

Roasted pigeon pea meal replacement to soybean meal and its effect on external and internal egg quality parameters of Bovans Brown layers

Abstract

Aims: The objective of the research was to evaluate egg quality of Bovans Brown (BB) layers by replacing soybean meal with roasted pigeon pea (*Cajanuscajan*) seed meal in the diet.

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Study design: The experimental rations were formulated to be iso-caloric and iso-nitrogenous. A total of 105 commercial BB egg laying hens of 6 months old with body weight of (1412.04g ± 71.095g) were used.

Place and Duration of the study: The study took place at Shire Endaslasie, district, north western Tigray, Ethiopia located at 14°6'18" North and 34° 17' 4" East (Satellite Map) for 90 days with 10 days of adaptation period.

Methodology: The experiment was designed using complete randomized design (CRD) with 5 treatments and replicated 3 times. Treatments were contain 0% (control), 25%, 50%, 75% and 100 % level of *RPSM* replaced to soybean meal for *T1, T2, T3, T4 and T5*, respectively. The treatment rations were containing a range of 2882.28 - 2899.53 kcal/kg *DM* of *ME* and 16.63 – 16.92% CP. Data were analyzed using general linear model (GLM) of the SAS software.

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Results: There was no significant ($p>0.05$) difference in egg quality parameters among treatments. The average result in the experiment was egg weight 58.34 - 59.04 gram, shell weight 5.49 - 5.78g, egg shape index 71.70 ± 3.6 . Similarly the range of albumin weight, albumin height and yolk weight in the experiment was 34.62 - 35.14g, 7.72 - 8.06 mm and 15.2 - 15.9g, respectively. Yolk color result using *RYCF* was in the range of 4.96 - 5.07.

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Concussion:It is concluded that roasted pigeon pea seed meal can replace soybean meal up to 75% in layers diet without negative effect of external and internal egg quality parameters.

Key words: Egg quality-parameters, locally available- feeds, pigeon pea seed, soybean meal

Introduction

Feed cost for poultry production is increasing due to limited availability of cereals and oil cakes [1] and since the demand for the cereals is increasing highly resulted by the alarming population growth of the developing countries. As a result, the commercial layer feed is expensive and not easily available by smallholder poultry producers which causes feed problem (both quality and quantity) in developing countries like in Ethiopia [2]. Therefore, there is need to reduce the

competition between human and livestock for the same feedstuffs by turning to unconventional feedstuffs and it is better to utilize locally available non-conventional feed resources.

In poultry industry Soybean meal is widely used as high protein feed ingredient with close to ideal in amino acid profile. However, there is no access of soybean meal for smallholders in the country in general and Tigray region in particular. Hence, based on [3] and [4] searching of alternative feed sources is required to reduce the feed cost. As a result one of the important but less known plant protein sources is pigeon pea seed meal [5] and can be used as alternative to replace protein sources like soybean meal.

Pigeon pea (*Cajanuscajan*) has numerous uses in animal feeding and the seed can be fed to poultry [6]. As reported by [7]; [8] pigeon pea is good source protein, energy and considerable amount of minerals which makes close to that of soybean. This directs that pigeon pea seed meal can replace the commercial plant protein sources like soybean meals which are used as poultry protein source feed ingredient. Raw Pigeon pea seeds like other legume seeds contain Anti-Nutritional Factors (ANFs) that can positively treat by heat treatments [9]. Generally, even though, pigeon pea seed is promising to the poultry feed industry; there is limited information on the effect of roasted pigeon pea seed meal on internal egg quality parameters of Bovans Brown (BB) layers by replacing to soybean meal in their diet. Therefore, the objective of the study was to see the effect of replacing soybean meal with roasted pigeon pea seed meal and to see its effect on the egg quality parameters of Bovans Brown layers.

Comment [LS7]: Mention recent researches for this point

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Comment [LS11]: mention more details on pigeon pea with some recent researches

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Materials and methods

Description of the study area

The experiment was conducted in Shire Endaslassie district which is located in North-western zone of Tigray. Tigray regional state is located in the northern part of Ethiopia. North-western zone is one of the six administrative zones in Tigray region which is located at 307 km west of

Mekelle and 1087 km from Addis Ababa via Mekelle. Shire Enda lassie district (Shire Enda lassie city) has an average altitude of 1923 m.a.s.l as well as it is found at 14°6'18" North and 34° 17' 4" East (Satellite Map). It is surrounded by Tahtay-Koraro district and it is the capital city of North Western Zone administration as well it serves as the center of administration for Tahtay-Koraro district. Shire Enda lassie has savannah climatic condition with average rain fall reaching 800-1000 mm and mean annual temperature of 15-20 °C [10].

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Feed ingredients and experimental ration

The feed ingredients used in the formulation of the different experimental rations for the study were maize grain (MG), Sorghum Grain, pigeon pea seed meal, wheat bran, noug seed cake, sesame seed cake, soybean meal, salt, limestone, and L-lysine Hcland Di-calcium phosphate. All the feed ingredients for the experiment were purchased from available market of Shire Enda lassie except Soybean meal, L-lysine Hcland Di-calcium phosphate. Soybean meal was purchased from Addis Ababa and both the L-lysine Hcland Di-calcium phosphate was purchased from Mekelle. Maize grain, Sorghum Grain, pigeon pea seed meal, sesame seed cake, noug seed cake, and soybean meal were hammer milled to 5 mm sieve size and stored until required for formulation of the experimental rations. The five treatment rations used in this study were formulated to be iso-caloric and iso-nitrogenous with 2,800 – 2,900 kcal ME/kg DM and 16 - 17% CP [11] to meet the nutrient requirements of layers and the ration was formulated using the feed- win ration formulation software. In the treatments soybean meal was replaced with pigeon pea level by level.

Comment [LS15]: redefine the sentence

Comment [LS16]: kindly mention how soybean meal was replaced?

Source and roasting method of pigeon pea seeds

The white type of pigeon pea seed was collected from Shire-Maitsebri agricultural research center. The pigeon pea seed was roasted for 3-5 minute at about 80 °C [12] using locally available frying pan until some color change comes then it was coarsely grind before incorporation into the diets.

Table 1. Percentage composition of the feed ingredients

Feed stuff %	T1 (control)	T2	T3	T4	T5
Maize grain	20.5	24	25.9	25.5	24
Sorghum grain	25.3	24.5	23	21	20
SBM	15	11.3	7.5	3.75	0
RPSM	0	3.75	7.5	11.3	15
Sesame seed cake	2.75	4.25	7.5	12	17
Noug seed cake	8	11.2	12	11	9.4
Wheat bran	15	8.5	4	3.9	3
Limestone	8	8	8	8	8
L-lysine Hcl	0	0.1	0.1	0.1	0.1
Salt	0.5	0.5	0.5	0.5	0.5
Di-calcium phosphate	5	4	4	3	3
Total (%)	100	100	100	100	100

SBM= Soybean meal; RPSM= roasted pigeon pea seed meal; T1= 100% soybean meal (control); T2=25% roasted pigeon pea seed meal+ 75% soybean meal; T3= 50% roasted pigeon pea +50% soybean meal; T4=75% roasted pigeon pea+ 25% soybean meal; T5= 100% roasted pigeon pea replacement to soybean meal

Experimental chickens and their management

A total of 105 commercial Bovans Brown egg laying hens of 6 month old with similar body weight and husbandry were purchased from the local private chicken distributor. Before the commencement of the actual experiment, the experimental pens, watering and feeding troughs were thoroughly cleaned, disinfected and sprayed. The layers were kept in deep litter experimental house which was partitioned in to 2.25 m² pens by wire-mesh. The birds were supplied artificial light for about four o'clock in addition to the natural day light throughout the whole experiment. Sawdust litter material of 07 cm up to 10 cm depth was used. The wet litter was changed with dry, disinfected, and clean sawdust in the middle of the experiment. All health precautions and disease control measures was carefully followed throughout the study period. Feed was offered in hanging tubular feeders, which was suspended approximately at a height of the backs of the birds and water was provided in plastic fountains freely. The feeding and watering troughs were cleaned every morning.

Comment [LS17]: Are they vaccinated? Or any other feed were given to them before? Kindly mention

Comment [LS18]: What is the light and dark cycle (mention in hrs)

Comment [LS19]: Did you weigh the feed before giving the birds?

Experimental design and treatments

The experimental design was Completely Randomized Design (CRD), with five treatments having 3 replications and 7 birds per replicate. Birds were randomly allocated to dietary treatment. The experiment was conducted for 90 days and 10 days of adaptation period.

The treatments were pigeon pea replacement to soybean meal at the rate of 0%, 25%, 50%, 75% and 100 % level in the rations to be formulated for T1, T2, T3, T4 and T5, respectively. Because different authors [13],[14]have recommend different percentage of pigeon pea replacement to soybean meal and different level of inclusion in the layers diet.

Comment [LS20]: What is the male to female ratio?

Table 2. Layout of the experiment

Treatment	No of Rep	Number of birds per replication		
		R ₁	R ₂	R ₃
T ₁ : No RPSM replacement (100% soybean meal)	3	7	7	7
T ₂ : 25% RPSM replacement to soybean meal	3	7	7	7
T ₃ : 50% RPSM replacement to soybean meal	3	7	7	7
T ₄ : 75% RPSM replacement to soybean meal	3	7	7	7
T ₅ : 100% RPSM replacement to soybean meal	3	7	7	7

RPSM: roasted pigeon pea seed meal; Rep: Replication; R1, R2, R3: replication 1, 2, 3, respectively.

Chemical analysis of the feed ingredients

Representative samples was taken randomly from each of the feed ingredients that were used in the experimental diet for laboratory analysis except the premixes and salt before formulating the actual dietary treatments. Samples were also taken for chemical analysis from each treatment ration. Feed samples were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF) and ash according to Weende or proximate analysis method [15]. Nitrogen (N) content were determine by Kjeldahl procedure and crude protein (CP) were calculated as Nx6.25. Chemical analyses of feeds were done in the nutrition laboratory of Hawasa

University. Metabolizable energy (ME) of the experimental diets was determined by indirect method according to [16] as follows:

$$\text{ME (Kcal/kg DM)} = 3951 + 54.4 \text{ EE} - 88.7 \text{ CF} - 40.8 \text{ Ash}$$

Egg quality measurements

For the measurement of egg quality parameters 03 eggs per replication (09 eggs per treatment) were randomly taken weekly and the average value from each replication was computed for each quality parameters.

Egg weight and egg mass

The average egg weight was computed using the sensitive balance of 0.005- 5kg weighing capacity by dividing the total weight of egg mass to the number of eggs laid. Egg mass = HDEP* Average egg weight in gram

Eggshell quality

Egg shell is one of the major quality parameters to evaluate the egg quality and viability in poultry production and has high influence in the acceptance of consumers. Both the egg shell weight and the eggshell thickness were measured by destruction of the egg. The average of the blunt, middle and sharp points of the egg was measured using the digital caliper and was summarized to get the shell thickness. The eggshell weight was measured using a sensitive balance that have 0.005- 5kg weighing capacity.

Shape index

The shape index was calculated based on the commonly used formula of [17]by using the ratio of egg width to egg length. The width and the length were taken carefully from the sample eggs used for quality parameters measurements.

Egg albumen quality

The eggs were break carefully to allow passage of albumin and yolk easily. Then after, albumin and yolk were separated each other in their own pre weighed Petri dish to take their weight separately. Then weight of Petri dish containing the albumen was measured and then the weight

difference of the container before and after will be the weight of albumin. Simultaneously, the height of albumen was measured using the Electronic digital caliper.

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Haug unit (HU) is one of the most significant measures of egg quality like the other egg quality measurements and the higher the Haugh unit number, the better the quality of the egg (fresher, higher quality eggs have thicker whites) [18]. $HU = 100 \log (ALH + 7.6 - 1.7W^{0.37})$

Where: ALH = Observed height of the albumen in mm, and W = Weight of egg in grams

Egg yolk quality

After breaking the egg, yolk and albumin were separated each other in their own pre weighed Petri dish. Then the egg yolk with its Petri dish was weighed using the sensitive balance. Then the weight difference of Petri dish with yolk and empty Petri dish will be taken as yolk weight. Soon after taking the weight, the egg yolk color was compared with Roche yolk color fan (RYCF) which consists of a series of fifteen colored plastic strips, with one rated as very pale yellow to a deep intense reddish orange [19]. During yolk color measurement, first the yolk membrane was removed, the whole yolk was thoroughly mixed and yolk sample was taken on a piece of white paper and compared with Roche yolk color fan measurement strips. RYCF is important to define the desired yolk color and helps to formulate the hens' feed according the target yolk color.

Comment [LS22]: Rephrase the sentences for better understanding

Statistical model and data analysis

The collected data was analyzed using General Linear Model of SAS software [20]. The Significant differences ($p \leq 0.05$) between treatments mean were separated using Tukey's Studentized Range Test (HSD) method. The following model was used for the experiment (Gomez and Gomez 1984). $Y_{ij} = \mu + i + e_{ij}$; Where, Y_{ij} = Response variable; μ = overall mean; i = i^{th} treatment effect (1, 2, 3, 4, 5); E_{ij} = Random error effect

UNDER PEER REVIEW

Results

Chemical composition of feed ingredients and treatment diets

The result for the chemical composition of each ingredient used in the formulation of dietary rations for each treatment is described in Table 3.

Table 3. Proximate chemical composition of ingredients used in formulation of dietary treatments (% DM Basis)

Comment [LS23]: Add the p value

Composition	Feed ingredients							
	Maize	Sorghum	SBM	RPSM	SSC	NSC	WB	Limestone
Dry matter	91.03	90.9	92.7	92.7	91.5	92.7	90.9	99

(%)								
ME (Kcal/kg DM)	3,880.7	3,651.6	3,373	2,956	3,141	1,690.6	3,010.8	
Crud protein (%)	6.12	12.9	42.9	19.7	34.5	34	15.3	
Ether extract (%)	3.85	1.71	6.45	0.69	9.25	1.8	2.66	
Crud fiber (%)	2.68	3.32	7.85	10.1	10.5	21.4	9.78	
Ash (%)	1.03	2.4	5.7	3.45	9.38	6.5	5.33	
Ca (%)	0.04	0.03	1.02	2.83	2.31	0.90	0.91	32
P (%)	0.3	0.3	0.24	0.53	1.29	1.21	1.21	

DM= Dry matter, SBM=Soybean meal, RPSM=Roasted pigeon pea seed Meal, ME= Metabolizable energy NSC= noug seed cake, WB=Wheat bran, Ca= Calcium, P= Phosphors

Table 4. Chemical composition of treatment diets containing different level of processed pigeon pea seed meal on DM basis

Chemical composition	Treatments				
	T1	T2	T3	T4	T5
Dry matter (%)	92.4	92.4	92.5	92.4	92.4
ME(kcal/kg DM)	2896.8	2897.9	2882.3	2899.5	2892.1
Crud Protein (%)	16.9	16.9	16.6	16.7	16.6
L-Lysin HCL (%)	0.72	0.78	0.76	0.76	0.74
Methionin (%)	0.3	0.32	0.34	0.38	0.42
M+C (%)	0.61	0.63	0.67	0.72	0.77
Ether Extract (%)	2.99	2.91	2.94	3.07	3.19
Crud Fiber (%)	6.03	6.38	6.54	6.8	6.9
Ca (%)	4.08	3.97	4.11	4.03	4.2
P (%)	1.38	1.2	1.21	1.08	1.12

T1= 100% soybean meal (control); T2=25% roasted pigeon pea seed meal+ 75% soybean meal; T3= 50% roasted pigeon pea seed meal +50% soybean meal; T4=75% roasted pigeon pea seed meal + 25% soybean meal; T5= 100% roasted pigeon pea seed meal replacement to soybean meal; ME= Metabolizable energy; M+C= Methionin + Cystine; Ca= Calcium; P= Phosphors

Egg quality parameters

There was no significant ($p>0.05$) difference in all external and internal egg quality parameters among treatments by replacing soybean meal with RPSM in layer's diet as indicated in Table 5.

Table 5. External and internal egg quality parameters of Bovans brown layers fed dietary treatments

Parameters	Treatments					SEM	p-value
	T1	T2	T3	T4	T5		
Egg Weight (g)	58.74	58.91	58.79	58.34	59.04	0.16	0.7446

Comment [LS24]: Mention the p value

Comment [LS25]: add units for all parameters

Egg Width (mm)	40.42	39.98	40.19	40.10	40.31	0.06	0.1379
Egg Length (mm)	56.41	56.41	56.56	56.30	56.71	0.11	0.785
Shape Index (SHI)	72.22	72.44	71.33	71.25	71.26	0.31	0.5973
Shell weight (g)	5.63	5.55	5.49	5.64	5.78	0.035	0.0846
Shell Thickness (mm)	0.39	0.37	0.37	0.37	0.39	.004	0.2408
Albumin Weight (g)	34.79	34.62	35.14	35.14	35.07	0.16	0.773
Albumin Height	7.95	8.03	7.96	7.72	8.06	0.05	0.2456
Haugh Unit Score	89.54	90.00	89.60	88.23	90.10	0.28	0.1079
Yolk Weight (g)	15.48	15.42	15.28	15.20	15.93	0.09	0.217
Yolk Color	4.96	5.07	4.89	4.96	4.96	0.07	0.9385

T1= 100% soybean meal (control); T2=25% roasted pigeon pea+ 75% soybean meal; T3= 50% roasted pigeon pea +50% soybean meal; T4=75% roasted pigeon pea+ 25% soybean meal; T5= 100% roasted pigeon pea replacement to soybean meal; SEM = Standard Error of mean

Discussion

Chemical composition of feed ingredients and treatment diets

The Chemical Composition result of RPSM used in this study was 92.7% DM, 2956.3 kcal ME, 19.7% CP, 0.69% EE, 10.1% CF and 3.45% Ash as indicated in Table 3. The CP content of the RPSM in this experiment is found in the range of 17.9 - 24% CP content reported by [21] and [22]. In comparison with the result of [23]; [5]; [24] the current experiment shows the lowest crude protein content. The result of these authors was reported in the range of 22-27 % CP content by [23]; [24]. Similarly 25.4- 27.3 % of CP content was reported by [5]. The CF% of RPSM in the current experiment was similar with the proximate composition of RPSM results reported by [24] and [25] but higher value than the report of ([23];[5]). The reason for different results of nutritional value of pigeon pea seen by different authors may be come from the difference in

Comment [LS26]: Mention some recent researches

variety, growth condition, storage duration, soil, processing method and other reasons. These differences in results are supported by [26] that shows the protein content of pigeon pea cultivars vary between 17.9% up to 24.3% of CP. Feeds with $\geq 18\%$ protein content is classified as protein feeds. As a result pigeon pea seed meal is classified in the protein feed ingredients.

Comment [LS27]: Mention the references for it

There was no difference between the experimental diets in their proximate composition as they were formulated to be iso-caloric and iso-nitrogenous based [11] as indicated in Table 4. The experimental diets was prepared in the range of 2882.28 – 2899.53 kcal/kg DM metabolizable energy and 16.6% – 16.9% crude protein content with the recommended ratio of 2:1 up to 4:1 Ca:P ratio (FAO 2003). Both the ether extract and crude fiber percentages were thoroughly prepared to be less than the maximum tolerable level ($<8\%$) and the minimum requirement of layers for those critical essential amino acids and minerals was considered [11].

Comment [LS28]: Check for corrections

Comment [LS29]: correction

Egg quality parameters

The current result shows non-significant ($p>0.05$) difference in all external and internal egg quality parameters as indicated in Table 5. The current result was in contrast with the finding of [27] that describes feeding of 30% toasted pigeon pea seed meal to layers positively affect to produce eggs with better external and internal egg quality characteristics than the control diets. Generally, the differences in external and internal parameters among the present study and findings from different authors could be associated with the feed type, age of birds and strain of layers used in the experiment [28].

Egg weight

The average egg weight in this finding was 58.3-59.04 gram with a mean of 58.76 ± 1.96 gram which satisfy the standard for large size in United State and the medium size in Africa, respectively as presented in Table 5. The average egg weight of the present study was similar with the result obtained by [29] and [27]. But the result was higher than those obtained from autoclaved pigeon pea and soybean seed meal [25], Pigeon pea inclusion [5], Brewery Spent Grain Inclusion [30] and it was lower than from the result obtained by diet of pigeon pea seed

meal in layers [14], egg quality traits of Bovans Brown managed intensively [31] and rapeseed expeller cake inclusion [31] in layers diet. The different result of egg weight by different authors in different time could be due to environmental and nutritional factors [28]. Egg weight of the birds is significantly increased as the result of high protein consumption [32]; [33]. The standard check for eggs based on the weight varies from country to country.

Comment [LS30]: give more detailed discussion with recent researches

Egg shell quality

There was no significant difference in egg shell quality among the treatments as presented in Table 5. The shell weight in the current experiment was with the range of 5.49-5.78g and with a mean of $5.62 \pm 0.4g$ which shows similar result with [30] reported as 5.58-5.9 g and ([27]; [5]). But this result was below the finding of [29] which was in the range of 6.4-6.5 g. The environmental conditions, feed quality and breed could be the main factors for difference in egg shell quality [34]. The range of egg shell thickness in the current study was between 0.37-0.39 mm which is non-significant among treatments ([5]; [14]). This result was higher than the result found by Girma et al (2011) and [30] reported in the range of 0.29-0.3 mm and 0.32-0.34 mm, respectively. The differences in these results could be associated with the fact that shell thickness is closely correlated with the deposition of calcium which is metabolized from the skeleton of the birds and the dietary feed used.

Comment [LS31]: kindly give more clarity in discussion

Egg shape index

There was no statistically significant difference in egg shape index among the treatments since difference in egg width and length among the treatments was not present. It is because the shape index is the ratio of egg width to egg length [17] The standard eggs from hens had a shape index of 74% with blunt and pointed ends and elongated eggs have lower shape index while rounded eggs have higher value [36]. The average egg shape index result for this experiment was 71.70 ± 3.6 which is categorized in the sharp type but it approximate to the range of normal shape index value of 72-76% [36].

Comment [LS32]: Mention the p value

Egg albumen quality

There was no significant difference in albumen weight, albumen height and HU among the treatments as indicated in Table 5. Nutrition has minor impact on albumen quality, and the change in egg albumen quality is mostly related to age of the hen and egg storage conditions ([37]; [38]). As pointed by [38] albumen quality decreases as the age of egg increases and albumen height can be influenced by the age and strain of hen [39]. The range of albumen weight and albumen height in the present experiment was 34.62-35.14g and 7.72-8.06 mm, respectively. The result was in line with [29], but greater than the [30]. In case of albumen height similar range with [30] and greater result from [32] was observed. The higher average value of the albumen height in the experiment (7.95 ± 0.6) shows the better internal quality. The range of HUS in this result was 88.23 - 90.1 which was lower when compared with [29]; [30] and higher compared to the result from rapeseed replaced to soybean in layers feed [32]. The Average HU'S result from the current experiment was 89.49 ± 3.24 which shows rank of a good quality (70 -100HU) and ensures the USDA standard for AA quality and the higher the HUS, the better the quality of the egg (fresher, higher quality eggs have thicker whites) [18].

Comment [LS33]: Mention recent researches
With more detailed discussion

Egg yolk quality

There was no significant ($p>0.05$) differences in egg yolk weight, height and egg yolk color among treatments as presented in Table 5. Yolk weight obtained in the present experiment (15.20-15.93g) was higher than those reported by ([27] and [5]; [14]). Similar average yolk weight result of Bovans Brown layers was reported by [30] but the current yolk weight result was lower than the finding of [29]. The yolk color result of the current experiment in references to the RYCF was in the range of 4.96-5.07 (with average of 4.97 ± 0.76) which shows no significant ($p>0.05$) differences in yolk color among treatments. Since yolk color mainly influenced by diet [40], in the current experiment yellow maize was used as a major feed ingredient which contains carotenes and xanthophylls which can help in producing yellow colored yolk.

Comment [LS34]: Give more detailed information

Conclusion

To conclude, 75% of SBM could be replaced by RPSM in layers diet without any adverse effect on internal and external egg quality parameters. Therefore it can be concluded that RPSM would be a good alternative solution when the price of SBM become very expensive and limited access.

Comment [LS35]: Give more details of your findings

Reference

1. Ponnuel P, Narayanankutty K, Jalaludeen A, Anitha P. Economics of phytase enzyme supplementation in low energy-protein layer chicken diet. *International Journal of Livestock Production*. 2014 Jul 31; 5(7):113-6.
2. Sebho HK. Exotic chicken status, production performance and constraints in Ethiopia: a review. *Asian Journal of Poultry Science*. 2016; 10(1):30-9.
3. Girma M, Tamir B, Dessie T. Effects of replacing peanut seed cake with brewery dried yeast on laying performance, egg quality and carcass characteristics of rhode island red chicken. *Int. J. Poult. Sci*. 2012; 11:65-72.
4. Swain BK, Naik PK, Chakurkar EB, Singh NP. Effect of feeding brewers' dried grain on the performance of Japanese quail layers. *Indian Journal of Animal Nutrition*. 2013; 30(2):210-3.
5. Amaefule KU, Oguike MA, Ironkwe MO, Obioha FC. Pigeon pea [*Cajanuscajan* (L.) Millsp.] Seed meal in layer diets: 3. Effect of higher inclusion level and prolonged feeding of raw or processed pigeon pea seed

Comment [LS36]: Check the references

- meal diets from pullet chick stage on the laying performance of pullets. *International Journal of Poultry Science*. 2007; 6(6):452-8.
6. Orwa C. Agroforestry Database: a tree reference and selection guide, version 4.0. <http://www.worldagroforestry.org/sites/treedbs/treedatabases>. Asp. 2009.
 7. Odeny DA. The potential of pigeonpea (*Cajanuscajan* (L.) Millsp.) In Africa. In *Natural resources forum* 2007 Nov (Vol. 31, No. 4, pp. 297-305). Oxford, UK: Blackwell Publishing Ltd.
 8. Amaefule KU, Onwudike OC. Evaluation of processing methods of pigeonpea seeds (*Cajanuscajan*) as protein source for broiler starters.
 9. Onu PN, Okongwu SN. Performance characteristics and nutrient utilization of starter broilers fed raw and processed pigeon pea (*Cajanuscajan*) seed meal. *International Journal of Poultry Science*. 2006; 5(7):693-7.
 10. BOANR unpublished report 2020
 11. National Research Council, Subcommittee on Poultry Nutrition. *Nutrient requirements of poultry*: 1994. National Academies Press; 1994 Feb 1.
 12. Akande KE, Abubakar MM, Adegbola TA, Bogoro SE, Doma UD. Chemical evaluation of the nutritive quality of pigeon pea [*Cajanuscajan* (L.) Millsp.].
 13. Amaefule KU, Onwuchuruba CF, Okereke OC. Replacement value of soybean meal and maize with raw or boiled pigeon pea seed meal in exotic pullet diets. *Nigerian Journal of Animal Science*. 2013 Sep 11; 15:37-48.
 14. Amaefule KU, Oke UK, Obioha FC. Pigeon pea (*Cajanuscajan* (L.) Mill sp) seed meal in layer diets: 2. Laying performance and egg quality characteristics of pullets fed raw or processed pigeon pea seed meal diets during grower and layer stages of life. *International Journal of Poultry Science*. 2007; 6(6):445-51.
 15. Chemists AO. *Official Methods of Analysis: Changes in official methods of analysis made at the annual meeting*. Assoc. Off. Anal. Chem. 1990; 15:132-7.
 16. Wiseman J, Salvador F, Craigon J. Prediction of the apparent metabolizable energy content of fats fed to broiler chickens. *Poultry Science*. 1991 Jul 1; 70(7):1527-33.
 17. Anderson KE, Tharrington JB, Curtis PA, Jones FT. Shell characteristics of eggs from historic strains of single comb white leghorn chickens and the relationship of egg shape to shell strength. *International journal of poultry science*. 2004 Mar 24; 3(1):17-9.
 18. Haugh RR. The Haugh unit for measuring egg quality.
 19. Vuilleumier JP. The 'Roche Yolk Colour Fan'—An Instrument for Measuring Yolk Colour. *Poultry Science*. 1969 May 1; 48(3):767-79.
 20. Lohr SL. *SAS® Software companion for sampling: design and analysis*. Chapman and Hall/CRC; 2021 Nov 29.
 21. Emefiene ME, Joshua VI, Nwadike C, Yaroson AY, Zwalnan ND. Profitability analysis of Pigeon pea (*Cajanuscajan*) production in Riyom LGA of Plateau State. *International Letters of Natural Sciences*. 2014; 13(2).
 22. Emefiene ME, Joshua VI, Nwadike C, Yaroson AY, Zwalnan ND. Profitability analysis of Pigeon pea (*Cajanuscajan*) production in Riyom LGA of Plateau State. *International Letters of Natural Sciences*. 2014; 13(2).
 23. Akande KE, Abubakar MM, Adegbola TA, Bogoro SE, Doma UD. Chemical evaluation of the nutritive quality of pigeon pea [*Cajanuscajan* (L.) Millsp.].
 24. Arif M, Rehman A, Saeed M, EL-HACK ME, Alagawany M, Abbas H, Arian MA, Fazlani SA, Hussain AI, AYASAN T. Effect of different processing methods of pigeon pea (*Cajanuscajan*) on growth performance, carcass traits, and blood biochemical and hematological parameters of broiler chickens. *Turkish Journal of Veterinary & Animal Sciences*. 2017; 41(1):38-45
 25. Agwunobi LN. EFFECT OF FEEDING HEAT TREATED SOYBEANS (*Glycine Max*) AND PIGEON PEA (*Cajanus Cajan*) AS MAJOR SOURCES OF PROTEIN ON LAYER PERFORMANCE. *Global Journal of Pure and Applied Sciences*. 2014 Dec 5; 6(1):1-3.
 26. Akande KE. Dietary effects of increasing levels of pigeon pea meal on rabbit performance.

Comment [LS37]: 12 and 23 references are same

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Comment [LS42]: 21 and 22 are the same reference

Comment [LS43]: check

Comment [LS44]: check for format

Comment [LS45]: check

27. Amaefule KU, Ironkwe MC, Obioha FC. Pigeon pea [Cajanuscajan (L.) Millsp.] Seed meal in layer diets: 1. Performance of point of lay pullets fed raw or processed pigeon pea seed meal diets. *International Journal of Poultry Science*. 2006; 5(7):639-45.
28. Islam MA, Bulbul SM, Seeland G, Islam AB. Egg quality of different chicken genotypes in summer and winter. *Pakistan Journal of Biological Sciences*. 2001 Oct 15; 4(11):1411-4.
29. Girma M, Urge M, Animut G. Ground Prosopisjuliflora pod as feed ingredient in poultry diet: Effects on laying performance and egg quality. *International Journal of Poultry Science*. 2011; 10(11):879-85.
30. Gebremedhn B, Niguse M, Hagos B, Tesfamariam T, Kidane T, Berhe A, Gebresilassie L, Gebreegziabher L, Gebremariam T, Gebremeskel Y. Effects of dietary brewery spent grain inclusion on egg laying performance and quality parameters of bovans brown chickens. *Brazilian Journal of Poultry Science*. 2019 Aug 19; 21(02): eRBCA-2018.
31. Tadesse D, Esatu W, Girma M, Dessie T. Comparative study on some egg quality traits of exotic chickens in different production systems in East Shewa, Ethiopia. *African Journal of Agricultural Research*. 2015 Feb 26; 10(9):1016-21.
32. Swiatkiewicz S, Koreleski J, Arczewska-Wlosek A. Egg performance, egg quality, and nutrient utilization in laying hens fed diets with different levels of rapeseed expeller cake.
33. Almeida VR, Dias AN, Bueno CF, Couto FA, Rodrigues PA, Nogueira WC, Faria Filho DE. Crude protein and metabolizable energy levels for layers reared in hot climates. *Brazilian Journal of Poultry Science*. 2012; 14:203-8.
34. Rashid MM. Nutritional status of scavenging chickens with special emphasis on energy and protein supplementation under rural conditions in Bangladesh. Master of Science). Royal Veterinary and Agricultural University: Denmark. 2003.
35. Zita L, Tůmová E, Štolc L. Effects of genotype, age and their interaction on egg quality in brown-egg laying hens. *ActaVeterinaria Brno*. 2009; 78(1):85-91.
36. Ezenwosu C, Onyimonyi AE, Anizoba NW, Ugwu CM, Machebe NS, Yusuf M, Ezeokonkwo RC. Using sodium bentonite to reduce litter ammonia gas production and improve hen laying performance.
37. Şekeroğlu A, Sarıca M, Demir E, Ulutaş Z, Tilki M, Saatçi M. The effects of housing system and storage length on the quality of eggs produced by two lines of laying hens.
38. Rajkumar U, Sharma RP, Rajaravindra KS, Niranjan M, Reddy BL, Bhattacharya TK, Chatterjee RN. Effect of genotype and age on egg quality traits in naked neck chicken under tropical climate from India. *International Journal of Poultry Science*. 2009;8(12):1151-5.
39. Silversides FG, Scott AT. Effect of storage and layer age on quality of eggs from two lines of hens. *Poultry science*. 2001 Aug 1; 80(8):1240-5.
40. Galbert J, Sala R, Rincón-Carruyo X, Manzanilla EG, Vila B, Gasa J. Egg yolk color as affected by saponification of different natural pigmenting sources. *Journal of Applied Poultry Research*. 2004 Jul 1; 13(2):328-34.

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