers and eggs age is not expected. Hence, absence of difference in these parameters among the treatments indicates that replacing soybean meal with processed kidney bean meal up to 100% do not affect HU. The height of the albumen determines the HU of the egg. The higher the height of the albumen, the greater the HU and the better the quality of the eggs. But, albumen height did not significantly vary among treatments in the present study. The HU values recorded for all treatment groups were within the range of freshly laid eggs (Essien, 1990). In this study, all treatments scored HU within the recommended range of 70-100, which is an indication of good egg quality (Lewko and Gornowicz, 2009). This is an index ability of albumen to remain viscous indicating that processed kidney bean meal based rations did not have any adverse effect on HU. The difference in albumen weight among treatments was consistent with differences in egg weight, which is in line with that demonstrated by Suk and Park (2001) that albumen

Table 5. Yolk color points of egg samples from white leghorn layers fed diets containing processed kidney bean meal as a replacement for soybean meal.

	Roche color fan number								
Treatment	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
T1	2	10	20	26	7	4	1	2	72
T2	5	7	20	18	13	7	2	0	72
T3	1	7	19	26	13	6	0	0	72
T4	0	7	12	23	21	9	0	0	72
T5	4	7	17	24	18	2	0	0	72
Total	12	38	88	117	72	28	3	2	360

PKBM = processed kidney bean meal; SBM = soybean meal; T_1 = ration containing 0% PKBM; 100% SBM (control); T_2 = ration containing 25% of SBM substituted by PKBM; T_3 = ration containing 50% of SBM substituted by PKBM; T_4 = ration containing 75% of SBM substituted by PKBM; T_5 = ration containing 100% SBM substituted by PKBM.

weight is positively associated with egg weight.

Yolk weight, height, diameter and index

Yolk weight, height, diameter and index of layers are presented in Table 4. There was no difference (P>0.05) in yolk weight, yolk height, diameter and index among treatments. Since there was no difference in yolk weight among treatments, differences between yolks height is also not expected. The yolk index values of the eggs from the treatment groups in the present experiment ranged from 0.43-0.44, which is within the accepted range of 0.33-0.50 for fresh eggs (Ihekoronye and Ngoddy, 1985).

Yolk color

Values for yolk color of eggs are presented in Table 4. The logistic regression results for yolk color showed significant difference (pr>chisq <0.001 at α = 0.05) with Wald chi square value of 18.43 among the treatments (Appendix Tables 1 and 2). Mean of treatments from SAS output are presented in Appendix Table 3. The odd ratio value of T1 versus T5 shows that T1 has 2.260 times the odds of receiving a lower score than T5 and the other follows the same trend. The increased yellow color intensity of yolk at higher level of processed kidney bean as replacement of soybean meal could be attributed to higher beta-carotene content of kidney bean than soybean meal. The present experiment is in line with the finding of Fru-Nji et al. (2007) that faba bean (*Vicia faba*) resulted in increased yolk color. The roche color fan reading recorded during the experiment ranges from 1 (pale yellow) to 8, with majority of the eggs having 3 and 4 values on the yolk color point (Table 5). The roche color fan number of 7 to 8 (deeper yellow color) is appreciated by consumers in most areas (Leeson and Summer, 1997).

ACKNOWLEDGEMENTS

The corresponding author is very grateful to his advisors, Dr. Mengistu Urge and Dr. Getachew Animut, for their endless help in correcting, commenting, criticizing and encouraging him to accomplish this work. Also, he is greatly indebted to the Ministry of Education (MOE) for covering the research cost and his stipend. Haramaya University in general, and the School of Animal and Range Sciences in particular, are highly appreciated for the input to his career facilitating the course work and research undertaking. Finally, his gratitude goes to Ato Minayu Nega, who supported him during the chick quality assessment part of the research work.

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APPENDIXES

Appendix Table 1. Analysis of variance summary result for egg quality parameters of white leghorn layers fed on rations containing different proportions of processed kidney bean as a substitute for soybean meal.

Source	DF	Sum square	M. square	F value	Prob>F	CV%
Sample egg weight	4	11.31	2.82	1.21	0.36	2.93
Albumen weight	4	5.02	1.25	1.13	0.39	3.51
Yolk weight	4	2.65	0.66	3.30	0.06	2.95
Shell weight	4	1.14	0.28	3.64	0.04	4.93
Yolk index	4	0.00	0.00	0.54	0.71	2.05
Yolk diameter	4	0.01	0.00	1.30	0.33	1.16
Haugh unit	4	23.14	5.78	0.72	0.59	3.09
Shell thickness	4	0.00	0.00	1.91	0.18	2.65
Yolk height	4	0.42	0.10	1.04	0.43	2.01
Albumen height	4	0.78	0.19	0.69	0.61	6.67

Appendix Table 2. Results of logistic regression of yolk color in white leghorn chicken fed diet containing different proportions of processed kidney bean meal as a substitute of soybean meal.

		Wald	
Parameter	DF	Chi-Square	Pr> ChiSq
Yolk color	4	18.43	0.001

Appendix Table 3. Analysis of maximum likelihood estimates of yolk color of white leghorn chicken fed diet containing different levels of processed kidney bean meal as a substitute for soybean meal.

Parameter	DF	Estimate	Standard error	Wald Chi-Square	Pr > ChiSq	Exp (Est)
Intercept 8	1	-5.9233	0.7514	62.1377	<0.0001	0.003
Intercept 7	1	-5.5146	0.6308	76.4344	<0.0001	0.004
Intercept 6	1	-3.4930	0.3326	110.2686	<0.0001	0.030
Intercept 5	1	-2.1933	0.2775	62.4558	<0.0001	0.112
Intercept 4	1	-0.8813	0.2539	12.0481	0.0005	0.414
Intercept 3	1	0.7795	0.2539	9.5460	0.0020	2.180
Intercept 2	1	2.5543	0.3701	47.6214	<0.0001	12.862
Tre 1 vs 2	1	-0.1939	0.3363	0.3325	0.5642	0.26
Tre 1 vs 3	1	-0.5099	0.3369	2.2906	0.1302	0.744
Tre 1 vs 4	1	-0.4153	0.3366	1.5222	0.2173	1.763
Tre 1 vs 5	1	0.8154	0.3411	5.7137	0.0168	2.260
Tre 2 vs 3	1	-0.3160	0.3358	0.8851	0.3468	0.423
Tre 2 vs 4	1	-0.2214	0.3357	0.4348	0.5096	2.367
Tre 2 vs 5	1	1.0093	0.3424	8.6880	0.0032	2.744
Tre 3 vs 4	1	0.0946	0.3351	0.0797	0.7777	0.721
Tre 3 vs 5	1	1.3252	0.3451	14.7484	0.0001	3.763
Trt 4 vs 5	1	1.2306	0.3442	12.7827	0.0003	3.423