

## **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 study aims to determine whether layer hens could substitute their soybean meal with roasted pigeon pea (*Cajanuscajan*) seed meal.

**Study design:** Five treatments and three replications in a complete randomized design (CRD)

**Place and Duration of the Study:** The study was conducted in Ethiopia's northwest Tigray, in the Shire Enda lassie district.

**Methodology:** The experimental diets were designed to have the same calorie and nitrogen content. A group of 105 commercial Bovans Brown (BB) egg-laying hens, 6 months old with an average body weight of (1412.04g ± 71.095g), were utilized for 90 days with a 10-day adaptation period. The treatments included rations with 0% (control or without roasted pigeon pea), 25%, 50%, 75%, and 100% roasted pigeon pea seed meal replaced by soybean meal for T1, T2, T3, T4, and T5, respectively. These treatment diets contained a metabolized energy range of 2882.28 - 2899.53 kcal/kg dry matter and 16.63 – 16.92% crude protein content. Data analysis was performed using the general linear model (GLM) of the SAS software.

**Results:** The results of egg quality parameters did not exhibit any significant ( $p > 0.05$ ) variation between the treatments. The average result of the experiment was egg weight 58.34 - 59.04 grams, shell weight 5.49 - 5.78g, and egg shape index  $71.70 \pm 3.6$ . 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. The yolk colour result using roch yolk colour fun was in the range of 4.96 - 5.07 which indicates a normal result.

**Conclusion:** According to the findings, roasted Pigeon pea seed meal could be replaced up to 75% of Soybean meal in the layer's diet without an adverse effect on the parameters of internal and external egg quality.

**Key words:** Egg, internal and external quality, Available local- feeds, pigeon pea, soya bean

### **Introduction**

Worldwide the cost of the poultry industry is rising because of the limited resources of cereals and oil cakes [1]. The high demand for cereal crops is becoming highly competitive since population increment is high in developing countries. In Africa like Ethiopia [2], feed shortages

are the main obstacle for small- scale poultry producers since the commercial layer feeds are highly expensive.

Hence, competition minimization for similar feed ingredients among livestock and humans is mandatory by replacing conventional feeds with locally accessible non-conventional feed resources. Mostly Soybean meal is ideal in amino acid profile to produce high protein feed ingredients for layers.

In Ethiopia especially in the Tigray region, since there is a low supply of soya beans **searching for alternative feed sources is required to reduce the feed cost [3, 4]. Therefore, the problem can be solved using unconventional protein plants such as pigeon pea (PSM)[5] as an alternative to replace expensive protein sources like soybean meal [6].**

As indicated by [7], PSM can be a good alternative in the poultry feed industry and it has multi advantages in feeding animals. As reported by [8]; and [9] PSM is a promising protein and energy source with a good content of essential minerals that is comparable to soybean. It is also known as a source of lysine since its protein content is excellent [6].

This indicates that protein feed ingredients like soya bean which are commonly used as protein sources in the poultry feed industry can be substituted with PSM. The anti-nutritional factors found in raw PSM can be treated by heat treatment simply [10]. Although PSM in the poultry industry is promising, little is known about how to use it as a feed ingredient especially how can it replace soybean meal and its effect on egg quality parameters of Bovans Brown (BB) layer hens.

Therefore, the research was designed to evaluate the egg quality parameters of BB layers by replacing soya bean meal with roasted Pigeon pea seed meal (RPSM).

## **Materials and methods**

### **Description of the study area**

The experiment took place in the northwestern Tigray region of Ethiopia, specifically in the Shire Enda lassie district.

## Feed ingredients and experimental ration

The ingredients for the feed formulation of the treatment were maize, Sorghum, pigeon pea seed, wheat bran, nuge seed cake, sesame seed cake, soybean meal, salt, limestone, L-lysineHcl and di-calcium phosphate. Except for the few feed components, all the cereal crops for the research were obtained from the local markets of Shire-Endaslassie. The ingredient L-lysine Hcl and Di-calcium phosphate were bought from Mekelle while the soya bean meal was from Addis Abeba. Before preparation of experimental rations, all the ingredients were crushed to 5 mm size uniformly to mix with Limestone, salt, L-lysin HCL and Di-calcium phosphate carefully. All the treatment diets were 2,800 – 2,900 kcal ME/kg DM and 16 - 17% CP content to fulfil the minimum standard requirements of layers [11]. The ration formulation was prepared by feed-win software for ration formulation. The experimental diets were formulated level by level as 0%, 25%, 50%, 75% and 100% roasted PSM replacement to the soya bean in T1 (control), T2, T3, T4 and T5, respectively.

## Roasting method and Source of Pigeon pea seeds

Matured white PSM was harvested from Shire-Maitsebri agricultural research center and the seed was roasted for 3-5 minutes at about 80 °C[12] till reached the required stage of frying,

Table 1. %arrangement of the feedstuff

Feed ingredients %	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-ca-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**

One hundred five Bovans Brown egg-laying hens of Six-month-old pullets with the same management and body weight were bought from the isolated chicken distributors. Their vaccination and feeding history were carefully recorded and the necessary preparations were taken pre-starting of the practical experiment. The whole experimental house with its pens, feeding and watering troughs was disinfected and cleaned thoroughly before starting the experiment.

The experimental pens were partitioned by 2.25 m<sup>2</sup> using wire mesh in the deep litter housing system. Additional 4 o'clock light to the normal daylight was supplied in the full experimental period. Then the light and dark cycle in twenty-four hours was sixteen to eight hours, respectively.

The size of seven cm - ten cm sawdust by-products was used as litter. The old and dirty litter was changed with fresh and disinfected new ones every month throughout the experiment. In the whole study, strict health precautions and disease control measures were maintained. The ration was daily dispensed via hanging tubular feeders, and birds had access to water through plastic fountains. Every morning, the troughs were cleaned for feeding and watering.

### **Experimental design and treatments**

In the Completely Randomized Design (CRD), five treatments were replicated three times with seven birds per replicate. Birds were assigned randomly to different diets. The experiment lasted 90 days with a 10-day adaptation period.

In the rations for T1, T2, T3, T4, and T5, soybean meal was replaced by pigeon pea seed meal at the amounts of 0%, 25%, 50%, 75%, and 100% respectively. The recommended percentages of pigeon pea replacement for soybean meal and the levels of inclusion in layers' diet vary among different authors [13], [14].

Table 2. Experimental Layout

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

RPSM: Roasted Pigeon pea Seed Meal; Rep: Replication; R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>: replication 1, 2, 3, respectively.

### Chemical analysis of the feed ingredients

Random samples were taken from all feed ingredients except premixes and salt for laboratory analysis before diet formulation. Chemical analysis was performed on samples taken from each treatment ration. Samples were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF), and ash following the Weende or proximate analysis methods [15]. Nitrogen (N) content was determined by the Kjeldahl procedure and crude protein (CP) was calculated as  $N \times 6.25$ . Chemical analyses of each feed ingredient and samples from the formulated feeds were conducted at Hawasa University nutrition laboratory. The experimental diets' metabolizable energy was assessed using an indirect method as described in [16].

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

## **Egg quality measurements**

Egg quality measurements for each parameter were done using nine eggs per treatment (three eggs per replication) weekly and the average value from each replication was computed for each quality parameter.

### **Egg weight and egg mass**

The Average weight of eggs was computed using the commonly used sensitive balance of 0.005-5kg weighing capacity by dividing the total weight of egg mass by the number of eggs laid.

Egg mass = HDEP\* Average egg weight in gram

### **Eggshell quality**

In poultry production, eggshell quality significantly impacts consumer acceptance. The weight and thickness of the eggshell were determined through its destruction. The shell thickness was determined by averaging the measurements of the blunt, middle, and sharp points of the egg using a digital caliper. The sensitive balance with a capacity of 0.005- 5kg was used to measure the eggshell weight.

### **Shape index**

The shape index was calculated based on the standard formula of [17] involving the ratio of width to length. Egg width and length were taken carefully from the sample eggs used for quality parameter measurements.

### **Egg albumen quality**

The eggs were carefully broken to separate the albumin and yolk. Then after, albumin and yolk were placed in their own pre-weighed Petri dish for individual weight measurement. First, the weight of a petri dish containing the albumin was measured, and then the albumin weight was determined using the weight difference between a petri dish with and without albumin. Simultaneously, albumen height was measured using the Electronic digital caliper. The Haugh unit (HU) measurement indicates higher egg quality, with a more desirable number reflecting fresher, thicker-white eggs [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**

Yolk quality was compared after careful separating of yolk and albumin in their pre-weighed Petri dish. Then the egg yolk with its Petri dish was weighed using the sensitive balance. Then the weight difference between the Petri dish with yolk and the empty Petri-dish was taken as yolk weight. Soon after taking the weight, the color of the yolk was compared with the Roche yolk color fan (RYCF) which is used to isolate the original yolk color by comparing the plastic strips of fifteen colors ordered from very pale yellow to a deep intense reddish orange [19].

The yolk sample was taken on a piece of white paper for comparison with RYCF after removing the yolk membrane and homogenizing the yolk. RYCF is crucial for defining the target yolk color and formulating the hens' feed accordingly.

### **Statistical model and data analysis**

Data analysis was conducted using the General Linear Model in SAS version 9.2 [20]. Tukey's HSD test was used to distinguish the significant differences ( $p \leq 0.05$ ) among the treatments' means. The experiment utilized the Gomez and Gomez (1984) model.

$$Y_{ij} = \mu + i + e_{ij};$$

Where,  $Y_{ij}$  = Response variable;  $\mu$  = overall mean;  $i = i^{\text{th}}$  treatment effect (1, 2, 3, 4, 5);  $E_{ij}$  = Random error effect

## Results

### Chemical composition of feedstuffs and treatment diets

The chemical compositions of each ingredient in the dietary rations for each treatment are outlined in Table 3.

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

Composition	Feedstuffs								SEM	P-value
	Maize	Sorghum	SBM	RPSM	SSC	NSC	WB	Lime stone		
DM (%)	91.03	90.9	92.7	92.7	91.5	92.7	90.9	99	0.19	0.001
ME(Kcal/kg DM)	3,880.7	3,651.6	3,373	2,956	3,141	1,690.6	3,0108		0.31	0.001
CP (%)	6.12	12.9	42.9	19.7	34.5	34	15.3		0.05	0.001
EE (%)	3.85	1.71	6.45	0.69	9.25	1.8	2.66		0.02	0.001
CF (%)	2.68	3.32	7.85	10.1	10.5	21.4	9.78		0.04	0.001
Ash (%)	1.03	2.4	5.7	3.45	9.38	6.5	5.33		0.03	0.001
Ca (%)	0.04	0.03	1.02	2.83	2.31	0.90	0.91	32	0.21	0.001
P (%)	0.3	0.3	0.24	0.53	1.29	1.21	1.21		0.03	0.001

DM= Dry Matter, ME= Metabolizable Energy; Kcal= kilo calorie; kg= kilo gram; CP= Crude Protein; EE= Ether Extract;

CF= Crude Fiber; Ca= Calcium, P= Phosphorus; SBM= Soya bean Meal, RPSM= Roasted Pigeon pea Seed Meal, NSC= Noug Seed Cake, WB= Wheat bran

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

Chemical composition	Treatments					SEM	P- value
	T1	T2	T3	T4	T5		
DM (%)	92.4	92.4	92.5	92.4	92.4	0.144	0.98
ME(kcal/kg DM)	2896.8	2897.9	2882.3	2899.5	2892.1	0.063	0.001
CP( %)	16.9	16.9	16.6	16.7	16.6	0.052	0.005
L-Lysin HCL (%)	0.72	0.78	0.76	0.76	0.74	0.005	0.001
Methionine (%)	0.3	0.32	0.34	0.38	0.42	0.028	0.084
M+C ( %)	0.61	0.63	0.67	0.72	0.77	0.006	0.001
EE ( % )	2.99	2.91	2.94	3.07	3.19	0.000	0.001
CF (%)	6.03	6.38	6.54	6.8	6.9	0.029	0.001
Ca (%)	4.08	3.97	4.11	4.03	4.2	0.005	0.001
P (%)	1.38	1.2	1.21	1.08	1.12	0.023	0.001

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; DM= Dry Matter; ME= Metabolizable energy; CP = Crude Protein

Protein; M+C= Methionine + Cystine; EE= Ether Extract; CF= Crud Fiber; Ca= Calcium; P = Phosphors

### Egg quality parameters

According to Table 5, replacing soybean meal with RPSM in the layer's diet had no statistically significant impact on any internal or external egg quality parameter ( $p>0.05$ ).

Table 5. External and internal egg quality parameters of BB layer-fed dietary treatments

Parameters	Treatments					SEM	p-value
	T1	T2	T3	T4	T5		
EW (g)	58.74	58.91	58.79	58.34	59.04	0.16	0.7446
EWth (mm)	40.42	39.98	40.19	40.10	40.31	0.06	0.1379
EL (mm)	56.41	56.41	56.56	56.30	56.71	0.11	0.785
SI	72.22	72.44	71.33	71.25	71.26	0.31	0.5973
SW (g)	5.63	5.55	5.49	5.64	5.78	0.035	0.0846
ST (mm)	0.39	0.37	0.37	0.37	0.39	.004	0.2408
AW (g)	34.79	34.62	35.14	35.14	35.07	0.16	0.773
AH(cm)	7.95	8.03	7.96	7.72	8.06	0.05	0.2456
HUS	89.54	90.00	89.60	88.23	90.10	0.28	0.1079
YW (g)	15.48	15.42	15.28	15.20	15.93	0.09	0.217
Y C	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; EW= Egg weight; EWth= Egg width; EL= Egg length; SI= Shape index; SW= Shell weight; ST= Shell thickness; AW= Albumin weight; AH= Albumin height; HUS= Haugh Unit Score; YW= Yolk weight; YC= Yolk color

## **Discussion**

### **Chemical composition of feed ingredients and treatment diets**

The chemical composition of RPSM, as shown in Table 3, was 92.7% dry matter, 2956.3 kcal metabolic energy, 19.7% crude protein, 0.69% ether extract, 10.1% crude fibre, and 3.45% ash. According to [21] and [22], the RPSM used in this study contained CP levels between 17.9% and 24%. The current experiment shows the lowest crude protein content compared to the studies explored by [12], [5], and [23]. The experiments by [6], [12], and [23] reported CP content ranging from 22 to 29%. As reported by [5], the CP content of Pigeon pea can reach up to 25.4 to 27.3 %. The CF% of the RPSM used in the current study is in line with the result reported by [23] and [24], but exceeded the values from [12] and [5]. The nutritional value of pigeon peas can vary among studies due to different factors like variety, growth condition, storage duration, soil, processing method, and more. According to [25], the protein content of pigeon pea cultivars ranges from 17.9% to 24.3% which strengthens the idea that pigeon pea seed meal falls under the category of protein feed ingredients [5] since its protein content exceeding 18% [26]. 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 diets were ranged from 2882.28-2899.53 kcal/kg DM and 16.6%-16.9% CP, with a recommended Ca:P ratio of 2:1 to 4:1. The prepared amount of EE% and CF% was below the maximum tolerable level (<8%) while ensuring the minimum requirement for critical essential amino acids and minerals [11].

### **Egg quality parameters**

As indicated in Table 5, there were no significant ( $p>0.05$ ) differences for the internal and external egg quality parameters among all treatments. As reported by [27] 30% toasted Pigeon pea seed meal inclusion in layer diets enhances superior egg quality over control diets which contrasts with the current finding that shows no difference at all. Generally, the differences in external and internal egg quality parameters among the present study and findings from different authors may come from the type of feed, bird's age and varieties of layer hens used in the experiment [28].

### **Egg weight**

As indicated in Table 5, the average egg weight in this finding was 58.3-59.04 grams with a mean of  $58.76 \pm 1.96$  grams which satisfies the standard for large size in the United States and medium size in Africa, respectively. The current result of this finding is in line with the average egg weight reported by [29] and [27] and in contrast with the result of egg weight obtained from autoclaved pigeon pea and soybean seed meal [24], up to 30% inclusion of raw and processed Pigeon pea [5] and Brewery spent grain inclusion [30]. The egg weight of the current experiment was lower than the result obtained by feeding pigeon pea seed meal in layers [14], egg quality traits of Bovans Brown managed intensively [31] and rapeseed expeller cake inclusion [32] in layers diet. The different results of egg weight by different authors at different times could be due to environmental and nutritional factors [28]. Consuming a high-protein diet led to a significant increase in egg weight [33]; [34]. The acceptable standard weight of eggs varies from country to country.

### **Eggshell quality**

According to Table 5, eggshell quality did not vary significantly among the treatments. The eggshell weight in the current experiment was in the range of 5.49 -5.78g with a mean of  $5.62 \pm 0.4$ g which shows a similar result with [30] reported as 5.58-5.9 g and a similar result with

the finding of ([27]; [5]). But this result was below the finding of [29] which was in the range of 6.4-6.5g. The environmental conditions, feed quality and breed could be the main factors for differences in eggshell quality [35]. The range of eggshell thickness in the current study was between 0.37-0.39 mm which is in line with the findings of ([5]; [14]) but higher than the result found by [29] and [30] reported in the range of 0.29-0.3 mm and 0.32-0.34 mm, respectively. The correlation between shell thickness and calcium deposition from bird skeletons and diet affects the variation in eggshell quality.

### **Egg shape index**

The treatment groups did not vary significantly ( $p > 0.05$ ) in their resulting egg shapes due to a minor variation in egg width and length. The shape index, defined as the ratio of egg width to egg length, determines [17] the character of the eggs. As indicated by [36] 74% egg shape index with blunt and pointed ends is taken as standard while the elongated and rounded eggs have lower and higher shape index values, respectively.

The average egg shape index result for this experiment was  $71.70 \pm 3.6$  which is categorized in the sharp type but it approximates the range of normal shape index value of 72-76% [36].

### **Egg albumen quality**

According to Table 5, no significant ( $p > 0.05$ ) differences were observed in albumin quality, including weight, height, and HU among the treatments. Albumen quality is mostly affected by the hen's age and egg storage conditions, with nutrition playing a minor role ([37]; [38]). As pointed out by [38] albumen quality decreases as the age of the egg increases and albumen height can be influenced by the age and strain of hen [39]. The range of albumin weight and albumin 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 the case of albumin height similar range with [30] and greater result from [32] was observed. The higher average value of the albumen height in the experiment ( $7.95 \pm 0.6$ ) shows better internal quality. The result of HU in the finding was 88.23 - 90.1 which was lower when compared with [29]; [30] and higher compared to the result from rapeseed replaced with soybean in layers feed [32]. The Average HU result from the current experiment was  $89.49 \pm 3.24$  which shows a rank of good quality (70 -100HU) and ensures the standard for the United States Department of Agriculture for AA quality and the

higher the HU, the better the quality of the egg (fresher, higher quality eggs have thicker whites) [18].

### **Egg yolk quality**

As pointed out in Table 5, there were no significant ( $p>0.05$ ) differences in egg yolk quality (yolk weight, yolk height and egg yolk color) resulting from the difference in experiments diets. The Yolk weight obtained in the present experiment (15.20-15.93g) was higher than those reported by ([27] and [5]; [14]). A 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 about the Roch Yolk Color Fan (RYCF) was in the range of 4.96-5.07 (with an average of  $4.97\pm 0.76$ ) which shows non-significant ( $p>0.05$ ) differences in yolk color among treatments. Since yolk color is mainly influenced by diet [40], in the current experiment, yellow maize was used as a major feed ingredient which contains carotenes and xanthophyll which can help in producing yellow-colored yolk.

### **Conclusion**

Replacing 75% of soya bean meal with RPSM in the treatment diet yields equivalent egg quality parameters as the fully soya bean meal diet (controlled treatment), without any negative impact on either internal or external egg quality. When soybean meal prices are high and access is limited, roasted pigeon pea seed meal can function as a suitable replacement. Poultry feed producers can save costs by using up to 75% roasted pigeon pea seed meal instead of soybean meal in layer diets. Finally, it is essential to carry out additional research on using roasted pigeon pea seed meal as a replacement for soybean meal in chicken diets, from chick stage to layers.

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