

Effects of lupin seed supplementation on egg production performance, and qualitative egg traits in laying hens

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ABSTRACT: We investigated the effects of lupin (*Lupinus angustifolius* L.) seed supplementation on egg production performance and egg quality in laying hens. A total of 120 Hy-Line Brown laying hens (29-week old) were used in a nine week trial. Laying hens were sorted into pens with six birds per pen and five pens per treatment. Treatments were (1) control, corn-soybean meal-based control diets, (2) 11% lupin (control + 11% lupin), (3) 16.5% lupin (control + 16.5% lupin), and (4) 22% lupin (control + 22% lupin). The average daily feed intake of laying hens fed with lupin diets was increased compared with control at Week 6 ($P < 0.05$). The egg production rate was higher in lupin diets than in the control diet at Week 3, 4, and 5 ($P < 0.05$). Supplementation with lupin improved the egg production rate linearly at Week 3, 4, 5, and 9, as dietary lupin increased from 11 to 22%. Laying hens fed with 11% and 16.5% lupin diets had higher rates of extra-large-sized egg production at Week 1 (quadratic, $P = 0.04$). Yolk colour was higher in hens fed lupin diets than in those fed with the control at Week 9 ($P < 0.05$). Supplementation of lupin in laying hen diets linearly improved the yolk colour ($P < 0.05$) at Week 5. In conclusion, supplementation of the diet with lupin can improve egg production and yolk colour with no apparent effect on average daily feed intake and eggshell quality in laying hens.

Keywords: *Lupinus angustifolius* L.; laying performance; egg quality; yolk colour

Due to limitations connected with the use of animal proteins, plant-based protein meals have attracted considerable attention in recent years as an alternative to animal protein meal. Due to its high protein and food energy content, soybean meal is a potentially suitable plant protein meal in poultry diets (Hove 1974; Watkins and Mirosh 1987; Nalle et al. 2011). However, due to the increasing price of soybean meal, many researchers have sought alternative plant protein sources.

Grain legumes have been used as alternatives to animal proteins in poultry feed. Among the various grain legumes, lupins can be used as an alternative to soybeans and are highly regarded as a stock feed, not only for poultry but also for pigs, ruminants, and as fish-feed (Donovan et al. 1993; Glencross et al. 2004; Laudadio and Tufarelli 2011a). Lupin seeds contain similar protein content to soybeans

and also harbour less fat. Moreover, lupin seeds are gluten-free, high in amino acids, dietary fibre, and antioxidants (Lampart-Szczapa et al. 2003). In a previous report, sweet lupin, at 6, 12, and 18% inclusion rates, elicited significant changes in fattening capacity, the percentage of fat, and the percentage of valuable parts in the carcasses of broilers (Schamsscharch et al. 1994). However, dietary supplementation with up to 20% of white lupin had adverse effects on performance indicators, carcass value, and meat quality, including average daily gain, protein deposition in the carcass, and energy retention in broilers (Rothmaier and Kirchgessner 1994).

To our knowledge, the effects of Australian sweet lupin on egg production performance, eggshell parameters, and egg quality have not been systematically investigated in laying hens. Thus, the aim

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of the present study was to evaluate the effects of lupin supplementation on egg production performance, eggshell parameters, and egg quality in laying hens.

MATERIAL AND METHODS

The Animal Care and Use Committee of Dankook University approved all experimental protocols used in the current study.

Table 1. Basal diet composition (as-fed basis)

Item	Control	Lupin (%)		
		11	16.5	22
Ingredients (%)				
Corn	48.71	57.15	56.04	54.48
Soybean meal, 45% CP	25.08	17.50	13.30	9.24
Lupin, 31% CP	–	11.00	16.50	22.00
Wheat	11.00	–	–	–
Limestone	9.70	9.71	9.73	9.75
Animal fat	3.10	2.10	1.80	1.90
Monocalcium phosphate	1.59	1.61	1.60	1.58
Salt	0.35	0.35	0.35	0.35
Methionine	0.17	0.19	0.20	0.21
Choline	0.10	0.10	0.10	0.10
Mineral ¹	0.10	0.10	0.10	0.10
Vitamin ²	0.06	0.06	0.06	0.06
Lysine	0.04	0.13	0.22	0.23
Chemical composition ³				
ME (MJ/kg)	11.94	11.86	11.76	11.77
Crude protein	16.89	16.95	16.88	16.93
Crude fat	5.15	4.88	4.81	4.78
Crude fibre	2.22	2.14	2.09	2.06
Crude ash	14.28	14.08	13.95	13.86
Ca	4.08	4.08	4.09	4.07
P	0.41	0.40	0.38	0.37
Lysine	0.72	0.73	0.71	0.70
Methionine	0.41	0.40	0.39	0.38

¹Provided per kg of complete diet: Cu – 25 000 mg, Fe – 40 000 mg, Zn – 60 000 mg, Mn – 80 000 mg, I – 1500 mg, Co – 300 mg, Se – 150 mg

²Provided per kg of complete diet: vitamin A – 12 500 000 IU, vitamin D3 – 2 500 000 IU, vitamin E – 10 000 IU, vitamin K3 – 2000 mg, biotin – 50 mg, folic acid – 500 mg, niacin – 35 000 mg, Ca pantothenate – 10 000 mg, vitamin B6 – 1000 mg, vitamin B2 – 5000 mg, vitamin B1 – 1000 mg, and vitamin B12 – 15 mg

³Analysed values

Experimental design, animals and diets. A total of 120 Hy-Line Brown laying hens (29-week old) were used in a nine week trial. Laying hens were sorted into pens with six birds per pens and five pens per treatments. Treatments were (1) control, corn-soybean meal-based control diets, (2) 11% lupin (control + 11% lupin), (3) 16.5% lupin (control + 16.5% lupin), and (4) 22% lupin (control + 22% lupin). All the diets were formulated to meet or exceed the NRC (1994) nutritional requirements (Table 1). The nutrient composition of lupin seed is shown in Table 2. Dietary calcium (Ca), phosphorus (P), and crude protein (CP) were analysed according to the procedures described by the AOAC (2012). Dietary Ca was assayed using atomic absorption spectrophotometry after wet ash procedures and P was determined using colorimetry. Amino acid contents were measured using an amino acid analyser (Beckman 6300, Beckman Coulter, Inc., Fullerton, USA) after 24 h 6 N HCl hydrolysis at 110 °C (AOAC 2012). Energy was determined by using a Parr 6100 oxygen bomb calorimeter (Parr instrument Co., Moline, USA). Laying hens were raised in a temperature-controlled house, maintained at 21 °C with a light regime of 16-h light/8-h darkness. Laying hens were reared in adjacent steel cages, equipped with a nipple drinker, trough, and egg collecting plate. Birds had *ad libitum* access to water and feed.

Sampling and measurements. Daily records of egg production and feed intake were kept throughout the experimental period. Egg production is expressed as an average production of hen per day, calculated from the total number of eggs divided by the experimental time (unit: days), and summarised on an average basis. The average daily feed intake was calculated on a weekly basis during the trial

Table 2. Nutrient composition of lupin (%)

Moisture	9.74
Crude protein	31.07
Ether extract	5.48
Nitrogen free extract	37.75
Crude fibre	14.95
Ash	2.54
Ca	0.20
P	0.30
Lysine	2.02
Methionine	0.24
Threonine	1.04
Cysteine	0.39

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Table 3. The effects of lupin supplementation on feed intake (g) in laying hens

Week	Control	Lupin (%)			SEM	P-value		
		11	16.5	22		control vs lupins ¹	linear ²	quadratic
1	109.8	110.8	112.2	111.6	1.5	0.36	0.32	0.70
2	109.2	111.2	111.4	112.0	2.7	0.46	0.45	0.84
3	111.2	112.8	109.8	110.6	2.3	0.98	0.71	0.87
4	111.0	112.6	112.4	113.4	1.3	0.23	0.25	0.75
5	110.8	113.8	114.2	112.4	2.0	0.29	0.59	0.27
6	109.8	114.0	115.2	112.8	1.7	0.04	0.17	0.06
7	111.6	113.6	116.0	113.4	2.1	0.30	0.45	0.28
8	111.0	113.2	114.6	114.0	1.4	0.12	0.15	0.36
9	112.0	114.7	113.0	112.0	1.7	0.66	0.79	0.42
1–9	110.7	113.0	113.2	112.5	1.8	0.27	0.34	0.64

¹Orthogonal contrast to test control diet vs all lupin supplementation diets²Orthogonal polynomial contrast coefficients were used to determine the linear and quadratic effect of increasing dietary lupin supplementation levels

period. In total, 20 saleable eggs (no shell defects, cracks, or double yolks) were collected randomly from each treatment on a weekly basis and used to determine the egg shell parameters and quality. Egg weight was measured using an egg multi tester (Touhoku Rhythm Co. Ltd., Tokyo, Japan), and extra large-sized eggs (60–68 g) were recorded. The total average egg weight was calculated based on weekly egg weight measurements. Eggshell colour was determined using a colour fan (Eggshell colour fan, Samyang Feed Co., Korea) with a range from 1 to 15. A dial gauge (Ozaki Mfg. Co. Ltd., Tokyo, Japan) was used to measure egg shell thickness, which was determined based on the average thickness of the

rounded end, pointed end, and the middle of the egg, excluding the inner membrane. Egg yolk colour and Haugh units were evaluated using an egg multi-tester (Touhoku Rhythm Co. Ltd., Tokyo, Japan). These eggs were immersed in a series of chloride solutions (0.010 increments between 1.060 and 1.110) to determine specific gravity (Yan et al. 2011).

Statistical analysis. All data were subjected to statistical analyses as a randomised complete block design using the GLM procedure of the SAS software (SAS Institute 1996), with the pen as the experimental unit. Orthogonal contrasts used to separate treatment means were control vs lupins (11, 16.5, and 22%). The means were separated us-

Table 4. The effects of lupin supplementation on egg production (%) in laying hens

Week	Control	Lupin (%)			SEM	P-value		
		11	16.5	22		control vs lupins ¹	linear ²	quadratic
1	93.2	93.8	94.4	94.0	0.9	0.50	0.60	0.58
2	94.2	95.0	94.6	94.6	0.7	0.48	0.81	0.53
3	93.2	96.2	97.0	97.4	1.1	0.02	0.02	0.33
4	93.0	95.0	96.4	96.8	0.9	0.01	0.01	0.41
5	94.2	94.8	96.6	96.2	0.6	0.03	0.01	0.37
6	95.2	96.0	96.8	97.6	0.8	0.14	0.06	0.97
7	96.6	96.4	97.0	98.0	0.6	0.62	0.15	0.29
8	94.2	94.8	96.0	95.4	0.6	0.09	0.08	0.28
9	93.0	94.6	95.4	96.0	1.1	0.07	0.05	0.70
1–9	94.1	95.2	96.0	96.2	0.9	0.35	0.18	0.45

¹Orthogonal contrast to test control diet vs all lupin supplementation diets²Orthogonal polynomial contrast coefficients were used to determine the linear and quadratic effect of increasing dietary lupin supplementation levels

Table 5. The effects of lupin supplementation on broken egg rate (%) in laying hens

Week	Control	Lupin (%)			SEM	P-value		
		11	16.5	22		control vs lupins ¹	linear ²	quadratic
1	5.2	3.8	5.1	3.1	0.6	0.07	0.05	0.57
2	4.7	3.8	4.0	3.3	0.6	0.20	0.20	0.86
3	4.3	4.2	3.9	3.6	0.5	0.52	0.33	0.84
4	3.9	3.7	3.6	3.6	0.4	0.71	0.69	0.89
5	4.7	4.2	4.3	4.0	0.5	0.39	0.38	0.89
6	3.8	3.3	3.7	3.3	0.4	0.49	0.59	0.90
7	4.2	3.8	3.6	3.3	0.9	0.57	0.51	0.98
8	5.6	5.0	3.7	4.4	0.6	0.10	0.08	0.32
9	4.4	3.8	3.8	3.3	0.9	0.46	0.42	0.95
1–9	4.5	4.0	4.0	3.5	0.5	0.28	0.45	0.78

¹Orthogonal contrast to test control diet vs all lupin supplementation diets

²Orthogonal polynomial contrast coefficients were used to determine the linear and quadratic effect of increasing dietary lupin supplementation levels

ing orthogonal polynomial contrasts to examine the effects of lupin diets. Variability in the data was expressed as the pooled standard error of the mean (SEM). *P*-values < 0.05 were considered to indicate statistical significance.

RESULTS

Egg production performance

Laying hens fed with lupin diets had higher average daily feed intake (*P* = 0.04) than laying hens fed the control diet at six weeks (Table 3). Laying hens

fed with lupin-supplemented diets had higher egg production rates than laying hens fed the control diet at three weeks (*P* = 0.02), four weeks (*P* = 0.01), and five weeks (*P* = 0.03). Further, laying hens fed with lupin-supplemented diets exhibited linearly improved egg production at three weeks (*P* = 0.02), four weeks (*P* = 0.01), five weeks (*P* = 0.01), and nine weeks (*P* = 0.05; Table 4). During the experimental period, no difference (*P* > 0.05) was observed in broken egg production among the treatments (Table 5). After one week, the diets supplemented with 11% and 16.5% lupin had a higher ratio (quadratic, *P* = 0.04) of extra-large-sized eggs than those fed with the 22% lupin diet (Table 6).

Table 6. The effects of lupin supplementation on the ratio of extra large-sized eggs (%) in laying hens

Week	Control	Lupin (%)			SEM	P-value		
		11	16.5	22		control vs lupins ¹	linear ²	quadratic
1	54.2	59.4	59.4	55.0	2.2	0.16	0.81	0.04
2	52.4	59.7	56.7	58.7	2.6	0.06	0.19	0.31
3	54.2	57.3	55.8	55.9	3.3	0.59	0.82	0.65
4	55.6	59.4	57.8	58.1	4.4	0.58	0.77	0.69
5	52.8	59.0	58.3	56.9	3.8	0.25	0.50	0.33
6	54.5	56.3	59.2	56.7	3.1	0.44	0.51	0.50
7	54.5	60.0	59.7	58.9	2.3	0.08	0.23	0.19
8	57.6	60.1	59.7	57.0	1.9	0.57	0.78	0.19
9	56.3	59.4	57.2	55.6	3.4	0.78	0.78	0.50
1–9	54.7	59.0	58.2	57.0	2.8	0.25	0.67	0.48

¹Orthogonal contrast to test control diet vs all lupin supplementation diets

²Orthogonal polynomial contrast coefficients were used to determine the linear and quadratic effect of increasing dietary lupin supplementation levels

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Egg quality

No differences were observed in colour, strength, or thickness of the eggshell (Table 7) or specific gravity or weight (Table 8) of the egg during the experimental period ($P > 0.05$).

Laying hens fed with lupin-supplemented diets had higher yolk colour ($P = 0.02$) than laying hens fed the control diet at nine weeks. Also, laying hens fed with lupin-supplemented diets exhibited

linearly improved yolk colour at five weeks ($P = 0.05$). No differences were observed in yolk height or Haugh units during the experimental period ($P > 0.05$; Table 9).

DISCUSSION

Soybean meal, as an alternative to animal protein meal in poultry diets, has a high-protein content, a

Table 7. The effects of lupin supplementation on eggshell colour, strength (kg/cm²) and thickness (mm²) in laying hens

	Week	Control	Lupin (%)			SEM	P-value		
			11	16.5	22		control vs lupins ¹	linear ²	quadratic
Eggshell colour	1	11.3	11.3	11.0	11.0	0.3	0.57	0.40	0.93
	2	11.7	11.7	11.5	11.6	0.5	0.78	0.75	0.87
	3	11.8	11.7	11.9	11.8	0.5	1.00	0.93	1.00
	4	11.4	12.0	11.9	11.8	0.4	0.23	0.53	0.31
	5	11.7	12.0	11.8	12.0	0.3	0.43	0.54	0.78
	6	11.9	12.1	12.2	12.3	0.2	0.30	0.22	0.90
	7	12.1	12.0	12.1	12.2	0.3	0.92	0.65	0.73
	8	12.1	12.1	12.1	12.2	0.3	0.82	0.72	0.84
	9	11.9	12.0	11.8	11.8	0.2	0.90	0.63	0.83
	1–9	11.8	11.9	11.8	11.9	0.3	0.66	0.45	0.87
Eggshell strength	1	3.372	3.414	3.373	3.394	0.120	0.88	0.96	0.93
	2	3.473	3.515	3.529	3.597	0.120	0.59	0.47	0.91
	3	3.561	3.592	3.603	3.583	0.120	0.82	0.89	0.83
	4	3.597	3.687	3.642	3.620	0.131	0.73	0.96	0.67
	5	3.694	3.714	3.740	3.681	0.116	0.89	0.98	0.73
	6	3.574	3.503	3.563	3.521	0.108	0.72	0.84	0.89
	7	3.701	3.669	3.634	3.524	0.146	0.57	0.38	0.80
	8	3.515	3.485	3.564	3.620	0.134	0.79	0.51	0.75
	9	3.494	3.653	3.634	3.643	0.138	0.35	0.49	0.59
	1–9	3.553	3.581	3.587	3.576	0.125	0.70	0.78	0.82
Eggshell thickness	1	39.24	39.04	39.21	39.07	0.55	0.61	0.88	0.57
	2	39.44	39.24	39.41	39.27	0.54	0.72	0.99	0.62
	3	39.74	39.54	39.71	39.57	0.50	0.42	0.61	0.71
	4	39.84	39.64	39.81	39.67	0.56	0.99	0.84	0.92
	5	39.94	39.74	39.91	39.77	0.51	0.73	0.87	0.51
	6	40.41	40.13	40.39	40.52	0.41	0.89	0.75	0.60
	7	40.09	39.61	39.75	40.19	0.41	0.60	0.81	0.25
	8	39.92	39.68	39.63	40.17	0.29	0.78	0.58	0.18
	9	39.72	39.82	40.12	40.18	0.28	0.33	0.18	0.98
	1–9	39.82	39.60	39.77	39.82	0.45	0.64	0.72	0.56

¹Orthogonal contrast to test control diet vs all lupin supplementation diets

²Orthogonal polynomial contrast coefficients were used to determine the linear and quadratic effect of increasing dietary lupin supplementation levels

Table 8. The effects of lupin supplementation on egg gravity and egg weight in laying hens

	Week	Control	Lupin (%)			SEM	P-value		
			11	16.5	22		control vs lupins ¹	linear ²	quadratic
Egg gravity	1	1.091	1.089	1.089	1.088	0.002	0.19	0.19	0.75
	2	1.091	1.094	1.091	1.092	0.002	0.61	0.89	0.55
	3	1.095	1.093	1.094	1.092	0.002	0.19	0.14	0.86
	4	1.092	1.094	1.094	1.094	0.001	0.17	0.22	0.59
	5	1.095	1.094	1.095	1.096	0.001	0.82	0.72	0.43
	6	1.095	1.094	1.094	1.096	0.001	0.66	0.73	0.25
	7	1.095	1.094	1.095	1.096	0.001	0.82	0.47	0.23
	8	1.094	1.095	1.095	1.095	0.001	0.50	0.61	0.70
	9	1.095	1.095	1.095	1.095	0.001	0.92	0.94	0.86
	1–9	1.095	1.094	1.094	1.094	0.001	0.54	0.57	0.62
Egg weight	1	60.7	59.6	60.5	59.1	1.2	0.47	0.47	0.94
	2	59.6	60.4	60.0	59.8	1.3	0.74	0.94	0.70
	3	59.6	59.9	60.1	59.7	1.2	0.80	0.89	0.77
	4	59.8	59.9	60.4	60.1	0.9	0.78	0.75	0.85
	5	59.8	59.9	60.4	60.1	0.9	0.78	0.75	0.85
	6	60.1	59.1	60.3	60.2	0.7	0.81	0.62	0.54
	7	59.7	60.5	60.1	59.4	0.6	0.72	0.55	0.20
	8	59.8	60.4	60.1	60.0	0.6	0.57	0.94	0.49
	9	59.8	60.2	60.2	59.6	0.6	0.76	0.77	0.38
	1–9	59.9	60.0	60.3	59.8	0.9	0.75	0.71	0.64

¹Orthogonal contrast to test control diet vs all lupin supplementation diets

²Orthogonal polynomial contrast coefficients were used to determine the linear and quadratic effect of increasing dietary lupin supplementation levels

balanced amino acid profile, and represents an adequate source of essential fatty acids (Hammershoj and Steenfeldt 2005). However, increasing demand for soybean meal, associated with the increase in poultry production worldwide, has resulted in increased prices (Laudadio and Tufarelli 2010). Due to low protein content, amino acid imbalances, and the presence of anti-nutritional factors, few plant protein sources are suitable as replacements for soybean meal in poultry diets (Rubio et al. 2003). Lupins have attracted considerable attention for poultry diets, due to their high levels of protein and unsaturated fatty acids, favourable price, and market availability (Ravindran and Blair 1992; Martinez-Villaluenga et al. 2006; Boschini et al. 2008; Vicenti et al. 2009). In the present study, we evaluated the effects of lupin diets in replacing soybean meal on egg production and egg quality in laying hens. It is well documented that lupin diets, as an alternative to soybean meal, have positive effects on performance, carcass

value, and meat quality in broilers (Suchy et al. 2010; Laudadio and Tufarelli 2011b). Diets with different concentrations of lupin seeds influenced the average daily feed intake in the present study. Unlike our results, Rubio et al. (2003) reported that the body weight and average daily feed intake of chickens fed diets containing whole lupin seed meal (400 g/kg) were lower than controls. Also, lupin-supplemented laying hen diets reduced feed consumption and weight gain, but increased feed-to-gain ratio (Viveros et al. 2007).

In the present study, lupin-fed laying hens had higher egg production rates than those receiving the control diet. Also, laying hens fed with 11% and 16.5% lupin diets had higher ratios of extra-large-sized eggs than the control and hens fed the 22% lupin diet. The effects of lupin diets on egg production performance in laying hens that we observed are inconsistent with some previous studies. In a previous report, 25% lupin supplementation in layer diets resulted in higher performance in a 40-

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Table 9. The effects of lupin supplementation on yolk colour, yolk height (mm) and haugh unit in laying hens

	Week	Control	Lupin (%)			SEM	P-value		
			11	16.5	22		control vs lupins ¹	linear ²	quadratic
Yolk colour	1	8.6	8.3	8.8	8.8	0.1	0.99	0.07	0.16
	2	8.7	8.6	8.8	8.8	0.1	0.88	0.42	0.80
	3	8.7	8.7	8.7	8.9	0.1	0.57	0.25	0.55
	4	8.6	8.6	8.7	8.8	0.1	0.94	0.41	0.41
	5	8.6	8.6	8.8	9.0	0.1	0.31	0.01	0.22
	6	8.5	8.7	8.6	8.8	0.1	0.12	0.16	0.79
	7	8.5	8.3	8.4	8.5	0.1	0.73	0.65	0.32
	8	8.4	8.3	8.4	8.6	0.1	0.90	0.30	0.14
	9	8.4	8.7	8.7	8.7	0.1	0.02	0.06	0.12
	1–9	8.6	8.5	8.7	8.8	0.1	0.54	0.26	0.37
Yolk height	1	9.12	9.04	8.89	9.14	0.15	0.55	0.88	0.25
	2	9.22	9.01	9.29	9.15	0.14	0.63	0.92	0.78
	3	9.08	8.96	9.19	9.24	0.09	0.62	0.08	0.36
	4	9.05	8.93	9.14	9.04	0.06	0.81	0.52	0.81
	5	9.02	8.93	9.14	9.04	0.07	0.86	0.42	0.97
	6	9.05	8.88	9.06	9.00	0.07	0.36	0.93	0.39
	7	9.00	8.95	9.06	9.16	0.07	0.48	0.06	0.25
	8	8.95	8.87	8.84	9.04	0.08	0.71	0.49	0.07
	9	9.01	9.15	9.15	9.07	0.08	0.19	0.61	0.14
	1–9	9.06	8.97	9.08	9.10	0.09	0.57	0.52	0.42
Haugh unit	1	95.1	94.8	94.4	94.8	0.7	0.57	0.68	0.57
	2	95.1	94.8	94.4	94.8	0.7	0.57	0.68	0.57
	3	94.8	94.3	95.0	94.9	0.9	0.99	0.79	0.86
	4	95.2	95.3	94.9	95.0	0.8	0.84	0.74	0.98
	5	95.2	95.3	95.0	95.1	0.6	0.95	0.81	0.94
	6	94.7	94.8	94.4	94.1	0.7	0.70	0.42	0.73
	7	94.6	94.5	95.2	95.4	0.4	0.40	0.12	0.70
	8	95.0	94.9	95.6	95.8	0.7	0.61	0.33	0.81
	9	94.4	95.3	94.6	94.7	0.5	0.41	0.89	0.43
	1–9	94.9	94.9	94.8	95.0	0.7	0.72	0.61	0.72

¹Orthogonal contrast to test control diet vs all lupin supplementation diets²Orthogonal polynomial contrast coefficients were used to determine the linear and quadratic effect of increasing dietary lupin supplementation levels

week experiment: hen per day egg production was higher than 90% and feed intake was approximately 120 g/hen per day (Perez-Maldonado et al. 1999). In another study, feed supplemented with up to 30% white lupin in diets resulted in good egg production (Prinsloo et al. 1992). However, negative effects of lupin diets on egg production performance have also been reported. Laying hens fed diets with 25 and 30% white lupin had a lower egg mass compared with hens on a lupin-free diet (Watkins and Mirosh 1987). Moreover, a 25% lupin diet exerted significant negative effects on performance, egg

weight, and daily egg mass (Hammershoj and Steenfeldt 2005).

The results of the present study showed that laying hens fed different concentrations of lupin exhibited linearly improved egg yolk colour at five weeks ($P = 0.01$; Table 9). Egg yolk colour in laying hens is one of the major parameters for the quality of an egg and is an important criterion for consumers (Teoh et al. 2015). Egg yolk colour is correlated with pigment content and ranges from yellow (e.g., lutein, zeaxanthin, apo-ester) to red (e.g., canthaxanthin, citraxanthin, astaxanthin)

due to carotenoids or xanthophylls in the hen's diet (Beardsworth and Hernandez 2004; Roberts 2004). Various efforts have focused on the use of suitable feeds to enhance egg yolk colour. The substitution of soy bean meal with low-fibre alfalfa (*Medicago sativa* L.) meal improved yolk colour, yolk percentage, yolk cholesterol, and β -carotene contents in hen diets (Laudadio et al. 2014). The use of yellow lupin seed meal in the feeding mixture resulted in a more distinct yellow/red egg yolk (Dvorak et al. 2007). In addition, defatted diatom microalgal biomass diet in laying hens increased the egg albumen weight and height compared with 7.5% defatted diatom microalgal biomass diets (Leng et al. 2014).

In conclusion, the present study indicates that diets supplemented with lupin could improve egg production performance and egg yolk quality, while it apparently had no effect on eggshell quality in laying hens. Further investigations are necessary to explore the effects of lupin on the immune response in laying hens.

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