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Potential of heat-treated dehulled seed of *Lupinus angustifolius* on egg production, feed intake and egg quality characteristics in commercial chickens

Potencial de la semilla de *Lupinus angustifolius*, descascarada y tratada con calor, para la producción de huevos, consumo de alimento y calidad del huevo en pollos comerciales

Alem Dida[1,2], Aberra Melesse[1*]

[1]*Hawassa University, College of Agriculture, School of Animal and Range Sciences, Ethiopia*
[2]*Selale University, College of Agriculture and Natural Resource, Department of Animal Science, Ethiopia*

[*]*Corresponding author: a_melesse@uni-hohenheim.de*

This study was conducted to investigate the effect of substitution of soybean meal as partial replacement of heat-treated-dehulled lupin (*Lupinus angustifolius* meal, HDL) on the productivity and egg quality from Lohmann Brown laying hens. One hundred sixty laying hens were randomly assigned to four treatment diets replicated four times with ten birds each. The dietary treatments were the control diet without HDL (HDL0) and diets containing HDL at 9 % (HDL9), 18 % (HDL18) and 27 % (HDL27). The results indicated that egg production, egg weight, egg mass and feed conversion ratio did not change due to the effect of the experimental groups, except for feed intake. Hens fed HDL0 had higher (P<0.05) feed intake than those fed HDL27 diet. Hen-housed egg production was recorded at 48.3 %, 47.3 %, 50.5 %, and 47.0 % for the HDL0, HDL9, HDL18, and HDL27 diets, respectively. The corresponding average daily feed intake for hens fed on HDL0, HDL9, HDL18, and HDL27 diets was 107 g, 106 g, 105 g, and 102 g. Egg mass per hen for these diets measured 2.84 kg, 2.74 kg, 2.97 kg and 2.72 kg, in that order. The feed conversion ratio (kg feed/kg egg mass) ranged from 3.94 with HDL18 to 4.23 with HDL27 diets. Internal egg qualities were not significantly influenced by the dietary treatments. Dry shell weight, eggshell ratio, and density for hens receiving HDL9 and HDL18 diets were comparable to those observed in the control group. Egg weight, egg length, egg width, yolk height, yolk width, albumen height, and Haugh unit score increased with advancing age (P<0.05). In conclusion, replacing soybean with up to 18 % HDL resulted in observable effects on the nutritional quality and performance metrics of hens.

Keywords: *egg production, egg quality, feed intake, laying hen, processed sweet lupin*

Este estudio se realizó para investigar el efecto de la sustitución de harina de soja, como reemplazo parcial de lupino (harina de *Lupinus angustifolius*, HDL) tratado con calor y descascarado, en la productividad y calidad de los huevos de gallinas ponedoras Lohmann Brown. Se asignaron aleatoriamente ciento sesenta gallinas ponedoras a cuatro dietas de tratamiento, replicadas cuatro veces con diez aves cada una. Los tratamientos fueron la dieta control sin HDL (HDL0) y dietas que contenían HDL al 9 % (HDL9), 18 % (HDL18) y 27 % (HDL27). Los resultados indicaron que la producción de huevos, el peso de los huevos, la masa de huevos y la relación de conversión alimenticia no cambiaron debido al efecto de los grupos experimentales, excepto el consumo de alimento. Las gallinas alimentadas con HDL0 tuvieron un mayor (P<0.05) consumo de alimento que aquellas alimentadas con la dieta HDL27. La producción de huevo por gallina en el galpón se registró en 48.3 %, 47.3 %, 50.5 % y 47.0 % para las dietas HDL0, HDL9, HDL18 y HDL27, respectivamente. El consumo de alimento promedio diario de las gallinas alimentadas con dietas HDL0, HDL9, HDL18 y HDL27 fue de 107 g, 106 g, 105 g y 102 g, respectivamente. La masa de huevo por gallina para estas dietas fue de 2.84 kg, 2.74 kg, 2.97 kg y 2.72 kg, en ese orden. La relación de conversión alimenticia (kg de alimento/kg de masa de huevo) varió de 3.94 con la dieta HDL18 a 4.23 con la dieta HDL27. Las cualidades internas del huevo no tuvieron influencia significativa de los tratamientos dietéticos. El peso seco de la cáscara, la proporción de cáscara y la densidad en las gallinas que recibieron dietas HDL9 y HDL18 fueron comparables a los observados en el grupo control. El peso del huevo, la longitud del huevo, el ancho del huevo, la altura de la yema, el ancho de la yema, la altura de la clara y la puntuación de la unidad Haugh aumentaron con la edad (P<0.05). En conclusión, reemplazar la soja con hasta un 18 % de HDL resultó en efectos observables en la calidad nutricional y los parámetros de rendimiento de las gallinas.

Palabras clave: *calidad del huevo, consumo de alimento, gallina ponedora, producción de huevos, lupino dulce procesado*

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Introduction

Poultry feed accounts for over 70 % of production costs, with many developing countries facing higher expenses due to reliance on imported protein sources like soybean meal (Juodka *et al*. 2017, Melesse *et al*. 2019 and Struti *et al*. 2021). Soybean meal is valued for its balanced amino acids and typically comprises 20-33 % of poultry diets, though rising global demand has made it less affordable for smallholders (Mabusela *et al*. 2018 and Struti *et al*. 2020). This warrants exploration of alternative protein sources with comparable nutrition that could reduce costs (Melesse *et al*. 2019 and Al-Sagan *et al*. 2020). Lupin seed is a promising substitute but has been limited by antinutritional factors, primarily quinolizidine alkaloids such as lupinine and sparteine (Abraham *et al*. 2019). Advances in breeding have produced low-alkaloid varieties, including sweet blue lupin (*L. angustifolius*), which contains less than 0.05 % alkaloids and can replace soybean meal in poultry diets without harming egg production (Kasprowicz-Potocka *et al*. 2016 and Abraham *et al*. 2019). Lupins offer higher levels of several essential amino acids than soybean, though they contain less methionine and cysteine (Amir *et al*. 2012, Tadele 2015 and Kasprowicz-Potocka *et al*. 2016).

Removing the hull using heat treatment has been considered a suitable strategy to increase the supplementation level of lupin in the laying hens' diet (Struti *et al*. 2020, 2021, Ferchichi *et al*. 2021 and Ayalew *et al*. 2024). The combination application of heating and dehulling of sweet lupin seed could degrade non-structural polysaccharides and anti-nutritional properties while increasing the protein and crude fat levels up to 51.08 % and 11.90 %, respectively and the fiber content has considerably decreased by 4.40 % (Timová *et al*. 2020 and Struti *et al*. 2020, 2021). Uzun and Agma Okur (2023) reported that heat treatment at 130 °C for 20 min were applied to white and blue lupin seeds without causing any denaturing effect on protein content of processed lupin. Al-Amrousi *et al*. (2022) reported that roasting of both seat and bitter lupin seeds at 180 °C for a short time (10 min) enhanced the minor bioactive components. Straková *et al*. (2021) revealed that the replacement of soybean with lupin has resulted in a significant reduction of total cholesterol and triglyceride levels in the chicken's blood plasma making it suitable feed source with a positive impact on consumers' health. Moreover, egg yolks with low cholesterol levels were produced due the feeding of white lupine seeds meal (Timová *et al*. 2020).

Several research works have evaluated the nutritional value of sweet lupin seed in the growth and feed consumption performances of broiler chickens (Struti *et al*. 2020, 2021, Siger *et al*. 2023 and Dida and Melesse 2024). However, there are limited information available in the literature reporting on the potential of heat-treated dehulled sweet lupin (*L. angustifolius*) seed meal on feed intake and egg production and egg quality characteristics under tropical production environments. It was hypothesized that substituting soybean meal with varying proportions of heat-treated dehulled lupin seed meal will not significantly impact feed intake, egg production, or egg quality characteristics. Therefore, the current study aimed at determining the effect of replacement of soybean meal with heat-treated dehulled sweet blue lupin on feed consumption, egg production and egg quality traits of Lohmann brown layer chicken breeds reared in tropical environment.

Materials and Methods

Preparation of sweet lupin seeds: The raw seed was roasted using a pan for 15 minutes at a temperature of 130 °C and then cooled and dehulled using a roller mill and exposed to the air for completed separation of the cotyledons from hulls. Hammer mill was used to grind the roasted seed as described by Laudadio and Tufarelli (2011) to produce the meal which is referred hereafter as heat-treated dehulled lupin (HDL).

Research design and birds' management: Total of 160 Lohmann pre-lay stage pullets of 16 weeks age was obtained from the Alema Farms PLC (Bishoftu, Ethiopia). They were kept at the experimental site until they start laying eggs during which they were fed with pre-layer commercial ration. The actual experiment commenced when the pullets were 19 weeks age. Four dietary treatments were formulated to contain 0 % HDL (HDL0), 9 % HDL (HDL9), 18 % HDL (HDL18) and 27 % HDL (HDL27) (table 1). The hens were then randomly distributed to each treatment diets replicated four times containing 10 chicks each. The treatment diets were formulated to be isocaloric and isonitrogenous with similar energy density and protein levels (table 2).

The experimental pens were cleaned, disinfected, and watering and feeding equipment were cleaned and disinfected as well. The birds were kept in 1.5 m x 1.65m wire mesh partitioned pens and the concrete floor was covered with sawdust of about 5 cm deep. The wet litter was replaced with dried and cleaned sawdust whenever necessary. Sunlight was used as a source of natural light during the day period, and additional supplementary fluorescent light were used at evening for a total of about 16 hours. Water was provided *ad libitum*. A 20 % amprolium powder solution (12 g per 20 litres) was administered through drinking water for 5 to 7 days as a prophylactic measure against coccidiosis.

Data collection procedures: Data collection on egg production commenced when the birds were 22 weeks old and continued until they reached 37 weeks of age, spanning 16 consecutive weeks. The birds were provided with their respective dietary treatments twice daily: once in the morning at 08:00 and again in the afternoon at 16:00. The daily weighed allowance was divided into two equal portions. Feed refusal was collected the following day before the next feeding is offered. Feed intake was calculated by subtracting the feed refusal from the offered feed.

Eggs were collected twice a day from each pen at 08:00 and 17:00. As there was no mortality recorded during the experimental period, egg production was determined on a hen-housed basis, considering the number of hens initially housed. The weight of each egg was recorded daily and averaged. Total egg mass output was computed by multiplying the total number of eggs produced during the experimental period with the average weight of these eggs. The feed conversion ratio (kg feed/kg egg mass) was then calculated by dividing the total feed consumed by the total egg mass produced.

The egg quality characteristics were measured at four age points. The first, second, third and fourth egg quality characteristics were determined at the ages of 24, 28, and 32 and 36 weeks, respectively. Eggs were collected for five consecutive days at each age point and stored in a cool room. Individual eggs and dry shell weight were measured using a triple beam balance. The egg length, egg width, and yolk diameter were measured with a digital calliper. A tripod micrometre gauge was used to measure the heights of albumen and yolk. The yolk colour was determined using the Rosche Yolk Colour Fan. The Haugh unit (HU) score was calculated according to Eisen *et al.* (1962) by applying the average albumen height and egg weight into the equation: HU = 100log (albumen height + 7.57 − 1.7 * egg weight$^{0.37}$). The yolk index was calculated by dividing the yolk height by its width.

The egg dimensions, length (L) and width (W) were used to compute the following geometrical parameters: Egg shape index was calculated as the ratio of W to L multiplied by 100. The surface area of the egg was computed using the method of Narushin *et al.* (2021a) as 0.933W (W+2.343L). The proportion of shell was determined by dividing the dried shell weight by the egg weight. The eggshell density was calculated as follows:

$$\text{Egg shell density}\left(\text{g/cm}^2\right) = \frac{\text{dry shell weight}}{\text{egg surface area}}$$

Table 1. Inclusion rates of feed ingredients in the formulation of the experimental diets (%)

Feed ingredients	Levels of heat-treated dehulled lupin			
	HDL0	HDL9	HDL18	HDL27
Maize grain	34.5	31.0	30.0	26.1
Wheat grain	6.00	6.00	6.00	6.00
Wheat short	25.1	27.1	27.1	29.0
Soybean meal	25.5	18.0	10.0	3.0
Heat-treated dehulled lupin	0.0	9.0	18.0	27.0
Limestone powder	7.5	7.5	7.5	7.5
Vitamin premix	0.40	0.40	0.40	0.40
Salt	0.50	0.50	0.50	0.50
Lysine	0.32	0.32	0.32	0.32
Methionine	0.35	0.35	0.35	0.35
Total	100	100	100	100

Table 2. Analysed proximate composition of feed ingredients and calculated nutrient concentration of dietary treatment diets (%, as fed basis)

Items	Ash	CP	EE	CF	Ca	P	ME (kcal/kg)
Analysed ingredients							
Maize	4.30	8.01	4.30	3.10	0.03	0.42	2800
Soybean meal	10.3	43.1	12.5	7.80	0.23	0.62	3500
Wheat grain	4.10	13.4	9.10	5.20	0.07	0.34	2947
Wheat short	8.80	15.6	3.30	13.0	0.04	0.70	2621
Heat-treated dehulled lupin	8.40	37.1	12.9	11.2	0.09	0.40	3316
Limestone	-	-	-	-	38.0	0.04	-
Vitamin premix	-	-	-	-	7.12	-	-
Calculated diets							
HDL0	6.56	18.5	4.96	6.63	3.12	0.59	2756
HDL9	6.57	18.6	6.18	7.21	3.11	0.49	2775
HDL18	6.46	18.4	6.30	7.56	3.10	0.47	2814
HDL27	6.50	18.7	6.48	8.15	3.09	0.46	2830

CP: crude protein, EE: ether extract, CF: crude fiber, Ca: calcium, P: phosphorous, ME: metabolizable energy

Chemical composition analysis: Samples of the soybean meal, the heat-treated dehulled lupin seed meal and feeds offered were analysed for dry matter, ash, ether extract, and crude fiber using the procedures of AOAC (1995) at the Animal Nutrition Laboratory of Hawassa University. The crude protein, calcium, and phosphorus were analysed at the Holeta Agricultural Research Centre (Ethiopia). The crude protein content was analysed using the micro-Kjeldahl method while the concentrations of calcium and phosphorous were determined by atomic absorption spectrophotometer (dry ashing) as described by AOAC (1995). The metabolizable energy of diets was estimated based on the feed composition tables of tropical feeds for poultry, while that of heat-treated dehulled lupin was computed using the equation of Wiseman (1987).

Statistical Analysis: Data on feed intake, egg production, egg weight, egg mass and feed conversion ratio were analysed using one-way ANOVA by considering the effects of treatment diets as a single fixed factor. Since the interaction effects of treatment by age was not significant for all egg quality characteristics, it was dropped from the statistical analysis. Accordingly, the data for egg qualities measured at four age points were pooled and subjected to one-way ANOVA by fitting treatment diets as a single factor effect. The significance of mean differences was analysed using the post-hoc Tukey test. To check the age effect, data on treatment diets for egg qualities were further pooled and subjected to General Linear procedure (due to missing values) by fitting age as a single factor. The Tukey-Kramer test for adjusted means was then applied to compare the least square means of the age effect. Additionally, the orthogonal polynomial contrast trend analysis was conducted to determine the linear and quadratic effects on increasing levels of the dietary lupin substitutions. All statistical analysis was performed using the Statistical Analysis System (SAS 2016, ver. 9.4) and results are presented in least square means along with pooled standard error of the mean.

Results and Discussion

Feed intake and egg production: As presented in table 2, the fat content and metabolizable energy concentration in HDL was comparable to that of soybean meal (12.9 % in HDL vs. 12.5 % in soybean meal and 3316 kcal/kg in HDL vs. 3500 kcal/kg in soybean meal). As shown in table 3, the mean values for total egg number, egg weight and egg mass were similar (P>0.05) across dietary treatments which accords with the reports of Lee *et al.* (2016a). Furthermore, the findings align with those of Struti *et al.* (2021), who reported that incorporating dehulled lupin seeds into the diet of quails did not affect egg production compared to the control group. Kowalska *et al.* (2020) reported that a 10-20 % inclusion of lupin seeds with a 10 % inclusion of peas had no negative effect on most egg production traits. However, they reported that the highest dose of narrow-leaved lupin (25 %) in the diet was associated with reduced weight of eggs.

The egg mass has linearly decreased (P=0.03) with increased substitution levels of soybean meal with HDL the highest reduction being observed at 27 %. Egg mass is the product of total egg number and average egg weight, and the observed reduction might be associated with low levels of essential amino acids in the lupin seed. Researchers have revealed that most lupin species exhibit a significant deficiency in sulphur-containing amino acids, primarily methionine, which is essential for the synthesis of cysteine and phenylalanine (Roman *et al.* 2023 and Kponouglo *et al.* 2024).

On the other hand, diets containing graded levels of heat-treated dehulled lupin seed meal had affected the total and average daily feed intake of laying hens. Consequently, the substitution of soybean meal at 27 % of heat-treated dehulled lupin seed meal has resulted in a decrease (P<0.039) of feed intake in comparison to the remaining treatments. Similarly, total feed intake linearly decreased across treatment groups (P=0.044).

Table 3. Least square means of egg production, feed intake and feed conversion ratio of hens fed with graded heat-treated dehulled lupin seed meal as partial substitution for soybean meal

Variables	Substitution levels (%)				SEM (±)	P-values		
	HDL0	HDL9	HDL18	HDL27		ANOVA	Linear	Quadratic
Total egg number (per hen)	54.1	52.9	56.5	52.6	1.46	0.278	0.895	0.090
Hen-housed egg production (%)	48.3	47.3	50.5	47.0	1.30	0.275	0.882	0.890
Egg weight (g)	52.5	51.9	52.5	51.7	0.33	0.276	0.767	0.145
Total egg mass (kg/hen)	2.84	2.74	2.97	2.72	0.08	0.178	0.030	0.065
Total feed intake (kg/hen)	11.8[a]	11.8[a]	11.7[ab]	11.4[b]	0.09	0.039	0.044	0.256
Average daily feed intake (g/hen)	107[a]	106[ab]	105[ab]	102[b]	2.34	0.045	0.056	0.398
FCR (kg feed/kg egg mass)	4.18	4.29	3.96	4.23	0.12	0.317	0.741	0.130

[a-c]Means with different superscript letters across treatment diets are significant at p<0.05. FCR: feed conversion ratio, SEM: standard error of the mean

None of the variables showed a significant trend of a quadratic effect. Consistent with the current results, Rutkowski *et al.* (2017) observed depressed feed intake and poor feed conversion ratio in Hy-Line Brown hens fed diet with 25 % yellow lupin seeds. The reduction in feed consumption may be attributed to the presence of alkaloids and oligosaccharides in lupine seeds because high levels of alkaloids can markedly reduce protein digestibility (Jeroch *et al.* 2016). Moreover, Pham *et al.* (2020) reported that *L. angustifolius* seeds contained higher levels of structural carbohydrates, which may have negatively influenced the feed consumption of the hens.

On the other hand, Park *et al.* (2016) reported that the increased levels of non-processed lupin seeds in the diets of laying hens had positively influenced the average daily feed intake. They revealed that the daily feed intake of birds fed with lupin diets has increased at week six as compared with the control group. In another study, the substitution of soybean meal at 7, 10, and 15 % for dehulled lupin seed meal improved egg production and egg weight output per hen as compared to control diet (Andrianova *et al.* 2019). The differences reported by varies researchers on the effect of lupin seed on feed consumption might be associated with the seed variety, physical activity of the chickens, types of housing system used and the environmental conditions in which the birdy were raised.

Egg quality characteristics: As shown in table 4, egg width, dry shell weight, shell ratio and shell density were affected ($P<0.05$) by treatments diets being higher in hens fed with HDL0 diet than that of HDL27. No significant differences were observed between chickens fed with HDL0, HDL9, and HDL18 diets in terms of the same characteristics. However, egg width was significantly higher ($P<0.05$) in hens fed with the HDL0 diet than those fed the HDL18 and HDL27 diets. A linear decrease ($P<0.015$) was observed in shell weight, eggshell ratio, and eggshell density across the treatment diets.

In the current study, egg shape index was not affected by treatment diets which disagrees with the observations of Kowalska *et al.* (2021) who reported that the egg shape index in narrow-leaved lupin fed group was higher than the control group. The observed reduction in the dry shell weight in chickens fed with treatment diets is in line with that of Rutkowski *et al.* (2017) who reported a deterioration trend of egg quality traits with the increased levels of non-processed yellow lupin (*Lupinus luteus* L.) inclusion in poultry diets. They observed that a 10-25 % inclusion of lupin reduced the proportion of eggshell in each nutritional group. Eggshell is made almost entirely of calcium carbonate ($CaCO_3$) crystals. The supply of adequate amounts of mineral calcium in layer hens' diet is thus essential. The calcium content in heat-treated dehulled lupin was lower than that of soybean meal and this may be attributed to the dehulling process, as calcium is predominantly deposited in the hull, resulting in reduced concentration after dehulling (Park *et al.* 2016). The observed egg shape index score agrees with the findings of Kponouglo *et al.* (2024) who reported comparative values for hens fed fermented lupin seed. In the poultry industry, egg shape index is considered as one of the important characteristics for uniformed packages of eggs during transportation and delivery by decreasing the potential of breakage eggs. An egg shape index of 75 is regarded as the most satisfactory which accords to the present findings.

The quality of albumen and yolk is of particular interest to the consumers of poultry products (Tolimir *et al.* 2017). The quality of both albumen and yolk is expressed by proportion and index (Kraus *et al.* 2019 and 2021). Haugh unit scores are a crucial parameter for assessing albumen quality, which helps to determine the overall quality of egg content and its freshness (Narushin *et al.* 2021b). As presented in table 5, no significant difference was observed between treatment diets in all internal egg quality characteristics which is consistent with the observations of Park *et al.* (2016) and Kponouglo *et al.* (2024). Similarly, the orthogonal polynomial contrast analysis did not show any linear or quadratic effects on treatment diets for internal qualities.

Table 4. Least square means of external qualities in layer hens fed with different levels of heat-treated dehulled lupin by partially substituting the soybean meal

Egg quality traits	Substitution levels (%)				SEM (±)	P-values		
	HDL0	HDL9	HDL18	HDL27		ANOVA	Linear	Quadratic
Egg length (mm)	55.0	54.6	54.4	54.5	0.25	0.385	0.950	0.642
Egg width (mm)	42.1[a]	41.7[ab]	41.4[b]	41.5[b]	0.18	0.049	0.267	0.382
Egg shape index	77.5	77.0	76.3	76.0	0.47	0.522	0.578	0.347
Dried shell weight (g)	6.28[a]	6.10[ab]	5.95[ab]	5.75[b]	0.09	0.014	0.015	0.823
Eggshell ratio (%)	11.5[a]	11.2[ab]	11.0[ab]	10.6[b]	0.18	0.039	0.014	0.874
Egg surface area (cm^2)	67.0	66.8	66.5	66.4	0.49	0.884	0.646	0.894
Eggshell density (g/cm^2)	19.6 [a]	19.0[ab]	18.5[ab]	17.8[b]	0.36	0.097	0.038	0.903

[a-c] Means with different superscript letters across treatment diets are significant at $p<0.05$. SEM: standard error of the mean

Table 5. Least square means of internal egg qualities of layer hens fed with different levels of heat-treated dehulled lupin by partially substituting the soybean meal

Egg quality traits	Substitution levels (%)				SEM (±)	P-values		
	HDL0	HDL9	HDL18	HDL27		ANOVA	Linear	Quadratic
Egg weight (g)	54.8	54.4	54.2	54.1	0.57	0.847	0.647	0.939
Yolk width (mm)	39.6	39.3	39.2	39.1	0.18	0.293	0.562	0.961
Yolk height (mm)	16.7	16.4	16.2	16.3	0.20	0.375	0.728	0.778
Yolk index	0.42	0.42	0.41	0.42	0.04	0.601	0.781	0.632
Yolk colour	4.60	4.63	4.81	5.19	0.41	0.731	0.360	0.629
Albumen height (mm)	7.21	6.82	6.71	6.84	0.14	0.115	0.943	0.547
Haugh unit score	86.1	83.7	82.9	83.6	0.83	0.114	0.918	0.532

SEM: standard error of the mean

Rutkowski *et al.* (2017) reported that a 15 % inclusion of lupin was associated with a higher proportion of thin albumen in eggs. However, the current study did not reveal any significant differences in the albumen quality between treatment diets. In contrast to the current study, Kowalska *et al.* (2021) reported that in narrow-leaved lupin fed group the height of thick albumen, Haugh score, and yolk colour was higher than the control group. Such differences might be due to the variety of lupin used by various researchers.

Consistent with the current findings, Kowalska *et al.* (2020) and Kponouglo *et al.* (2024) reported that diets containing narrow-leaf and fermented lupin seed had no effect on the albumen height and Haugh unit scores. In another study, Park *et al.* (2016) reported higher Haugh unit scores than observed in the current study for brown laying hens fed unprocessed different levels of lupin seed (*Lupinus angustifolius* L.) supplementation. Although not significant, yolk colour linearly increased with increased treatment diets which is in accordance with the findings of Park *et al.* (2016) who reported that supplementation of unprocessed lupin seed supplementation in laying hen diets linearly improved the yolk colour. Rutkowski *et al.* (2017) also revealed a significant improvement of yolk colour in the group fed diet containing 25 % yellow lupin seeds (*Lupinus luteus* L.).

Inclusion of blue lupin seed meals in the diets of layer hens has been reported to improve the yolk fat profiles which is essential for the health of consumers (Kowalska *et al.* 2020). Research conducted by Timová *et al.* (2020) demonstrated that replacing extracted soybean with white lupin seed meal in feed mixtures, at both 50 % and 100 % levels as a source of protein, significantly decreased the saturated fatty acids and increased the mono- and polyunsaturated fatty acids, which are essential for consumers' health, especially for prevention of coronary diseases. The cholesterol levels of eggs produced from hens fed with fermented lupin seed (*Lupinus angustifolius*) was significantly lower than those fed on the control diet (Kponouglo *et al.* 2024). Similarly, Lim and Choi (2023) observed significantly higher levels of polyunsaturated fatty acids in broiler chickens fed with 10 % and 20 % lupin seed (*Lupinus angustifolius*) meal than in those fed the control diet.

Age effect on egg quality: As presented in table 6, dry shell weight and yolk colour were not affected by age while egg weight, egg length, egg width, yolk height and width, albumen height and Haugh unit score significantly and linearly increased with age. These observations are in line with those reported by Yurtseven *et al.* (2021) for chickens reared in the free-range production system. Similarly, Manyeula *et al.* (2021) reported that egg weight, egg length, egg width, egg yolk, egg content, egg volume, shell percentage, albumen weight, and egg surface area increased with the age of the hen. On the other hand, indices of egg shape and yolk significantly decreased with the advanced hens' age and these observations are in line with those reported by Kraus *et al.* (2021) and Tainika *et al.* (2024). The latter authors reported a significant decline of shell breaking strength with the age of then hens while shell thickness showed inconsistent pattern. In another study, Lee *et al.* (2016b), Kowalska *et al.* (2020) and Sam (2023) reported a significant decline in albumen height, Haugh unit score, and yolk color with increasing hens' age.

An increase of egg shape index with the age of the hen has been reported by Manyeula *et al.* (2021) which contrasts with the present observation. Such variations might be due to the chicken breed and type of diet used and the age of the hens when the data were collected. For instance, Lee *et al.* (2016b) and Alo *et al.* (2024) have revealed that eggs from 60-week-old hens had lower albumen height,

Table 6. Least square means of external and internal egg qualities as affected by hen´s age

Egg qualities	Age of the hens (wks.)				SEM (±)	P-value
	24	28	32	36		
Egg weight	51.1[c]	52.2[b]	56.4[a]	56.8[a]	0.416	<0.001
Egg length	52.4[c]	54.6[b]	54.6[b]	56.9[a]	0.291	<0.001
Egg width	40.9[c]	41.5[b]	41.9[ab]	42.4[a]	0.185	<0.001
Egg shape index	78.1[a]	76.0[b]	77.8[a]	73.9[c]	0.004	<0.001
Dried shell weight	6.02	6.25	5.79	6.17	0.126	0.057
Yolk width	35.5[d]	40.6[b]	39.6[c]	41.5[a]	0.264	<0.001
Yolk height	15.6[c]	16.3[b]	16.3[b]	17.4[a]	0.161	<0.001
Yolk index	0.44[a]	0.40[c]	0.41[bc]	0.42[b]	0.004	<0.001
Yolk colour	4.42	4.95	4.39	4.72	0.276	0.419
Albumen height	6.53[c]	6.61[b]	7.13[a]	7.35[a]	0.139	<0.001
Haugh unit	82.6[b]	83.0[b]	84.9[ab]	86.3[a]	0.849	<0.001

and Haugh unit score values compared to eggs from 30-week-old hens. Chung and Lee (2014) reported that egg quality was not affected by the age of the hen. In contrary, Kowalska *et al.* (2021) found a significant difference in egg quality characteristics between age of hens. Egg weight, shell density, shell weight, breaking strength and thickness significantly increased with the age of hens (from 19 to 39 weeks of age). The same authors reported a significant decline in height at albumen and Haugh unit score which disagrees with the results of the current study.

The Haugh unit score has been widely accepted as the "gold standard" for quantifying the internal quality and freshness of eggs. The Haugh unit score quickly declines over time, making it a sensitive measure of egg deterioration soon after being laid. On the other hand, the yolk index quantifies the structural integrity of the egg yolk, which gradually decreases over the storage period enabling the identification of quality differences among eggs that have deteriorated over time (Yurtseven *et al.* 2021). In contrast, the effect of age on yolk colour in the current study was found to be insignificant. Yolk colour is affected by the diet of the birds mainly by the presence of xanthophyll resulting in variations in the depth of the yolk colour (Lordelo *et al.* 2017).

Conclusions

Except feed intake, increased substitution levels of heat-treated dehulled lupin did not affect the egg production variables and feed utilization efficiency. The current observations suggest that incorporating heat-treated dehulled lupin into layer hen diets may be beneficial when used as a substitute for up to 18 % of soybean meal. This study further highlights on the significance of heat-treating and dehulling the lupin seed to improve its nutritive value and palatability.

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