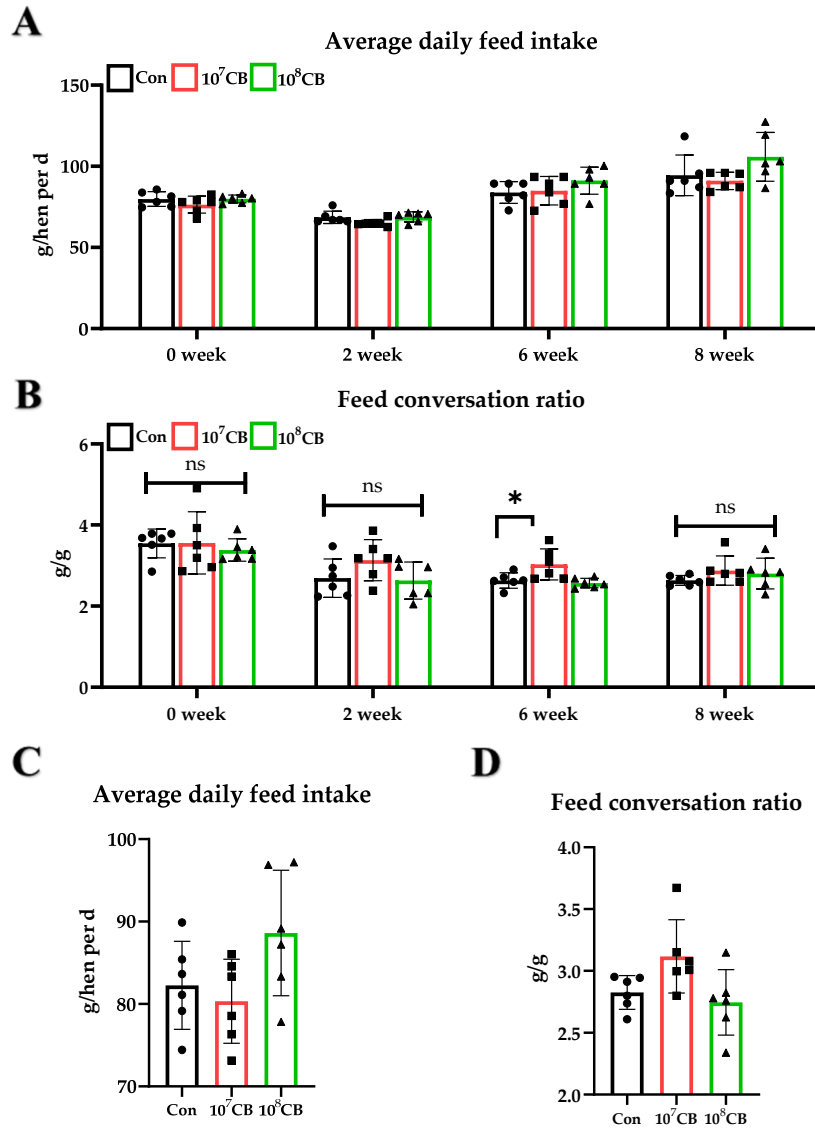
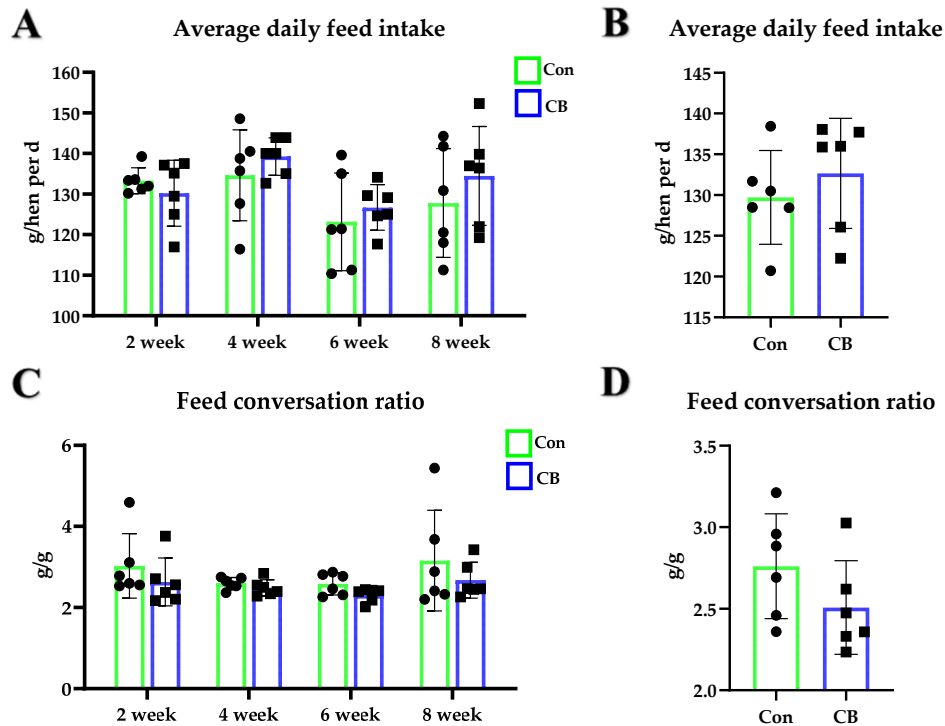


Supplementary Materials:



**Figure S1.** Effects of *Clostridium butyricum* (CB) on feed intake and feed conversion ratio of Luhua layer hens. (A, B) Changes in feed intake and feed conversion ratio of laying hens over time. (C) Feed intake from 1-8 weeks. (D) Feed conversion ratio from 1-8 weeks. Con: Control group fed basal diet;  $10^7$ CB: basal diet supplemented with  $1 \times 10^7$  CFU/kg CB;  $10^8$ CB: basal diet supplemented with  $1 \times 10^8$  CFU/kg CB. The data are presented as the mean  $\pm$  SD. \*,  $P < 0.05$ , ns, no difference.



**Figure S2.** Effect of *Clostridium butyricum* (CB) on feed intake and feed conversion ratio of Hy-line Brown layer hens. (A, B) Changes in feed intake and feed conversion ratio of laying hens over time. (C) Feed intake from 1-8 weeks. (D) Feed conversion ratio from 1-8 weeks. Con: Control group fed basal diet; CB: basal diet supplemented with  $1 \times 10^8$  CFU/kg CB. The data are presented as the mean  $\pm$  SD.

**Table S1.** Effects of *Clostridium butyricum* (CB) on feed intake and growth of Green-shell layer hens<sup>1</sup>

Productive performance	Treatments			P =	F =
	Con	10 <sup>8</sup> CB	10 <sup>9</sup> CB		
Body weight (g)					
28 days	305.75 $\pm$ 5.72	302.13 $\pm$ 4.85	305.38 $\pm$ 5.93	0.8784	F(2,21) =0.13
Feed intake (g/hen/day)					
0-28 day	207.16 $\pm$ 0.86 <sup>a</sup>	199.75 $\pm$ 2.11 <sup>b</sup>	199.61 $\pm$ 1.97 <sup>b</sup>	0.0206	F(2,9) =6.16

<sup>1</sup> The data are presented as the mean  $\pm$  SD. Con: Control group, fed basal diet; 10<sup>8</sup>CB: basal diet supplemented with  $1 \times 10^8$  CFU/kg CB; 10<sup>9</sup>CB: basal diet supplemented with  $1 \times 10^9$  CFU/kg CB.

<sup>a,b</sup> Means sharing different letters in the same row are significantly different ( $P < 0.05$ ).

**Table S2.** Effects of *Clostridium butyricum* (CB) on organ development of Green-shell layer hens<sup>1</sup>

	Control	10 <sup>8</sup> CB	10 <sup>9</sup> CB	P-value	F-value
Liver index, %	3.02 $\pm$ 0.17	2.71 $\pm$ 0.07	2.81 $\pm$ 0.06	0.1606	F(2,21) =2.00
Spleen index, %	0.27 $\pm$ 0.03	0.30 $\pm$ 0.03	0.28 $\pm$ 0.02	0.8175	F(2,21) =0.20
Bursa of Fabricius index, %	0.48 $\pm$ 0.03	0.56 $\pm$ 0.08	0.45 $\pm$ 0.03	0.2993	F(2,21) =1.28
Thymus index, %	0.8 $\pm$ 0.061	0.95 $\pm$ 0.153	0.843 $\pm$ 0.051	0.5739	F(2,21) =0.57

Duodenum length, cm	18.59±0.6	17.83±0.7	17.43±0.5	0.3831	F <sub>(2,21)</sub> =1.00
Jejunum length, cm	37.78±1.9	36.36±1.8	34.71±0.8	0.4017	F <sub>(2,21)</sub> =0.95
Ileum length, cm	42.44±1.8	42.45±1.0	40.83±0.9	0.6045	F <sub>(2,21)</sub> =0.51
Cecum length, cm	9.26±0.34	9.25±0.21	9.31±0.35	0.9886	F <sub>(2,21)</sub> =0.01

<sup>1</sup> The data are presented as the mean ± SD. Con: Control group, fed basal diet; 10<sup>8</sup>CB; basal diet supplemented with 1×10<sup>8</sup> CFU/kg CB; 10<sup>9</sup>CB: basal diet supplemented with 1×10<sup>9</sup> CFU/kg CB.

**Table S3.** Effect of *Clostridium butyricum* (CB) on production performance of Luhua layer hens <sup>1</sup>.

Productive performance	Treatments			P =	F =
	Con	10 <sup>7</sup> CB	10 <sup>8</sup> CB		
Egg production					
rate (%)					
0 week	63.24±2.25	59.67±3.48	64.43±2.20	0.4523	F(2,15) =0.84
2 week	63.84±3.39	51.64±4.02	63.99±4.80	0.0815	F(2,15) =2.98
4 week	70.08±2.84 <sup>b</sup>	64.48±1.74 <sup>b</sup>	84.28±1.74 <sup>a</sup>	0.0001	F(2,15)=22.68
6 week	77.93±3.32 <sup>a</sup>	65.96±3.20 <sup>b</sup>	82.97±2.00 <sup>a</sup>	0.0026	F(2,15) =9.05
8 week	78.17±3.27 <sup>a</sup>	67.74±3.08 <sup>b</sup>	80.40±2.09 <sup>a</sup>	0.0153	F(2,15) =5.59
overall period	67.13±1.40 <sup>a</sup>	58.15±2.63 <sup>b</sup>	71.92±0.97 <sup>a</sup>	0.0003	F(2,15)=14.95
Average egg weight (g)					
0 week	35.80±0.54	36.87±0.69	36.83±0.22	0.2920	F(2,15) =1.34
2 week	40.64±0.48 <sup>b</sup>	41.25±0.32 <sup>ab</sup>	41.93±0.30 <sup>a</sup>	0.0798	F(2,15) =3.01
4 week	42.25±0.72	43.50±0.65	44.08±0.40	0.1259	F(2,15) =2.39
6 week	44.24±0.59 <sup>b</sup>	46.12±0.54 <sup>a</sup>	45.97±0.29 <sup>a</sup>	0.0287	F(2,15) =4.54
8 week	45.80±0.63	47.13±0.50	47.00±0.40	0.1729	F(2,15) =1.98
overall period	43.39±0.53 <sup>b</sup>	44.63±0.42 <sup>ab</sup>	44.93±0.33 <sup>a</sup>	0.0562	F(2,15) =3.51
Average daily feed intake(g/hen per d)					
0 week	79.80±1.85	76.35±2.13	79.88±1.00	0.2881	F(2,15) =1.35
2 week	68.53±1.55	65.03±0.91	68.82±1.27	0.0946	F(2,15) =2.77
6 week	83.85±2.74	84.95±3.57	91.13±3.41	0.2655	F(2,15) =1.45
8 week	94.44±5.08	91.01±2.18	105.85±6.12	0.1024	F(2,15) =2.66
overall period	82.27±2.18 <sup>ab</sup>	80.33±2.08 <sup>b</sup>	88.60±3.10 <sup>a</sup>	0.0801	F(2,15) =3.00
Feed conversion ratio(g/g)					
0week	3.55±0.14	3.56±0.31	3.38±0.11	0.8051	F(2,15) =0.22
2 week	2.69±0.19	3.13±0.21	2.63±0.19	0.1756	F(2,15) =1.96
6 week	2.63±0.08 <sup>b</sup>	3.03±0.16 <sup>a</sup>	2.57±0.05 <sup>b</sup>	0.0138	F(2,15) =5.78
8 week	2.64±0.05	2.88±0.15	2.80±0.15	0.3984	F(2,15) =0.98
overall period	2.83±0.06 <sup>ab</sup>	3.12±0.12 <sup>a</sup>	2.75±0.11 <sup>b</sup>	0.0437	F(2,15) =3.89

<sup>1</sup> The data are presented as the mean ± SD. Con: Control group, fed basal diet; 10<sup>7</sup>CB; basal diet supplemented with 1×10<sup>7</sup> CFU/kg CB; 10<sup>8</sup>CB: basal diet supplemented with 1×10<sup>8</sup> CFU/kg CB.

<sup>a,b</sup> Means sharing different letters in the same row are significantly different (*P* < 0.05).

**Table S4.** Effect of *Clostridium butyricum* (CB) on egg quality of Luhua layer hens <sup>1</sup>.

Items	Con	10 <sup>7</sup> CB	10 <sup>8</sup> CB	P =	F =
<b>2Week</b>					
Egg shape index, %	1.32±0.009	1.32±0.035	1.29±0.02	0.5512	F(2,15) =0.62
Eggshell thickness, 0.01mm	31.71±1.2	32.43±0.83	33.04±0.82	0.5189	F(2,15) =0.69
Eggshell index, %	10.83±0.23	11.09±0.16	11.26±0.22	0.4251	F(2,15) =0.91
Eggshell hardness, N	37.55±1.91	35.53±1.58	39.48±1.13	0.2125	F(2,15) =1.72
Albumen height, mm	4.3±0.10	4.1±0.18	4.35±0.19	0.4989	F(2,15) =0.73
Egg yolk color	8.66±0.11	9.01±0.20	8.59±0.30	0.1930	F(2,15) =1.84
Yolk Index, %	31.73±0.52	28.83±1.33	30.92±0.65	0.3395	F(2,15) =1.16
Haugh unit	71.91±0.98	70.03±1.75	72.19±1.9	0.5704	F(2,15) =0.58
<b>4Week</b>					
Egg shape index, %	1.3±0.017	1.08±0.063	1.29±0.01	0.0739	F(2,15) =3.11
Eggshell thickness, 0.01mm	29.98±0.57	30.8±0.82	32.27±1.24	0.4521	F(2,15) =0.84
Eggshell index, %	9.71±0.16	9.82±0.23	9.95±0.18	0.6010	F(2,15) =0.53
Eggshell hardness, N	39.11±0.95	36.74±2.15	38.71±1.74	0.6341	F(2,15) =0.47
Albumen height, mm	5.14±0.28	4.56±0.28	4.59±0.20	0.1914	F(2,15) =1.85
Egg yolk color	8.56±0.14	9.12±0.36	9.12±0.47	0.5145	F(2,15) =0.69
Yolk Index, %	31.03±0.63	30.41±0.49	29.56±0.48	0.1014	F(2,15) =2.68
Haugh unit	76.49±2.21	69.52±2.33	71.63±1.59	0.0880	F(2,15) =2.87
<b>6Week</b>					
Egg shape index, %	1.3±0.013	1.32±0.015	1.3±0.09	0.05757	F(2,15) =0.57
Eggshell thickness, 0.01mm	30.67±0.073	31.61±0.7	31.31±0.79	0.7100	F(2,15) =0.35
Eggshell index, %	11.94±0.36	12.32±0.29	12.27±0.19	0.5484	F(2,15) =0.63
Eggshell hardness, N	37.22±2.66	37.44±1.88	39.85±1.76	0.7031	F(2,15) =0.36
Albumen height, mm	5.02±0.28	4.94±0.44	5.03±0.19	0.9196	F(2,15) =0.08
Egg yolk color	8.79±0.30	9.54±0.13	9.11±0.09	0.2112	F(2,15) =1.73
Yolk Index, %	30.04±0.50	29.64±0.56	29.49±0.43	0.3530	F(2,15) =1.12
Haugh unit	74.82±2.36	72.01±2.9	74.26±1.39	0.9463	F(2,15) =0.06
<b>8Week</b>					
Egg shape index, %	1.34±0.008	1.33±0.014	1.31±0.013	0.3542	F(2,15) =1.11
Eggshell thickness, 0.01mm	30.31±0.60 <sup>b</sup>	33.3±0.67 <sup>a</sup>	31.26±0.50 <sup>b</sup>	0.0033	F(2,15) =8.54
Eggshell index, %	10.43±0.26	10.53±0.26	10.26±0.19	0.6912	F(2,15) =0.38
Eggshell hardness, N	31.25±2.5	37.53±1.73	36.65±1.84	0.2443	F(2,15) =1.55
Albumen height, mm	4.22±0.21	4.46±0.19	3.73±0.13	0.1445	F(2,15) =2.21
Egg yolk color	8.85±0.09	8.72±0.18	8.79±0.06	0.3768	F(2,15) =1.04
Yolk Index, %	33.21±0.59	32.71±0.44	32.26±0.60	0.2735	F(2,15) =1.42
Haugh unit	68.13±1.75 <sup>a</sup>	68.37±1.94 <sup>ab</sup>	61.98±1.44 <sup>b</sup>	0.0722	F(2,15) =3.15

<sup>1</sup> The data are presented as the mean  $\pm$  SD. Con: Control group, fed basal diet; 10<sup>7</sup>CB: basal diet supplemented with 1 $\times$ 10<sup>7</sup> CFU/kg CB; 10<sup>8</sup>CB: basal diet supplemented with 1 $\times$ 10<sup>8</sup> CFU/kg CB.

<sup>a,b</sup> Means sharing different letters in the same row are significantly different ( $P < 0.05$ ).

**Table S5.** Effect of *Clostridium butyricum* (CB) on production performance of Hy-line Brown layer hens <sup>1</sup>.

Productive performance	Treatments		p values	F values
	Con	CB		
Egg production rate (%)				
2 week	81.55 $\pm$ 6.80	86.31 $\pm$ 6.08	0.6130	F(1,10) =0.27
4 week	87.50 $\pm$ 3.02	91.07 $\pm$ 3.02	0.4231	F(1,10) =0.70
6 week	81.55 $\pm$ 3.12	88.69 $\pm$ 3.62	0.1657	F(1,10) =2.24
8 week	73.81 $\pm$ 7.18	83.33 $\pm$ 5.95	0.3313	F(1,10) =1.04
overall period	82.98 $\pm$ 4.17	88.93 $\pm$ 2.84	0.2653	F(1,10) =1.39
Average egg weight (g)				
2 week	56.47 $\pm$ 0.62	59.26 $\pm$ 1.43	0.1035	F(1,10) =3.21
4 week	59.33 $\pm$ 0.83 <sup>b</sup>	61.90 $\pm$ 1.25 <sup>a</sup>	0.1185	F(1,10) =2.92
6 week	58.78 $\pm$ 0.67	62.14 $\pm$ 0.98	0.0175	F(1,10) =8.07
8 week	59.29 $\pm$ 0.87	61.65 $\pm$ 0.92	0.0903	F(1,10) =3.51
overall period	58.52 $\pm$ 0.56	61.26 $\pm$ 1.02	0.0399	F(1,10) =5.57
Average daily feed intake(g/hen per d)				
2 week	133.27 $\pm$ 1.31	130.21 $\pm$ 3.31	0.4096	F(1,10) =0.74
4 week	134.61 $\pm$ 4.57	139.26 $\pm$ 1.88	0.3698	F(1,10) =0.88
6 week	123.15 $\pm$ 4.91	126.70 $\pm$ 2.29	0.5284	F(1,10) =0.43
8 week	127.80 $\pm$ 5.47	134.46 $\pm$ 4.98	0.3889	F(1,10) =0.81
overall period	129.71 $\pm$ 2.34	132.66 $\pm$ 2.76	0.4345	F(1,10) =0.66
Feed conversionratio(g/g)				
2 week	3.029 $\pm$ 0.32	2.632 $\pm$ 0.24	0.3494	F(1,10) =0.96
4 week	2.598 $\pm$ 0.06	2.485 $\pm$ 0.08	0.2909	F(1,10) =1.24
6 week	2.581 $\pm$ 0.11	2.313 $\pm$ 0.07	0.0669	F(1,10) =4.22
8 week	3.158 $\pm$ 0.51	2.676 $\pm$ 0.18	0.3916	F(1,10) =0.80
overall period	2.761 $\pm$ 0.13	2.507 $\pm$ 0.12	0.1795	F(1,10) =2.08

<sup>1</sup> The data are presented as the mean  $\pm$  SD. Con: Control group, fed basal diet; 10<sup>7</sup>CB: basal diet supplemented with 1 $\times$ 10<sup>7</sup> CFU/kg CB; 10<sup>8</sup>CB: basal diet supplemented with 1 $\times$ 10<sup>8</sup> CFU/kg CB; SB: basal diet supplemented with 1% sodium butyrate.

<sup>a,b</sup> Means sharing different letters in the same row are significantly different ( $P < 0.05$ ).

**Table S6.** Effect of *Clostridium butyricum* (CB) on egg quality of Hy-line Brown layer hens <sup>1</sup>.

Items	Con	CB	P =	F =
<b>2Week</b>				
Egg shape index, %	1.3 $\pm$ 0.007	1.28 $\pm$ 0.009	0.1077	F(1,10) =3.12
Eggshell thickness, 0.01mm	36.73 $\pm$ 0.92	36.2 $\pm$ 0.68	0.8303	F(1,10) =0.05
Eggshell index, %	12.05 $\pm$ 0.17	11.85 $\pm$ 0.15	0.8194	F(1,10) =0.05
Eggshell hardness, N	48.82 $\pm$ 1.08	47.73 $\pm$ 1.04	0.3781	F(1,10) =0.85
Albumen height, mm	6.33 $\pm$ 0.32	7.36 $\pm$ 0.46	0.0984	F(1,10) =3.32
Egg yolk color	7.82 $\pm$ 0.11	7.12 $\pm$ 0.28	0.0820	F(1,10) =3.74
Yolk Index, %	24.48 $\pm$ 0.40	25.06 $\pm$ 0.40	0.7165	F(1,10) =0.14
Haugh unit	79.10 $\pm$ 2.23	83.87 $\pm$ 4.06	0.4189	F(1,10) =0.71
<b>4Week</b>				

Egg shape index, %	1.3±0.003 <sup>a</sup>	1.27±0.006 <sup>b</sup>	0.0003	F(1,10) =30.30
Eggshell thickness, 0.01mm	35.44±0.49	34.65±0.4	0.4094	F(1,10) =0.74
Eggshell index, %	11.64±0.11	11.45±0.09	0.6228	F(1,10) =0.26
Eggshell hardness, N	48.14±1.03	46.73±0.8	0.7504	F(1,10) =0.11
Albumen height, mm	7.3±0.15	7.5±0.25	0.3880	F(1,10) =0.81
Egg yolk color	7.56±0.20	7.94±0.19	0.3797	F(1,10) =0.84
Yolk Index, %	26.58±0.32	26.34±0.16	0.4831	F(1,10) =0.53
Haugh unit	85.41±0.85	85.15±1.81	0.9409	F(1,10) =0.01
<b>6Week</b>				
Egg shape index, %	1.3±0.005 <sup>a</sup>	1.27±0.007 <sup>b</sup>	0.0200	F(1,9) =7.64
Eggshell thickness, 0.01mm	35.97±0.72	36.54±0.5	0.6945	F(1,10) =0.16
Eggshell index, %	12.21±0.14	11.78±0.09	0.3965	F(1,10) =0.76
Eggshell hardness, N	45.67±1.95	43.32±1.77	0.4948	F(1,10) =0.50
Albumen height, mm	7.17±0.22	7.98±0.22	0.0928	F(1,10) =3.45
Egg yolk color	7.75±0.45	6.71±0.19	0.0652	F(1,10) =4.29
Yolk Index, %	26.00±0.30	26.78±1.05	0.2955	F(1,9) =1.23
Haugh unit	84.32±1.73	87.75±1.58	0.3023	F(1,10) =1.18
<b>8Week</b>				
Egg shape index, %	1.3±0.004 <sup>a</sup>	1.28±0.008 <sup>b</sup>	0.0468	F(1,10) =5.14
Eggshell thickness, 0.01mm	34.23±0.56	34.27±0.4	0.9665	F(1,10) =0.00
Eggshell index, %	11.56±0.15	11.39±0.09	0.3018	F(1,10) =1.19
Eggshell hardness, N	44.98±1.36	40.98±1.39	0.1514	F(1,10) =2.41
Albumen height, mm	7.28±0.16	7.68±0.25	0.1015	F(1,10) =3.25
Egg yolk color	7.26±0.24	7.37±0.10	0.6359	F(1,10) =0.24
Yolk Index, %	27.55±0.30	26.69±0.18	0.3545	F(1,10) =0.94
Haugh unit	79.88±1.10 <sup>b</sup>	84.90±2.02 <sup>a</sup>	0.0207	F(1,10) =7.52

<sup>1</sup> The data are presented as the mean ± SD. Con: Control group, fed basal diet; CB: basal diet supplemented with 1×10<sup>8</sup> CFU/kg CB.

<sup>a,b</sup> Means sharing different letters in the same row are significantly different (*P* < 0.05).