Effects of Dietary Protein Levels on Production and Characteristics of Japanese Quail Eggs

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Three different lines (normal, brown and crossbred) of Japanese quail were used in this study to examine the effects of dietary protein level on the production and quality of eggs. In all lines, poor egg production was evident throughout the experiment on a diet with 16% crude protein (CP). Egg production increased as the CP levels of diets increased. Until 32 weeks, normal (N) and brown (B) lines showed higher egg production at 24% CP, while the crossbred (BN) line showed higher egg production at 24% CP. The cumulative egg production at 24% CP was higher than or similar to that at 26% CP. A comparison of egg production among different lines showed that BN produced significantly (P<0.05) more eggs than N at the lower protein levels of 20% and 22% CP. Weight of eggs was lowest in all lines at 16% CP, and tended to increase with increasing protein level. No difference in egg weight was observed among lines. Egg weight and yolk color were significantly (P<0.05) affected by different levels of protein. It appears that 24% CP was optimal for higher egg production and egg weight for N and B lines and 22% CP for BN lines. Furthermore, our data indicate that heterosis was obtained in BN for egg production at the lower protein levels.

Key words : Japanese quail, line, dietary protein level, egg production, egg characteristics

Introduction

Japanese quails (*Coturnix coturnix japonica*) are, in addition to being raised for the purpose of producing eggs and meat, found useful as experimental animals because are small, arrive at puberty early, have a high breeding efficiency and are easily raised (Padgett and Ivey, 1959; Wilson *et al.*, 1961). The quail prefers and requires a high protein diet for optimum growth and reproduction (Howes, 1964; How and Beane, 1966; Shim and Vohra, 1984).

In recent years, with heightened interest in environmental issues, it has become apparent that a rearing method which reduces the burden on the environment is badly needed. Therefore, research to develop lower cost feed with reduced nitrogen excretion is important. Numerous quail studies have been conducted to investigate the effects of

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the protein content of normal and low-protein feed on the reduction of nitrogen excretion, and also the effects on egg production and egg weight. Reports of the protein requirements of laying quails have been published, but the reported values vary with the researcher (Table 1). Furthermore, the only quails used in these studies have been normal-line ones, and no other lines have ever been exanined. To address this, a comparison was made of the effects of different dietary protein levels on egg production and egg characteristics using normal-line, brown-line and a cross of brown and normal-line.

Materials and Methods

The Japanese quails used in this study were normal-line (N), brown-line (B), and crossbred (BN) female chicks. The BN line was produced by B males and N females. All animals were kept at the Laboratory of Animal Breeding and Genetics, Faculty of Agriculture, Okayama University. It is known that the inheritance of brown feathers is sex-linked, and all BN day-old female chicks had brown feathers (Wakasugi and Kondo, 1973). Hatched chicks were kept for 4 weeks in a 4-tier battery-type brooder equipped with heaters, then put in individual cages $(30 \times 40 \text{ cm}; 12 \text{ cm} \text{ high}, 16 \text{ chicks}$ per cage) and reared. The rearing density was 75.0 cm² per quail, and feed and water were supplied *ad libitum* throughout the experimental period. The rearing room was illuminated for 14 hours a day (6 : 00–20 : 00) and room temperature ranged from 22.5

CP (%)	Main protein sources	ME (kcal/kg)	CP/ME	Reference
20, 23	AM, BM, FM, SM	2,800,2,600	71.4,88.5	Tanaka <i>et al.</i> , 1966
25	AM, FM, SM	2,880	86.8	Vohra and Roudybush, 1972
20.4	AM, FM, SM	2,890	70.6	Begin and Insko, 1972
20.3	SM	2,690	75.5	Johri and Vohra, 1977
20	AM, FM, SM	2,600	76.9	Lee <i>et al.</i> , 1977
24.5	AM, FM, SM	3,000	81.7	Yamane et al., 1979
25	FM, SM	3,000	83.3	Sakurai, 1979
16	CSM, SM	3,150	50.8	Allen and Young, 1980
24	FM	3,004	79.9	Espinosa <i>et al.</i> , 1980
21	AM, SM	2,980	70.5	Arscolt and Pierson-Goeger, 1981
24	FM, SM	3,000	80.0	Sakurai, 1981
20	CSM, SM	3,250	61.5	Shwartz and Allen, 1981
20	BM, FM	2,600	76.9	Shim and Lee, 1982
20		3,000	66.7	NRC, 1985
24		2,800	85.7	JFS, 1992
19.4	FM, SM	2,900	65.8	Annaka <i>et al.</i> , 1993
22	FM, SM	2,870	76.7	Ohguchi et al., 1997
20	CGM, FM, SM	2,800	71.4	Minoguchi <i>et al.</i> , 2000

Table 1. Dietary CP and ME requirements for laying quails

AM : Alfalfa meal, BM : Blood meal, CGM : Corn gluten meal, CSM : Casein-soybean meal mixture, FM : Fish meal, SM : Soybean meal, SE : Sesame meal. to 32.0°C.

A commercial starter diet for quail (protein content 24% or more, Tokai-Kigyo Co., Ltd.) was fed to the quails until 5 weeks of age. From 5 weeks of age onward, diets with different protein levels (16, 18, 20, 22, 24, 26% CP) were fed until 32 weeks of age or until the end of this experiment. The protein levels of the above diets were adjusted based on the Standard Tables of Feed Composition in Japan (1995). The composition of diets is given in Table 2.

Egg production was monitored every day from 6 weeks of age onward, and was calculated as hen-day egg production. The egg weights were measured for a week using

	Protein level (%)					
Feed composition	16	18	20	22	24	26
Corn	67.2	64.5	61.8	58.7	55.2	50.8
Soybean meal	10.6	13.3	16.0	17.1	19.6	22.0
Corn gluten meal	4.0	4.0	5.0	5.0	6.0	6.0
Fish meal (65%)	1.0	2.0	2.5	4.0	4.5	5.5
Fish meal (60%)	1.0	2.0	2.5	4.0	4.5	5.5
Defatted rice bran	9.0	7.0	5.0	4.0	3.0	3.0
DL-methionine	0.1	0.1	0.1	0.1	0.1	0.1
Salt	0.2	0.2	0.2	0.2	0.2	0.2
Calcium carbonate	6.1	6.1	6.1	6.1	6.1	6.1
Calcium phosphate	0.7	0.7	0.7	0.7	0.7	0.7
Color-premix	0.1	0.1	0.1	0.1	0.1	0.1
Total content	100.0	100.0	100.0	100.0	100.0	100.0
Crude protein (%) ¹⁾	16.32	18.22	20.18	22.16	24.12	26.12
Crude fat (%)	3.22	3.23	3.23	3.23	3.22	3.18
Crude fiber (%)	2.68	2.62	2.57	2.50	2.50	2.56
Crude ash (%)	2.91	3.15	3.24	3.66	3.80	4.26
Calcium (%)	2.75	2.87	2.93	3.10	3.16	3.28
Phosphorus (%)	0.70	0.73	0.73	0.79	0.82	0.88
Metabolizable energy (kcal/kg)	2,809	2,821	2,823	2,822	2,817	2,792
Lysine (%) ²⁾	0.66	0.82	0.93	1.09	1.21	1.36
Methionine $(\%)^{2)}$	0.37	0.42	0.46	0.51	0.54	0.58
Threonine $(\%)^{2)}$	0.56	0.64	0.72	0.80	0.88	0.96

Table 2. Composition (upper panel) and ingredient contents (lower panel)

¹⁾ This is a result of measuring the protein level roughly $(16\sim26\%)$; the analyzed values turned out to be different : 15.91%, 17.93%, 19.90%, 21.42%, 23.28% and 25.48%, respectively.

²⁾ This is the analyzed value of lysine, methionine, and threonine : lysine 1.01%, 1.13%, 0.92%, 0.94%, 1.23% and 1.45%, methionine 0.55%, 0.67%, 0.58%, 0.47%, 0.54% and 0.73%, threonine 0.65%, 0.80%, 0.72%, 0.74%, 0.89% and 1.09% of dietary protein (16~26%).

an electronic scale (accuracy : 0.1 g) at 6, 10, 16, 20, 24, 28 and 31 weeks of age, and are presented as mean values. Also, at 20 weeks of age, egg characteristics such as the egg yolk ratio, albumen ratio, egg shell ratio, egg shape index, egg shell strength and egg yolk color were measured. After the measurement of the egg weight, the long and short axes of the eggs were measured using slide calipers (accuracy : 0.1 mm), and the egg shell strength was measured using an egg shell strength meter (accuracy : 0.1 kg/cm²; Fujihira Industry Co., Ltd.). The egg yolks were then taken out using scissors and tweezers, and weighed. The egg yolk color was determined macroscopically using a Roche Yolk Color Fan with 15 grades (Vuilleumier, 1969). The egg shells (including the egg shell membrane) were weighed after rinsing in water and drying for 24 hours at room temperature. The weight of the albumen was determined by subtracting the weight of the egg yolk and egg shell from the weight of the egg weight.

The significance of differences between dietary protein levels, and between lines was assayed by the Student's t-test.

Results

Cumulative egg production from 6 to 32 weeks of age is presented in Table 3. In all lines, poor egg production was evidient at 16% CP throughout the experimental period. Egg production increased throughout the experimental period. Egg production increased as the CP levels of diets increased. Consistently higher egg production was maintained at 24% CP in N and B lines, and at 22% for BN line. In this experiment, egg production at the highest protein level (26%) was not always higher than that at 24% CP. A comparison of cumulative egg production between lines shows that BN values at 20% CP are significantly higher than N or B values, and at 22% CP. BN values are significantly higher than N values. Differences in egg production between lines were observed at 20% and 22% CP. Egg production was highest in BN followed by B and then N lines.

Weights of eggs in different lines at different protein levels are presented in Table 4. In all lines, the egg weight was lowest at 16% CP and tended to increase with increasing protein level. At 24% CP, especially in B and BN, the egg weight always showed high values. There were no significant differences in egg weight between different lines at the same feed protein level.

Different egg characteristics at 20 weeks of age are summarized in Table 5. It was evident that 26% CP was not always best for egg quality. There were no significant differences in egg weight between different lines at the same feed protein level. No significant differences in egg white weight, egg shape index and egg shell strength were observed between protein levels and between lines, indicating that level of protein in diet did not have any effect on these egg characteristics. Egg shell weight and yolk color were significantly (P < 0.05) affected by different levels of protein in all lines. Egg yolk color increased as CP level increased, while no consistent changes in egg shell weight with protein level were observed. The brightness of the egg yolk color in N, B and BN was lowest at 16% CP.

Line	Protein level	Weeks of age					
Line	(%)	6~10	10~20	20~32	6~32		
	16	37.4^{aA}	50.3^{aA}	33.6^{aA}	45.6^{aA}		
	18	46.7^{bA}	61.6^{bAB}	42.8^{bA}	53.0^{aA}		
Ν	20	67.9^{cA}	$63.0^{\rm bcA}$	62.5^{cA}	63.7^{bA}		
IN	22	51.3^{bA}	71.7^{cA}	62.3^{cA}	64.6^{bA}		
	24	66.5^{cA}	81.7^{dA}	81.1^{dA}	78.7^{cA}		
	26	65.6^{cA}	81.6^{dA}	72.2^{cdA}	79.1^{cA}		
	16	53.3^{aAB}	49.7 ^{aA}	38.0^{aAB}	46.5^{aB}		
	18	64.1^{abB}	59.4^{bB}	56.5^{bB}	59.3^{bA}		
В	20	70.3^{bA}	72.1^{cB}	53.4^{bB}	64.6^{bA}		
D	22	75.9^{bB}	83.7^{dB}	71.4^{cdAB}	78.3^{cB}		
	24	67.2^{bA}	86.9^{dA}	80.4^{cA}	80.9^{cA}		
	26	73.9^{bA}	79.6^{cdA}	67.2^{dA}	74.8^{cB}		
	16	47.5^{aB}	52.4^{aA}	41.8 ^{aB}	47.4^{aB}		
	18	61.8^{bB}	68.3^{bA}	41.9^{aA}	59.0^{bA}		
BN	20	61.3^{bA}	74.3^{bB}	70.7^{bA}	70.5^{cB}		
DIN	22	73.7^{bB}	87.2^{cB}	76.5^{bB}	80.5^{dB}		
	24	69.6^{bA}	84.6^{cA}	78.0^{bA}	79.6^{dA}		
	26	66.7^{bA}	86.9^{cA}	$69.7^{\rm bA}$	76.3^{cdB}		

Table 3. Effects of protein levels on egg production (%)

 $^{\rm a\sim d)}$ Values having different small superscripts are significantly different (p<0.05) between protein levels.

 $^{A\sim B)}$ Values having different capital superscripts are significantly different (p<0.05) between lines.

Discussion

Numerous reports have been published on the effects of dietary protein level on egg production in the Japanese quail. In most of those reports, egg production was found to be low at low protein levels and high at high protein levels. In this study, too, at 16% CP, the lowest protein level, significantly lower egg production values were observed than at 24% or 26% CP, suggesting that at 16% CP protein is not adequate for egg production. Also, in the comparison of egg production at different levels of protein, 26% CP did not reveal any superiority among different lines. These results are in agreement with the report by Sakurai (1979 b), who showed that egg production did not increase as a result of feeding the animals more protein than required. BN and B quails produced significantly (P < 0.05) more eggs than N quails. It is known that in chickens, too, egg production differs between lines at different dietary protein levels (Harms and Waldroup, 1962; Moreng *et al.*, 1964; Deaton and Quisenberry, 1965; Sharpe and Morris, 1965; Harms *et al.*, 1966; Lillie and Denfon, 1967), and the results of this study also suggest that quail egg production differs between lines at different protein

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Line	Protein level (%)	Weeks of age					
Line		8~9	20~21	31~32			
	16	$7.7 \pm 0.6^{a1),2)}$	$8.3{\pm}0.6^{a}$	8.4 ± 1.2^{a}			
	18	$8.8{\pm}0.9^{\mathrm{b}}$	9.1 ± 0.9^{b}	9.4 ± 0.3^{a}			
Ν	20	$9.1 \pm 0.8^{\rm bc}$	$9.6 \pm 0.8^{\circ}$	$10.5 \pm 0.5^{ m b}$			
IN	22	9.2 ± 0.9^{b}	$10.1 \pm 0.8^{\circ}$	$11.0 \pm 1.0^{\rm bc}$			
	24	$8.8\pm0.7^{\mathrm{bc}}$	$9.8 \pm 0.9^{\circ}$	$11.1 \pm 0.6^{\circ}$			
	26	$9.2 \pm 0.9^{\circ}$	$9.9 \pm 1.1^{\circ}$	10.2 ± 1.1^{b}			
	16	7.9±0.9ª	8.1 ± 0.8^{a}	8.7±0.7ª			
	18	$8.6\pm0.6^{ m b}$	$8.5 {\pm} 1.0^{ab}$	$9.3 \pm 0.7^{\rm b}$			
В	20	$8.8{\pm}0.7^{ m b}$	$9.3 \pm 0.8^{\circ}$	$10.2 \pm 0.6^{\circ}$			
D	22	$8.8 \pm 0.6^{\mathrm{b}}$	$8.9\pm0.8^{\mathrm{bc}}$	9.4 ± 1.1^{b}			
	24	$9.5 \pm 0.9^{\circ}$	10.4 ± 1.1^{d}	11.1 ± 1.0^{d}			
	26	$9.3{\pm}0.7^{\circ}$	10.2 ± 1.0^{d}	$10.7 {\pm} 0.3^{d}$			
	16	$8.2{\pm}0.7^{a}$	$8.4{\pm}0.8^{a}$	$9.2{\pm}0.7^{a}$			
	18	$9.0 \pm 0.6^{\rm b}$	9.4 ± 0.9^{b}	$10.4 \pm 0.7^{\rm bc}$			
BN	20	$8.9{\pm}0.6^{\mathrm{b}}$	9.2 ± 1.1^{b}	$9.9\pm0.8^{\mathrm{b}}$			
DIN	22	$9.1 \pm 0.6^{\text{b}}$	$9.9 \pm 0.7^{\circ}$	$10.6 {\pm} 0.8^{\circ}$			
	24	$9.1 \pm 0.6^{\circ}$	$10.0 {\pm} 0.6^{\circ}$	$10.5 {\pm} 0.7^{\circ}$			
	26	9.0 ± 0.6^{b}	$9.9 \pm 0.6^{\circ}$	$10.5 {\pm} 0.6^{\circ}$			

Table 4. Effects of protein levels on egg weight (g)

¹⁾ Mean \pm SD.

 $^{2)}\,Mean$ values for each week of age having different superscripts are significantly different (p $\!<\!0.05\!)$ between protein levels.

levels.

Minvielle *et al.* (1995, 1999, 2000) observed a heterosis effect in terms of egg production in crossbreds from lines selected for high early egg production under low dietary protein conditions (metabolizable energy 2,500 kcal/kg at 19% CP). Also the authors of this study demonstrated that the percentage of heterosis during the three periods $(10\sim20, 20\sim32, 6\sim32$ weeks of age) ranged from 9.9 to 22.0% and from 12.2 to 14.4% at 20% and 22% CP, respectively, indicating that BN quails achieved better egg laying results at low protein levels. It is considered that increasing utilization of dietary protein is responsible for the heterosis effect in egg production. Further studies are necessary to compare the utilization of dietary protein in BN, N and B lines.

The reported optimum protein requirement for the Japanese quail varies with the researcher (Table 1), but the results of the present experiment agree with the values reported by Yamane *et al.* (1979), Espinosa *et al.* (1980), Sakurai (1981) and JFS (1992). These results demonstrated that a dietary protein level of 24% was satisfactory for egg production and egg weight during the laying period and showed evidence of the existence of line differences as related to protein levels. Tanaka *et al.* (1966) and

Line	Protein level (%)	Egg yolk weight (%)	Egg white weight (%)	Egg shell weight (%)	Egg shape index (%)	Egg shell strength (kg/cm ²)	Egg yolk color ¹⁾
	16	$31.7\!\pm\!1.79^{\text{a2),3)}}$	$60.1 {\pm} 2.53$	$8.2{\pm}0.74^{ab}$	76.6±3.0	$0.9 {\pm} 0.2$	$6.0{\pm}1.0^{a}$
	18	$30.3 {\pm} 0.93^{\rm ab}$	$60.8 {\pm} 1.00$	$8.6{\pm}0.06^{a}$	75.7 ± 2.4	1.0 ± 0.2	$7.0{\pm}0.9^{ab}$
Ν	20	$30.6 {\pm} 1.01^{\text{ab}}$	$61.3 {\pm} 1.08$	$8.2 \pm 0.24^{\text{bB}}$	$74.6 {\pm} 3.7$	1.0 ± 0.1	$7.0 {\pm} 1.2^{ab}$
IN	22	30.5 ± 1.06^{ab}	$60.8 {\pm} 1.42$	8.7 ± 0.49^{ab}	75.1 ± 2.4	1.0 ± 0.2	$7.0{\pm}0.7^{ab}$
	24	$29.6 \pm 0.00^{\circ}$	$61.9 {\pm} 0.59$	8.5 ± 0.59^{abAB}	$74.3 {\pm} 2.2$	1.0 ± 0.2	8.0 ± 1.3^{b}
	26	$30.0 {\pm} 0.70^{ab}$	$61.7 {\pm} 0.85$	$8.3{\pm}0.58^{ab}$	73.4 ± 3.8	1.0 ± 0.2	8.0 ± 1.3^{b}
	16	29.9 ± 0.90	$61.8 {\pm} 0.17$	$8.3{\pm}0.78^{\mathrm{b}}$	75.8 ± 3.7	1.0 ± 0.2	$5.0 {\pm} 0.9^{a}$
	18	29.7 ± 0.54	$61.8 {\pm} 0.97$	$8.5 \pm 0.43^{ m b}$	73.3 ± 3.2	1.0 ± 0.2	$6.0{\pm}0.6^a$
D	20	31.6 ± 0.69	61.1 ± 0.45	7.4 ± 0.35^{aA}	$73.5 {\pm} 2.6$	0.9 ± 0.2	$7.0 {\pm} 1.2^{ab}$
В	22	30.5 ± 0.42	$61.3 {\pm} 0.22$	$8.2{\pm}0.63^{ab}$	73.3 ± 3.2	1.0 ± 0.2	$7.0 {\pm} 1.0^{ab}$
	24	30.0 ± 0.39	61.0 ± 0.89	$8.9{\pm}0.50^{\scriptscriptstyle \rm bB}$	74.4 ± 3.3	1.1 ± 0.2	8.0 ± 1.1^{b}
	26	29.8 ± 1.09	$61.8 {\pm} 1.44$	$8.4{\pm}0.37^{ m b}$	71.5 ± 2.9	1.0 ± 0.2	8.0 ± 1.0^{b}
BN	16	30.6 ± 0.42	$60.7 {\pm} 0.47^{ab}$	$8.7 {\pm} 0.78^{\rm ab}$	73.3 ± 3.7	1.0 ± 0.2	$5.0 {\pm} 1.1^{a}$
	18	30.4 ± 1.71	61.1 ± 1.84^{ab}	$8.6{\pm}0.14^{\text{b}}$	$74.8 {\pm} 3.0$	1.1 ± 0.2	$5.0 {\pm} 1.4^{a}$
	20	$31.2 {\pm} 0.64$	$60.6 {\pm} 0.12^{a}$	$8.2{\pm}0.71^{abAB}$	76.9 ± 4.8	1.0 ± 0.2	$7.0 \pm 1.4^{\text{b}}$
	22	$30.1 {\pm} 0.51$	$61.5 {\pm} 0.66^{ab}$	8.4 ± 0.48^{ab}	$73.4 {\pm} 2.2$	1.0 ± 0.2	8.0 ± 1.0^{b}
	24	29.3 ± 1.76	$63.0 \pm 2.10^{\text{b}}$	7.7 ± 0.48^{aA}	74.8 ± 3.3	1.1 ± 0.2	8.0 ± 1.9^{b}
	26	$30.4 {\pm} 0.48$	$61.4{\pm}0.65^{ab}$	$8.3{\pm}0.22^{ab}$	73.8 ± 3.9	1.0 ± 0.2	8.0 ± 1.1^{b}

Table 5. Effects of protein levels on the egg characteristics at 20 weeks of age

¹⁾ The egg yolk-color is judged by Roche Yolk-Color Fan (1 \sim 15).

 $^{2)}$ Mean \pm SD.

^{3) a~b)} Values having different small superscripts are significantly different (p<0.05) between protein levels.

 $^{\rm A\sim B)}$ Values having different capital superscripts are significantly different (p < 0.05) between lines.

Sakurai (1979 a) reported that the egg production increased as the energy level of diets increased in the iso-protein diets. However, this experiment could not elucidate the precise effects of metabolizable energy content on the protein requirement.

Annaka, *et al.* (1993) reported that the egg weight increased with increasing protein level from 12.5 to 15.7% CP, after which it was nearly constant up to 21.9% CP, finally increasing slowly up to 28.9% CP. In the present experiment, the egg weight showed the lowest values at the low protein level of 16% CP, whereas it tended to show high values with increased CP content. This seems to show that at levels below 24% CP, the protein is used for egg laying in preference to an increase in egg weight, and at levels above 24% CP the protein is used for both, or excess protein after the achievement of egg production is used for increasing the egg weight.

No evident correlation was observed between protein levels and the egg yolk ratio, albumen ratio and egg shell ratio. In chickens, Gardner and Young (1972) reported that increasing the dietary protein level from 12% to 18% produced a significant

increase in the relative proportion of egg yolk, and a subsequent significant decrease in the proportion of egg shell. However, when comparisons were made among dietary protein levels from 9.3% to 20.5% (Fisher, 1969) and from 14% to 20% (Yamagami and Kobayashi, 1983), no significant differences were found in egg composition. The results of our experiment agree with the findings by Fisher (1969), and Yamagami and Kobayashi (1983) in chickens. The egg yolk color correlated with protein content; in all lines, the egg yolk color tended to lighten with decreasing protein level. It is known that egg yolk color is a result of dietary carotenoids transferred to the egg yolk, and most of the color is from xanthophylls, and partly carotene and cryptoxanthin (Guenther et al., 1973). Yellow corn, corn gluten meal, etc., are dietary sources of xanthophlls. Corn gluten meal contains 5-8 times the xanthophylls of yellow corn (Scott et al., 1968; Karunajeewa et al., 1984). In the present experiment, it is presumed that corn gluten meal intake contributed greatly to the lightening of the egg yolk color at low protein levels. Hereafter, in order to have laying quails exhibit excellent egg production even with low-protein feed, it seems crucial to select high egg production lines and supply them with low-protein feed that is supplemented with essential amino acids and coloring agents.

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