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Biological Effects of Dried Corn Steep Liquor on Egg Production Performance and Egg Quality of Laying Hens

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ABSTRACT This study evaluated the impact of supplementing dried corn steep liquor (CSL) as an alternative protein source on laying performance and egg quality in laying hens. A total of 108 Hy-Line Brown hens at 44 weeks of age were allocated into one of three treatments, including a control group (CON) that received the basal diet without CSL, while the treatments were supplemented with either 5 g/kg dried CSL (CSL 0.5) or 30 g/kg dried CSL (CSL 3.0) to partially replace soybean meal. Our trial lasted for four weeks. Results showed that CSL 3.0 exhibited higher (P<0.05) hen-day egg production and egg mass along with egg weight and feed efficiency than the CON from weeks 47−48. No differences (P>0.05) were found in eggshell thickness, eggshell strength, albumen height, Haugh units, eggshell color, or yolk color between the treatments at weeks 46 and 48. In conclusion, while supplementation with 0.5% CSL as an alternative protein source had no effect on production performance, 3.0% CSL exhibited potential benefits for laying performance in laying hens.

(Key words: corn steep liquor, egg production, egg quality, feed additives, laying hens)

INTRODUCTION

In the poultry industry, feed costs account for the largest proportion of total production expenses. Recently, the need for alternative protein sources that are more cost-effective and stable than conventional ingredients has been emphasized (Van Immerseel et al., 2017). Soybean meal, widely used as a primary protein source in poultry feed worldwide, is known for its high protein content and excellent amino acid composition. However, concerns over price fluctuations and supply instability have increased interest in alternative protein sources (Beski et al., 2015).

Corn steep liquor (CSL) is a liquid by-product of the corn wet-milling process that is rich in protein, lactic acid, and various other nutrients, making it a potential alternative feed ingredient (Liggett and Koffler, 1948; Zhou et al., 2022). While CSL has traditionally been used in ruminant feed or as a fermentation medium (Mirza and Mushtaq, 2006), recent studies have explored its role as a protein replacement ingredient in poultry feed. Previous studies suggest that supplementing CSL at optimal levels can maintain poultry production performance (Liggett and Koffler, 1948; Camp et al., 1957; Waldroup and Rutherford, 1971).

Lactic acid in CSL may improve gut health and nutrient utilization, further supporting its potential application in poultry diets (Jongbloed et al., 2000; Kim et al., 2015). Previous studies have shown that CSL can sustain growth performance in broilers (Waldroup et al., 1970; Yazdanyar et al., 2015), and when used at appropriate levels in layers, it does not deteriorate egg production performance (Waldroup and Rutherford, 1971; Lilburn and Jensen, 1984). However, excessive CSL supplementation may lead to imbalances in gut microbiota or metabolic processes, highlighting the importance of determining optimal inclusion levels (Ullah et al., 2018; Hamidifard et al., 2023). Notably, while Lilburn and Jensen (1984) investigated CSL as an alternative feed ingredient for soybean meal in layer diets, similar studies have been limited in recent years.

This study aimed to evaluate the effects of dried CSL as an alternative protein source in layer diets, hypothesizing that partially replacing soybean meal with CSL would maintain or even enhance production performance. Additionally, we tried

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to determine the optimal inclusion level of CSL that can effectively replace soybean meal in layer feed.

MATERIALS AND METHODS

1. Experimental Diets

The experimental diets were formulated to meet or exceed the nutritional requirements outlined in the Hy-Line Brown Management Guide (2024). Dried CSL, provided by ChungMi Bio Co., Ltd., was included in the diets at either 0.5% or 3% to partially replace soybean meal in the basal diet. The treatments were designated as follows: the control group (CON) received the basal diet without CSL, while the other treatments were supplemented with either 5 g/kg dried CSL (CSL 0.5) or 30 g/kg dried CSL (CSL 3.0). The diet composition used in the study is presented in Table 1, and the chemical compositions of soybean meal and dried CSL used in the experimental diets are presented in Table 2.

2. Experimental Birds and Design

This study was conducted following the guidelines of the Chungnam National University Institutional Animal Care and Use Committee (Approval No. 202501A-CNU-016). All procedures related to animal management and handling were approved by the University's Animal Ethics Committee and adhered to institutional regulations. The trial was performed at the Animal Resource Research Center of Chungnam National University. A total of 108 44-week-old Hy-Line Brown layers (1,888.77±12.73 g) were randomly assigned to one of three dietary treatments in a completely randomized design, with six replicates per treatment and six hens per replicate. The hens were housed in environmentally enriched cages (90 cm width × 90 cm depth × 90 cm height) equipped with perches and nest boxes within a climate-controlled facility with an automatic temperature and ventilation control system. Following a one-week adaptation period, the trial was conducted over four weeks. Each cage was equipped with four nipple drinkers to provide free access to water, while feed was offered 110 g per hen per day through metal troughs, based on the recommendations of the Hy-Line Brown Management Guide (2024). Eggs were collected daily from trays positioned beneath the metal troughs. The ambient temperature was

Table 1. Ingredients and calculated nutrient composition of the experimental diets[1]

Items	Dietary treatments[2]		
	CON	CSL 0.5	CSL 3.0
Ingredients			
Corn	53.85	53.75	53.45
Dried corn steep liquor	0.00	0.50	3.00
Wheat bran	7.44	7.48	7.48
Soybean meal (45%)	26.82	26.38	24.05
Vegetable oil	1.00	1.00	1.00
Limestone	9.60	9.60	9.60
Mono-calcium phosphate	0.68	0.68	0.75
Salt	0.30	0.30	0.30
Lysine-HCl	0.00	0.01	0.07
L-Threonine	0.01	0.00	0.00
Vitamin-mineral premix[3]	0.30	0.30	0.30
Calculated nutrient composition (%)			
ME (kcal/kg)	2,700	2,700	2,700
Crude protein	17.50	17.50	17.50
Calcium	4.00	4.00	4.00
Total phosphorus	0.58	0.58	0.58
SID lysine	0.82	0.82	0.82
SID methionine	0.24	0.24	0.24
SID threonine	0.57	0.57	0.57

[1] ME, metabolizable energy; SID, standardized ileal digestible.
[2] CON: Basal diet meets the recommended nutritional requirements for Hy-Line Brown; CSL 0.5: CON + 5 g/kg CSL; CSL 3.0: CON + 30 g/kg CSL.
[3] Provided per kilogram of diet: vitamin A, 12,000 IU; vitamin D_3, 2,500 IU; vitamin E, 30 IU; vitamin K_3, 3 mg; D-pantothenic acid, 15 mg; nicotinic acid, 40 mg; choline, 400 mg; and vitamin B_{12}, 12 μg; Fe, 90 mg from iron sulphate; Cu, 8.8 mg from copper sulphate; Zn, 100 mg from zinc oxide; Mn, 54 mg from manganese oxide; I, 0.35 mg from potassium iodide; Se, 0.30 mg from sodium selenite.

Table 2. Analyzed chemical composition of soybean meal and dried CSL used in experimental diets[1]

Ingredients	CP	CF	EE	Ash	Moisture
Soybean meal	45%	6%	<1%	–	<11.5%
Dried CSL	>40%	<0.5%	–	<20%	<15%

[1] CSL, corn steep liquor; CP, crude protein; CF, crude fiber; EE, ethyl ester.

maintained at 18−22℃ with a relative humidity of 50−70% throughout the experimental period. Lighting was programmed for 16 hours of light and 8 hours of darkness per day.

3. Laying Performance Measurements

Egg production parameters, including hen-day egg production (HDEP), egg mass, and feed conversion ratio (FCR), were recorded daily and analyzed weekly throughout the 4-week experimental period. Hen-day egg production (HDEP) was determined by calculating the percentage of eggs laid per day relative to the total number of hens. Egg mass was calculated by multiplying HDEP by the average egg weight. The FCR was calculated as the average daily feed intake divided by the egg mass, assessing feed efficiency across dietary treatments.

4. Egg Quality Measurements

Egg quality was investigated at weeks 46 and 48 using 90 eggs (30 per each treatment), excluding cracked or soft-shelled eggs. Internal egg quality parameters included yolk color measured with the DSM Yolk Color Fan, albumen height measured with the Egg Multitasker Instrument (TSS QCM+, Chessingham Park, York, UK), and Haugh unit (HU) calculated using the formula HU = 100 × log (H − 1.7 × $W^{0.37}$ + 7.57) (Haugh, 1937). External egg quality parameters included eggshell thickness and color evaluated with the Egg Multitasker Instrument (TSS QCM+) and eggshell strength measured using a Texture Analyzer (TA.XTplusC, Stable Micro Systems, Surrey, UK).

5. Statistical Analyses

All data were analyzed using the general linear model (GLM) of SPSS 29.0 (SPSS Inc., Chicago, USA). A battery cage was the experimental unit for statistical analysis. Statistical significance was determined at $P<0.05$. When significance was noticed for the treatment effects, means were separated using Tukey's Multiple Range Test.

RESULTS AND DISCUSSION

1. Laying Performance

The effects of dried CSL supplementation on laying performance are presented in Table 3. During weeks 44−45, no differences ($P>0.05$) were observed in HDEP among the CON (82.16%), CSL 0.5 (82.15%), and CSL 3.0 (82.22%). Similarly, no differences ($P>0.05$) were found in HDEP

Table 3. Effects of CSL on productive performance of laying hens[1]

Items	Dietary treatment[2]			SEM[3]	*P*-value
	CON	CSL 0.5	CSL 3.0		
Hen-day egg production (%)					
week 44−45	82.162	82.147	82.216	0.208	0.990
week 45−46	81.729	81.944	82.193	0.246	0.748
week 46−47	81.498	81.791	82.221	0.251	0.340
week 47−48	80.635[a]	81.514[ab]	82.147[b]	0.251	0.013
week 44−48	81.506	81.849	82.194	0.230	0.316
Egg weight (g)					
Week 44−45	60.79	61.26	61.98	0.518	0.644
Week 45−46	61.19	62.06	62.98	0.513	0.389
Week 46−47	61.32	62.88	63.84	0.476	0.128
Week 47−48	61.27[a]	63.25[ab]	65.00[b]	0.559	0.049
Week 44−48	61.14	62.36	63.45	0.504	0.208
Egg mass[4] (g/d/hen)					
Week 44−45	49.96	50.33	50.97	0.549	0.752
Week 45−46	50.03	50.87	51.77	0.563	0.468
Week 46−47	49.86	51.44	52.50	0.535	0.163
Week 47−48	49.30[a]	51.58[ab]	53.67b	0.606	0.033
Week 44−48	49.79	51.06	52.23	0.554	0.232
Feed conversion ratio[5] (g feed/g egg)					
Week 44−45	2.13	2.04	2.03	0.026	0.252
Week 45−46	2.08	2.05	2.02	0.029	0.696
Week 46−47	2.08	2.03	1.95	0.027	0.164
Week 47−48	2.18[a]	2.00[a]	1.96[b]	0.022	0.002
Week 44−48	2.12	2.03	1.99	0.023	0.098

[1] Values are mean of six replicates per treatment.
[2] CON: Basal diet meets the recommended nutritional requirements for Hy-Line Brown; CSL 0.5: CON + 5 g/kg CSL; CSL 3.0: CON + 30 g/kg CSL.
[3] SEM = standard error of mean.
[4] Egg mass = Average egg weight / Hen-day egg production.
[5] Feed conversion ratio = Total feed intake / Total egg mass.
[a,b] Means in a row with different letters are significantly different ($P<0.05$).

among the treatments during weeks 45−46 (CON: 81.73%, CSL 0.5: 81.94%, CSL 3.0: 82.19%), weeks 46−47 (CON: 81.50%, CSL 0.5: 81.79%, CSL 3.0: 82.22%), and throughout the overall experimental period (CON: 81.51%, CSL 0.5: 81.85%, CSL 3.0: 82.19%). However, during weeks 47−48, HDEP in the CSL 3.0 (82.15%) was higher (P=0.013) than in the CON (80.63%), while no difference (P>0.05) was observed between the CON and CSL 0.5.

Egg weight showed no differences (P>0.05) among the treatments during weeks 44−45 (CON: 60.79 g, CSL 0.5: 61.26 g, CSL 3.0: 61.98 g), weeks 45−46 (CON: 61.19 g, CSL 0.5: 62.06 g, CSL 3.0: 62.98 g), and weeks 46−47 (CON: 61.32 g, CSL 0.5: 62.88 g, CSL 3.0: 63.84 g). However, during weeks 47−48, egg weight was higher (P=0.049) in the CSL 3.0 (65.00 g) compared to the CON (61.27 g), while no difference (P>0.05) was found between the CON and CSL 0.5. Across the entire experimental period, egg weight did not differ (P>0.05) among the treatments (CON: 61.14 g, CSL 0.5: 62.36 g, CSL 3.0: 63.45 g).

Egg mass followed a similar trend, showing no differences (P>0.05) among the treatments during weeks 44−45 (CON: 49.96 g/hen/day, CSL 0.5: 50.33 g/hen/day, CSL 3.0: 50.97 g/hen/day), weeks 45−46 (CON: 50.03 g/hen/day, CSL 0.5: 50.87 g/hen/day, CSL 3.0: 51.77 g/hen/day), and weeks 46−47 (CON: 49.86 g/hen/day, CSL 0.5: 51.44 g/hen/day, CSL 3.0: 52.50 g/hen/day). However, during weeks 47−48, egg mass was higher (P=0.033) in the CSL 3.0 (53.67 g/hen/day) than in the CON (49.30 g/hen/day), while no difference (P>0.05) was found between the CON and CSL 0.5. No differences (P>0.05) in egg mass were observed across the overall experimental period (CON: 49.79 g/hen/day, CSL 0.5: 51.06 g/hen/day, CSL 3.0: 52.23 g/hen/day).

Feed conversion ratio (FCR) showed no differences (P>0.05) among the treatments during weeks 44−45 (CON: 2.13 g feed/g egg, CSL 0.5: 2.04 g feed/g egg, CSL 3.0: 2.03 g feed/g egg), weeks 45−46 (CON: 2.08 g feed/g egg, CSL 0.5: 2.05 g feed/g egg, CSL 3.0: 2.02 g feed/g egg), and weeks 46−47 (CON: 2.08 g feed/g egg, CSL 0.5: 2.03 g feed/g egg, CSL 3.0: 1.95 g feed/g egg). However, during weeks 47−48, FCR was lower (P=0.002) in the CSL 3.0 (1.96 g feed/g egg) than in the CON (2.18 g feed/g egg), while no difference (P>0.05) was found between the CON and CSL 0.5. Over the entire experimental period, no differences (P>0.05) in FCR were observed among the treatments (CON: 2.12 g feed/g egg, CSL 0.5: 2.03 g feed/g egg, CSL 3.0: 1.99 g feed/g egg).

The results of this study indicate that supplementing laying hen diets with 3% dried CSL to partially replace soybean meal improved laying performance, whereas 0.5% CSL had no effect. Similar findings have been reported in previous studies, demonstrating that CSL can enhance egg production when used as an alternative protein source in laying hen diets (Waldroup and Rutherford, 1971; Lilburn and Jensen, 1984). The improvement in laying performance may be attributed to the ability of CSL to enhance nutrient utilization by modulating gut microbiota and promoting digestive enzyme activity in the gut (Kim et al., 2015; Ebrahimpour et al., 2022). It has been reported that an optimal level of CSL inclusion in the diet may support gut health, as lactic acid helps regulate the microbial balance and improve nutrient digestibility (Waldroup et al., 1970; Hamidifard et al., 2023), contributing to improved laying performance. Since we did not analyze lactic acid content, this explanation remains a hypothesis. Meanwhile, excessive CSL supplementation has been reported to hamper laying performance, likely due to abrupt changes in gut acidity, which may impair the digestibility of the nutrients (Ullah et al., 2018; Atabak et al., 2021). Thus, it is essential to determine an optimal inclusion level when using CSL as an alternative protein source in laying hen diets. Based on the results of this study, supplementing 0.5% CSL in layers diet to partially replace soybean meal did not improve laying performance, whereas supplementing 3% CSL led to a significantly improved laying performance.

2. Egg Quality

The effects of dried CSL supplementation on egg quality are presented in Table 4. Eggshell thickness did not differ (P>0.05) among treatments at week 46, with values of 0.38 mm in the CON, 0.39 mm in the CSL 0.5, and 0.39 mm in the CSL 3.0. Similarly, no differences (P>0.05) were observed at week 48, with values of 0.38 mm, 0.39 mm, and 0.39 mm for the CON, CSL 0.5, and CSL 3.0, respectively. Eggshell strength at week 46 showed no differences (P>0.05) among treatments, with values of 5.78 kgf in the CON, 5.85 kgf in the CSL 0.5, and 5.84 kgf in the CSL 3.0. A similar

Table 4. Effects of CSL on egg quality of laying hens[1]

Items	Dietary treatment[2]			SEM[3]	*P*-value
	CON	CSL 0.5	CSL 3.0		
Eggshell thickness (mm)					
Week 46	0.38	0.39	0.39	0.003	0.075
Week 48	0.38	0.39	0.39	0.007	0.719
Egg-breaking strength (kgf)					
Week 46	5.78	5.85	5.84	0.049	0.838
Week 48	5.76	5.78	5.74	0.071	0.975
Eggshell color					
Week 46	20.51	20.93	20.45	0.306	0.787
Week 48	20.98	20.63	20.41	0.286	0.722
Albumen height (mm)					
Week 46	8.42	8.52	8.55	0.026	0.171
Week 48	8.38	8.47	8.54	0.034	0.210
Haugh units					
Week 46	91.40	91.68	91.86	0.210	0.674
Week 48	90.89	91.13	91.33	0.273	0.804
Yolk color					
Week 46	6.81	6.87	6.80	0.026	0.549
Week 48	6.75	6.87	6.82	0.032	0.288

[1] Values are mean of six replicates per treatment.
[2] CON: Basal diet meets the recommended nutritional requirements for Hy-Line Brown; CSL 0.5: CON + 5 g/kg CSL; CSL 3.0: CON + 30 g/kg CSL.
[3] SEM = standard error of mean.

trend (P>0.05) was observed at week 48, with eggshell strength values of 5.76 kgf, 5.78 kgf, and 5.74 kgf for the CON, CSL 0.5, and CSL 3.0, respectively.

Eggshell color did not differ (P>0.05) among the treatments at week 46, with values of 20.51 in the CON, 20.93 in the CSL 0.5, and 20.45 in the CSL 3.0. Similarly, no differences (P>0.05) were found at week 48, with values of 20.98, 20.63, and 20.41 for the CON, CSL 0.5, and CSL 3.0, respectively. At week 46, the yolk color was not affected (P>0.05) by dried CSL supplementation, with values of 6.81, 6.87, and 6.80 for the CON, CSL 0.5, and CSL 3.0, respectively. At week 48, yolk color was 6.75 in the CON, 6.87 in the CSL 0.5, and 6.82 in the CSL 3.0, with no differences (P>0.05) among treatments.

Albumen height was also not different (P>0.05) among the treatments at week 46, with values of 8.42 mm in the CON, 8.52 mm in the CSL 0.5, and 8.55 mm in the CSL 3.0. Similarly, at week 48, albumen height were 8.38 mm, 8.47 mm, and 8.54 mm in the CON, CSL 0.5, and CSL 3.0, respectively, with no differences (P>0.05) observed. Haugh units at week 46 were 91.40 in the CON, 91.68 in the CSL 0.5, and 91.86 in the CSL 3.0, with no differences (P>0.05). The same trend (P>0.05) was observed at week 48, where Haugh units were 90.89, 91.13, and 91.33 for the CON, CSL 0.5, and CSL 3.0, respectively.

The results of the study indicate that supplementing 0.5% or 3% dried CSL to partially replace soybean meal did not significantly affect egg quality in layers. Similar findings have been reported in previous studies (Waldroup and Rutherford, 1971; Lilburn and Jensen, 1984), suggesting that dried CSL does not have a direct impact on egg quality. However, the lactic acid content in CSL has been suggested to lower intestinal pH, thereby enhancing calcium utilization and potentially improving eggshell quality (Jongbloed et al., 2000; Kim et al., 2015). Additionally, CSL supplementation has been associated with increased xanthophyll intake, which could enhance yolk color (Waldroup and Rutherford, 1971). Nevertheless, in the present study, no changes in egg quality were observed following CSL supplementation, which may be attributed to differences in inclusion levels of CSL, ingredients in the diet, or experimental period. Based on the study, supplementing 0.5% or 3% CSL in layers diet to partially replace soybean meal did not improve egg quality.

SUMMARY

This study evaluated the effects of 0.5% or 3% dried CSL as an alternative protein source in layer diets, hypothesizing that partially replacing soybean meal with CSL would maintain or enhance production performance. The results demonstrated that supplementing 3% CSL improved laying performance, while 0.5% CSL had no effect. Additionally, despite the partial replacement of soybean meal, egg quality remained stable, suggesting that 0.5% or 3% CSL might be incorporated into layer diets without compromising egg quality. Therefore, supplementing 3% dried CSL partially replacing soybean meal in layer diets may enhance laying performance. However, as

this study did not analyze certain chemical compositions of CSL, such as lactic acid, the mechanisms underlying the observed improvements remain unclear. Moreover, reducing soybean meal in the diet could also affect the nitrogen content of the feed, which may have an environmental impact. Thus, future studies should consider not only production performance but also the environmental impact such as nitrogen excretion. In addition, studies with longer experimental periods and higher CSL inclusion levels are needed to better assess its efficacy and fully clarify its role in laying hen nutrition.

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