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Effects of Dietary Calcium Lactate Supplementation on Laying Performance, Blood Index, Shinbone Quality, Jejunal Immunity, and Egg Quality of Aged Laying Hens

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Abstract: This study aimed to verify the potential of calcium lactate for the performance, blood index, shinbone quality, jejunal immunity, and egg quality of aged laying hens. A total of 360 62-week-old Hy-Line Brown laying hens were randomly divided into four treatments, with six replicates and 15 chickens per replicate. Experimental groups were fed with 0.25%, 0.5%, and 1.0% calcium lactate to substitute limestone in the control group (maintaining the same amount of calcium). The feeding trial lasted for 12 weeks. The laying rate and daily egg mass of laying hens fed the diets supplemented with calcium lactate was increased relative to those of the control group. The dietary addition of calcium lactate for laying hens enhanced the eggshell ratio, eggshell thickness, eggshell strength, and albumen height of eggs, and the addition level of 0.5% had the best effect. Dietary calcium lactate increased the number of red blood cells, corpuscular hemoglobin, mononuclear leucocytes and basophilic granulocytes, and decreased heterophils in the blood of laying hens. The activities of serum alanine transaminase and creatine kinase in laying hens was reduced by the dietary addition of calcium lactate. Calcium lactate supplementation in diets increased the serum calcium and phosphorus contents of laying hens. The dietary inclusion of calcium lactate increased the contents of IgA, IgG, lysozyme, and sIgA in the jejunal mucosa, and the 0.5% addition level worked best, but the IL-2 content decreased. The addition of 0.5% calcium lactate to the diet reduced the maximal force of the shinbone and increased the work required for shinbone rupture in laying hens. In conclusion, the dietary addition of calcium lactate improved the performance and egg quality of laying hens, probably by its positive effects on body health, intestinal digestible ability, calcium bioavailability, and jejunal mucosal immunity. The optimum amount of calcium lactate in the diet of laying hens is recommended to be 0.5%.

Keywords: calcium lactate; organic element; nutrient bioavailability; egg quality; laying hens

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1. Introduction

Extending the laying period of hens is an important way to increase the economic benefits of laying-hen production, which is seriously limited by the decreased laying rate and deteriorated egg quality, especially the poor eggshell quality, in the later stage of the laying cycle. A long and intense laying period usually leads to a general deterioration in the health of the hens [1], such as the gradual loss of structural bone, specifically cortical bone, whose sacrifice promotes the formation of medullary bone to provide calcium for the formation of eggshells [2]. The decline in bone quality is manifested as bone deformation, fracture, osteoporosis, cage fatigue, etc., especially in the case of insufficient dietary calcium and phosphorus intake [3,4]. About 30% of laying hens with osteoporosis have fractures [5]. Compared with healthy ones, laying hens with severe osteoporosis lose weight, bone calcium reserves lose 15–20%, and egg production decreases by 18% [6].

Therefore, improving the quality of eggshells and bone by increasing the calcium utilization rate is the key to delaying the elimination of old laying hens and enlarging the economic benefits of egg production.

Based on the physiological and biochemical changes in laying hens, the demand for calcium in laying hens gradually increases along with age. Feeding a diet low in calcium (1.5%) for 4 weeks at the peak of egg laying increased the egg breaking rate, decreased the feed intake and laying rate after 8 weeks, and reduced the weight and bone weight of laying hens after 12 weeks [7]. The increase in dietary calcium content from 3.5% to 4.5% in the late laying period improved the eggshell quality and breaking strength of eggs [8]. During eggshell formation, mineral precursors of calcium ions and HCO_3^- ions are continuously supplied from plasma through the epithelial transport of uterine adenocytes [9]. Therefore, improving the calcium bioavailability of laying hens is one effective way to improve the quality of eggshells.

In the late laying phase of laying hens, inorganic trace elements can be effectively replaced by low levels of compound organic trace elements [10]. A positive effect was observed in the productive performance, eggshell quality, and eggshell ultrastructure of laying hens fed diets supplemented with organic trace minerals [11,12]. At low levels, the replacement of inorganic trace minerals with organic trace minerals could improve the bioavailability of copper, zinc, iron, manganese, chromium, and calcium and the immunity of laying hens [13,14]. The dietary inclusion of the organic forms of Zn, Mn, and Cu at a dosage of 50% to 75% lower than NRC recommendations is sufficient to maintain laying performance and can improve the eggshell and albumen qualities of the eggs from laying hens [15]. Appropriate organic zinc (40 mg/kg) was added to the diet of laying hens to promote the apparent digestion of minerals and nutrients, trace element deposition, and mitochondrial mRNA expression [16]. Organic ferrous glycinate replaced inorganic ferrous sulfate, which is more beneficial to egg quality and iron enrichment, and does not affect the production performance of laying hens [17]. Based on a quadratic regression analysis of egg production performance, egg quality, egg iron content, and blood biochemical indexes, the optimal level of Fe-lys-glu to replace $FeSO_4$ in laying-hen diets was 45 mg Fe/kg [18]. It can be seen that the use of organic mineral trace elements can effectively increase the bio-utilization rate compared with using the inorganic form. Therefore, the development of suitable and efficient organic calcium is of importance for improving eggshell quality.

During the later stages of the laying cycle, the addition of calcium propionate and calcium butyrate to the diet of laying hens could improve eggshell quality [19]. As another source of organic calcium, calcium lactate is often used as a food additive to improve antioxidant properties and extend the shelf life of food [20]. Meanwhile, the safety of lactic acid and calcium lactate at appropriate concentrations has been demonstrated with technological additives in feed for all animal species [21]. From the above, it can be hypothesized that calcium lactate has great potential to be used in layer feed to improve the bioavailability of calcium and improve the quality of eggshells, which will be verified in the present study from the perspective of the laying performance, blood index, shinbone quality, jejunal immunity, and egg quality of aged laying hens.

2. Material and Methods

2.1. Experimental Design and Bird Management

A total of 360 62-week-old Hy-Line Brown laying hens were randomly divided into 4 experimental treatments, with 6 replicates and 15 chickens per replicate. The birds were managed according to the Hy-Line International Online Management Guide (2011) and the farm's own experience. Prior to the trial, chicks were given free access to water and a commercial corn–soy-based layer diet. The control diet was prepared in accordance with the nutritional requirements of the National Research Council of the United States (NRC, 1994) and the Chinese Chicken Feeding Standards (NY/T 33-2004) [22]. The formula and nutritional levels of the basic diet are shown in Table 1. Calcium lactate, a commercial product with a purity of over 99%, was used in the present study. Each treatment group was

fed with 0.25%, 0.5%, and 1.0% calcium lactate to substitute limestone (maintaining the same amount of calcium) in the control diet, and the mass increase caused by different molecular weights of the two calcium sources during substitution was balanced by decreasing the amount of zeolite powder. The calcium sources of each treatment group are shown in Supplementary Table S1. The pre-test period was 1 week to allow the chicks to gradually adapt to the change from commercial layer feed to trial feed.

Table 1. The ingredient composition and nutritional content of the basic diet.

Ingredient, %	Contents	Nutrients [1]	Contents
Corn	62.32	Crude protein, %	16.50
Soya bean meal	25.68	Calcium, %	3.50
Limestone	9.15	Total phosphorus, %	0.49
Calcium hydrogen phosphate	0.95	AME, MJ/kg	11.11
Sodium chloride	0.30	SID Methionine, %	0.43
Zeolite powder	1.15	SID Met + Cys, %	0.65
DL-methionine	0.18	SID Lysine, %	0.80
Phytase	0.02	SID Tryptophan, %	0.18
Premix [2]	0.25	SID Threonine, %	0.56
Total	100	SID Isoleucine, %	0.67

[1] Calculated values. [2] Premix provided the following per kg of the diet: VA: 12,500 IU; VD3: 4125 IU; VE: 15 IU; VK: 2 mg; VB1: 1 mg; VB2: 8.5 mg; VB6: 8 mg; VB12: 5 mg; calcium pantothenate: 50 mg; niacin: 32.5 mg; biotin: 2 mg; folic acid: 5 mg; choline: 500 mg; Mn: 65 mg; I: 1 mg; Fe: 60 mg; Cu: 8 mg; Zn: 66 mg.

During the feeding trial, all birds were housed in three-tier A-type cages in an environmentally controlled room with 3 birds per cage (40 cm × 40 cm × 35 cm) with 16 h of light per day. The room temperature was kept at 15–25 °C. The feeding experiment was conducted for 12 weeks, and the egg number, egg weight, abnormal egg number, and mortality of hens per replicate were recorded every day. The feed consumed by each replicate was recorded weekly. Average daily feed intake, average daily egg mass, average egg weight, and feed conversion ratio were calculated.

2.2. Sample Collection and Organ Index Measurement

At weeks 2, 4, 8, and 12 of the trial, five normal eggs were selected for each replicate. One hen per replicate was taken at the end of the experiment. The chicks were fasted for 12 h, 5 mL of wing vein blood was taken in a vacuum blood-collecting vessel with heparin anticoagulant, 7 mL was taken in a vacuum blood-collecting vessel without anticoagulant, the latter was centrifuged at 3000× *g* r/min for 15 min at room temperature, and serum was taken and stored at −20 °C. Selected birds were dissected under sterile conditions after bleeding from the neck. We weighed the pancreas, spleen, leg muscle, and shinbone of the birds and calculated their relative weight based on the ratio of their weight (g) to BW (g). The small intestine was excised and frozen immediately. The jejunum mucosa was scraped and stored at −20 °C.

2.3. Quality Detection of Egg and Shinbone

We separated the albumen and yolk for each egg and weighed them separately. After natural drying for 3 days, the albumen residue was removed and the eggshell was weighed. The weight proportions of albumen, yolk, and shell in the whole egg were calculated. The thickness of the eggshell was detected on the egg surface with an Eggshell thickness Gauge (ESTG1, Orka Technology Ltd., Ramat Hasharon, Israel) at 3 locations to obtain an average value, including the air chamber, the equator, and the tip. The measurement of eggshell strength was performed with an Egg Force Reader (Orka Technology Ltd., Ramat Hasharon, Israel). An egg analyzer (Orka Technology Ltd., Ramat Hasharon, Israel) was used to detect albumen height, Haugh units, and yolk color. The force parameters of the shinbone were measured using a texture analyzer (TMS-PRO, Food Technology Corporation, Mecmesin Ltd., Brighton, UK).

2.4. Chemical Analysis

Routine blood examination of anticoagulant blood samples was conducted using a fully automatic hematology analyzer (XP-100, Kobe, Japan, Sysmex Corporation). Jejunal mucosa was homogenized before testing. Biochemical parameters of serum and jejunal mucosal were detected with the ELISA method by an automatic biochemical analyzer (Model 7020, Hitachi, Tokyo, Japan) using commercial kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The former included total protein, albumin, globulin, total cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein, creatinine, urea, aspartate aminotransferase, alanine transaminase, total bilirubin, creatine kinase, calcium, and phosphorus. The latter included immunoglobulin A (IgA), immunoglobulin M (IgM), immunoglobulin G (IgG), interleukin 2 (IL-2), interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), and secretory immunoglobulin A (sIgA). The product information of kits used in the current study is listed in Supplementary Table S2. And the detection was strictly performed according to the manufacturer's instructions.

2.5. Statistical Analysis

Experimental data were analyzed using one-way ANOVA and Duncan's multiple comparisons of SAS 9.2 (SAS institute. Inc., Cary, NC, USA). $p \leq 0.05$ means significance.

3. Results

3.1. Laying Performance and Egg Quality

The performance of the laying hens is shown in Table 2. Over a period of 1 to 4 weeks, the addition of 1.0% calcium lactate to the diet can improve ($p \leq 0.05$) the laying rate of hens, but 0.25% and 0.5% had no effects ($p > 0.05$). Over 5~8 and 1~12 weeks, the laying rate of hens fed diets supplemented with 0.25%, 0.5% or 1.0% calcium lactate was increased ($p \leq 0.05$) relative to that of the control group. The average daily egg mass of laying hens over the periods of 1–4, 5–8, and 1–12 weeks was increased ($p \leq 0.05$) by the dietary inclusion of 0.25%, 0.5%, or 1.0% calcium lactate. The laying rate and average daily egg mass over 9~12 weeks were not influenced ($p > 0.05$) by dietary treatments. During the whole test period, the addition of calcium lactate to the diet had no effects ($p > 0.05$) on the average daily feed intake, average egg weight, feed-to-egg ratio, or abnormal egg rate of laying hens.

Table 2. Effects of dietary calcium lactate supplementation on the performance of laying hens.

	Ctrl	Calcium Lactate, %			SEM	*p*-Value
		0.25	0.5	1.0		
Weeks 1–4						
Laying rate, %	90.84 ^{b}	92.40 ^{ab}	92.22 ^{ab}	93.07 ^{a}	3.15	0.050
Average egg weight, g	58.76	58.55	59.18	58.94	0.82	0.284
Average daily feed intake, g	107.14	110.05	109.20	108.67	5.28	0.544
Average daily egg mass, g	52.78 ^{b}	55.30 ^{a}	54.60 ^{a}	55.16 ^{a}	1.76	0.035
Feed conversion ratio	2.03	1.99	2.00	1.97	0.07	0.270
Abnormal egg rate, %	0.98	1.07	1.08	1.24	0.80	0.871
Weeks 5–8						
Laying rate, %	86.84 ^{b}	90.93 ^{a}	89.16 ^{a}	90.89 ^{a}	2.67	0.028
Average egg weight, g	58.92	58.48	58.98	58.77	0.95	0.570
Average daily feed intake, g	111.03	111.14	110.43	111.11	3.31	0.152
Average daily egg mass, g	51.17 ^{b}	53.18 ^{a}	52.59 ^{a}	53.42 ^{a}	1.86	0.049
Feed conversion ratio	2.17	2.09	2.10	2.08	0.11	0.064
Abnormal egg rate, %	0.89	0.59	0.66	1.15	0.63	0.164

Table 2. *Cont.*

	Ctrl	Calcium Lactate, %			SEM	*p*-Value
		0.25	0.5	1.0		
Weeks 9–12						
Laying rate, %	81.11	83.61	83.12	81.85	2.99	0.089
Average egg weight, g	58.36	57.71	57.96	57.80	1.12	0.484
Average daily feed intake, g	110.77	109.05	105.51	104.55	7.18	0.291
Average daily egg mass, g	47.34	48.25	48.18	47.31	3.11	0.390
Feed conversion ratio	2.34	2.26	2.19	2.21	0.19	0.172
Abnormal egg rate, %	2.02	1.47	1.87	1.70	1.44	0.800
Weeks 1–12						
Laying rate, %	86.26 ^{b}	88.98 ^{a}	88.17 ^{a}	88.60 ^{a}	2.35	0.017
Average egg weight, g	58.68	58.25	58.71	58.50	0.92	0.590
Average daily feed intake, g	109.65	110.08	108.38	108.11	3.21	0.473
Average daily egg mass, g	50.43 ^{b}	52.24 ^{a}	51.79 ^{a}	51.96 ^{a}	2.42	0.048
Feed conversion ratio	2.18	2.11	2.10	2.09	0.10	0.075
Abnormal egg rate, %	1.30	1.04	1.20	1.36	0.83	0.775

^{a},^{b} values in one row with no common superscripts are statistically different ($p \leq 0.05$). SEM: standard error of means.

The egg quality of laying hens is presented in Table 3. At weeks 2 and 4 of the trial, there were no effects ($p > 0.05$) of the dietary addition of calcium lactate on the egg quality of laying hens, including the eggshell strength, egg shape index, yolk ratio, albumen ratio, eggshell ratio, thick/thin albumen, albumen height, yolk color, Haugh unit, and eggshell thickness. At weeks 8 and 12, dietary supplementation with 0.25%, 0.5%, or 0.10% calcium lactate for laying hens enhanced ($p \leq 0.05$) the eggshell strength and eggshell ratio of eggs, and the addition level of 0.5% had the best effect ($p \leq 0.05$) on the eggshell ratio. At week 12, the inclusion of calcium lactate in the diets of laying hens increased ($p \leq 0.05$) the eggshell thickness, and the addition level of 0.5% also increased ($p \leq 0.05$) the albumen height.

Table 3. Effects of dietary calcium lactate supplementation on egg quality in laying hens.

	Ctrl	Calcium Lactate, %			SEM	*p*-Value
		0.25	0.5	1.0		
Week 2						
Eggshell strength, N	43.36	43.29	45.86	44.12	2.18	0.236
Egg shape index	1.26	1.27	1.27	1.26	0.02	0.601
Yolk ratio	23.85	24.09	24.24	24.71	0.65	0.272
Albumen ratio	66.16	65.94	65.70	65.21	0.71	0.257
Eggshell ratio	9.99	9.97	10.06	10.08	0.18	0.751
Thick/thin albumen	3.62	3.42	3.53	3.68	0.46	0.616
Albumen height, mm	7.56	7.95	7.60	7.53	0.78	0.809
Yolk color	6.33	6.23	6.07	6.73	0.67	0.494
Haught unit	86.23	87.78	87.24	86.44	5.73	0.952
Eggshell thickness, mm	37.53	39.05	38.65	38.96	1.86	0.547
Week 4						
Eggshell strength, N	44.09	43.83	44.51	43.35	2.63	0.834
Egg shape index	1.26	1.27	1.27	1.27	0.01	0.360
Yolk ratio	24.94	24.93	25.41	24.99	0.72	0.681
Albumen ratio	64.60	64.76	64.19	64.72	0.77	0.635
Eggshell ratio	10.46	10.32	10.40	10.29	0.29	0.770
Thick/thin albumen	3.31	2.83	3.15	3.18	0.51	0.547
Albumen height, mm	7.49	7.31	7.60	7.67	0.41	0.532
Yolk color	5.73	5.97	6.20	6.20	0.46	0.346
Haught unit	86.52	85.59	87.12	87.61	2.36	0.561
Eggshell thickness, mm	37.10	38.83	39.05	38.71	1.86	0.342

Table 3. *Cont.*

	Ctrl	Calcium Lactate, %			SEM	*p*-Value
		0.25	0.5	1.0		
Week 8						
Eggshell strength, N	39.88 [b]	43.02 [a]	45.27 [a]	42.72 [a]	1.51	0.019
Egg shape index	1.28	1.28	1.28	1.28	0.01	0.822
Yolk ratio	23.93	24.71	23.21	24.86	1.53	0.555
Albumen ratio	66.53	65.25	66.56	65.23	1.49	0.541
Eggshell ratio	9.54 [c]	10.04 [b]	10.23 [a]	9.90 [b]	0.32	0.007
Thick/thin albumen	2.51	2.33	2.58	2.68	0.41	0.641
Albumen height, mm	7.37	7.13	7.17	7.56	0.47	0.472
Yolk color	5.50	5.57	6.12	6.07	0.59	0.232
Haught unit	85.99	84.20	84.77	87.30	2.79	0.332
Eggshell thickness, mm	36.65	38.43	39.07	38.31	1.84	0.219
Week 12						
Eggshell strength, N	41.25 [b]	44.82 [a]	44.06 [a]	43.94 [a]	1.81	0.014
Egg shape index	1.28	1.27	1.27	1.28	0.02	0.705
Yolk ratio	23.74	23.39	23.89	23.79	0.73	0.750
Albumen ratio	66.97	66.85	66.23	66.57	0.73	0.419
Eggshell ratio	9.28 [c]	9.76 [ab]	9.88 [a]	9.64 [b]	0.30	0.019
Thick/thin albumen	3.03	3.31	3.84	3.59	1.07	0.674
Albumen height, mm	7.21 [b]	6.98 [b]	7.03 [b]	7.89 [a]	0.47	0.007
Yolk color	5.34	5.39	5.98	5.92	0.59	0.189
Haught unit	85.85	84.07	84.76	87.16	2.32	0.217
Eggshell thickness, mm	36.12 [b]	38.02 [a]	38.65 [a]	37.85 [a]	1.44	0.050

[a],[b],[c] values in one row with no common superscripts are statistically different ($p \leq 0.05$). SEM: standard error of means.

3.2. Routine Blood Examination and Blood Biochemical Index

The routine blood indexes of laying hens are shown in Table 4. Dietary supplementation with 0.25% calcium lactate increased ($p \leq 0.05$) the number of red blood cells in the blood of laying hens. The mean corpuscular hemoglobin of blood was improved ($p \leq 0.05$) in laying hens fed the diet with the addition of 1.0% calcium lactate. The inclusion of 0.5% or 1.0% calcium lactate in diets decreased ($p \leq 0.05$) the percentage of heterophils and increase ($p \leq 0.05$) that of mononuclear leucocytes and basophilic granulocytes in the blood of laying hens. The dietary addition of calcium lactate showed no effects ($p > 0.05$) on the other indexes, including the number of white blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, platelets, red cell distribution width-standard deviation, red cell distribution width-coefficient of variation, platelet distribution width, mean platelet volume, platelet large cell ratio, lymphocytes, eosinophilic granulocytes, and immature granulocytes.

As presented in Table 5, more than a dozen serum biochemical indicators of laying hens were detected. Compared with the control group, the addition of 0.25% or 0.5% calcium lactate to the feed of laying hens reduced ($p \leq 0.05$) the serum alanine transaminase activity of laying hens, but there were no changes ($p > 0.05$) in the 1.0% addition group. The serum creatine kinase activity of laying hens was reduced ($p \leq 0.05$) by the addition of 0.5% or 1.0% calcium lactate in feed, with the former decreasing even more ($p \leq 0.05$). Calcium lactate supplementation in diets increased ($p \leq 0.05$) the serum calcium content of laying hens, and the increases in the 0.5% and 1.0% addition groups were higher ($p \leq 0.05$) than that in the 0.25% addition group. Relative to the control group, the addition of 0.5% or 1.0% calcium lactate increased ($p \leq 0.05$) the serum phosphorus content of laying hens, and the former effect was better ($p \leq 0.05$), while there were no changes ($p > 0.05$) in the 0.25% addition group. There were no changes ($p > 0.05$) in the contents of total protein, albumin, globulin, total cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein, creatinine, urea, total bilirubin, and aspartate aminotransferase activity in the serum of laying hens fed the diet supplemented with calcium lactate.

Table 4. Effects of dietary calcium lactate supplementation on routine blood indexes of laying hens.

	Ctrl	Calcium Lactate, %			SEM	*p*-Value
		0.25	0.5	1.0		
WBC	23.68	21.46	27.19	25.92	6.00	0.530
RBC	2.15 ^{b}	2.42 ^{a}	2.24 ^{ab}	2.12 ^{b}	0.15	0.026
Hemoglobin, g/L	64.87	73.50	66.83	67.00	6.11	0.172
Hematocrit, %	26.62	29.28	27.58	26.42	1.84	0.091
MCV, fL	125.15	121.08	123.13	124.80	3.01	0.161
MCH, pg	29.95 ^{b}	30.38 ^{ab}	29.77 ^{b}	31.57 ^{a}	1.03	0.041
MCHC, g/L	241.50	251.33	242.00	253.00	9.09	0.117
Platelets	10.83	18.00	12.83	23.83	16.95	0.713
RDW-SD, fL	34.95	33.55	35.52	34.47	2.38	0.697
RDW-CV, %	8.13	8.03	8.42	7.98	0.54	0.716
PDW, fL	12.27	13.25	10.75	11.07	3.35	0.635
MPV, fL	11.22	11.33	10.55	10.67	0.96	0.523
P-LCR, %	33.88	35.35	29.88	28.12	5.95	0.225
PCT, %	0.01	0.02	0.02	0.03	0.02	0.731
Heterophils, %	49.95 ^{a}	49.48 ^{a}	38.62 ^{b}	37.30 ^{b}	4.17	<0.001
Lymphocytes, %	35.63	35.42	34.90	34.50	1.27	0.564
MONO, %	4.90 ^{b}	4.93 ^{b}	7.27 ^{a}	7.95 ^{a}	1.72	<0.001
EO, %	0.33	0.15	0.47	0.57	0.30	0.246
BASO, %	9.18 ^{b}	10.92 ^{b}	18.75 ^{a}	19.68 ^{a}	3.29	<0.001
IG, %	0.00	0.01	0.01	0.00	0.01	0.791

^{a,b} values in one row with no common superscripts are statistically different ($p \leq 0.05$). WBC: white blood cell, RBC: red blood cell, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW-SD: red cell distribution width-standard deviation, RDW-CV: red cell distribution width-coefficient of variation, PDW: platelet distribution width, MPV: mean platelet volume, PCT: platelet large cell ratio, MONO: mononuclear leucocyte, EO: eosinophilic granulocyte, BASO: basophilic granulocyte, IG: immature granulocyte, SEM: standard error of means.

Table 5. Effects of dietary calcium lactate supplementation on serum biochemical indexes of laying hens.

	Ctrl	Calcium Lactate, %			SEM	*p*-Value
		0.25	0.5	1.0		
Total protein, g/L	40.89	38.48	34.49	39.84	6.70	0.515
Albumin, g/L	12.57	11.77	9.82	11.70	1.58	0.111
Globulin, g/L	28.33	26.72	24.67	28.15	5.61	0.740
Total cholesterol, mmol/L	1.65	1.51	1.42	1.70	0.28	0.435
Triglyceride, mmol/L	4.93	4.88	3.38	4.10	1.25	0.237
High-density lipoprotein, mmol/L	0.67	0.57	0.51	0.65	0.11	0.169
Low-density lipoprotein, mmol/L	0.45	0.46	0.50	0.51	0.10	0.758
Creatinine, umol/L	30.38	28.03	25.90	30.02	5.09	0.563
Urea, mmol/L	0.94	0.99	0.96	1.03	0.11	0.640
Aspartate aminotransferase, U/L	211.42	206.68	176.61	210.65	32.16	0.315
Alanine transaminase, U/L	2.06 ^{a}	1.72 ^{b}	1.64 ^{b}	2.00 ^{a}	0.51	0.033
Total bilirubin, umol/L	0.18	0.16	0.24	0.18	0.11	0.194
Creatine kinase, U/L	3077 ^{a}	3104 ^{a}	2328 ^{c}	2793 ^{b}	360	0.012
Calcium, mmol/L	2.85 ^{c}	3.51 ^{b}	4.17 ^{a}	4.02 ^{a}	0.71	0.040
Phosphorus, mmol/L	1.03 ^{c}	1.02 ^{c}	1.24 ^{a}	1.11 ^{b}	0.12	0.036

^{a,b,c} values in one row with no common superscripts are statistically different ($p \leq 0.05$). SEM: standard error of means.

3.3. Organ Index, Jejunal Mucosal Immunity, and Shinbone Quality

As shown in Table 6, relative to the control group, the pancreas index of laying hens fed the diet supplemented with 1.0% calcium lactate was improved ($p \leq 0.05$), but no changes ($p > 0.05$) were observed for the 0.25% and 0.5% inclusion levels. No differences ($p > 0.05$)

existed between the experimental groups for the organ indexes of the spleens, leg muscles, and shinbones of laying hens.

Table 6. Effects of dietary calcium lactate supplementation on organ index of laying hens.

Item, %	Ctrl	Calcium Lactate, %			SEM	*p*-Value
		0.25	0.5	1.0		
Pancreas	0.19 ^{b}	0.17 ^{b}	0.18 ^{b}	0.23 ^{a}	0.03	0.034
Spleen	0.14	0.14	0.16	0.13	0.03	0.461
Leg muscle	9.07	7.75	9.40	8.86	1.35	0.497
Shinbone	0.55	0.57	0.61	0.57	0.04	0.339

^{a,b} values in one row with no common superscripts are statistically different ($p \leq 0.05$). SEM: standard error of means.

The jejunal mucosal immunity of laying hens is displayed in Table 7. The dietary inclusion of 0.25%, 0.5%, or 1.0% calcium lactate increased ($p \leq 0.05$) the contents of IgA and IgG in the jejunal mucosa of laying hens. Compared to the control group, the addition of calcium lactate in feed improved ($p \leq 0.05$) the contents of lysozyme and sIgA in the jejunal mucosa of laying hens, and the 0.5% addition level worked best ($p \leq 0.05$). The IL-2 content in the jejunal mucosa of laying hens was decreased ($p \leq 0.05$) by the addition of calcium lactate in feed, and the 1.0% addition level's effect was reduced the most ($p \leq 0.05$). The dietary inclusion of calcium lactate showed no effects ($p > 0.05$) on the contents of IgM, IL-6, and TNF-αin the jejunal mucosa of laying hens.

Table 7. Effects of dietary calcium lactate supplementation on jejunal mucosal immunity of laying hens.

	Ctrl	Calcium Lactate, %			SEM	*p*-Value
		0.25	0.5	1.0		
IgA, g/L	1.37 ^{b}	1.64 ^{a}	1.71 ^{a}	1.68 ^{a}	0.32	0.039
IgM, g/L	1.55	1.63	1.69	1.62	0.41	0.278
IgG, g/L	3.19 ^{b}	4.36 ^{a}	4.65 ^{a}	4.73 ^{a}	0.58	0.003
Lysozyme, mg/L	4.02 ^{c}	5.61 ^{ab}	5.88 ^{a}	5.42 ^{b}	0.64	0.026
IL-2, ng/mL	4.38 ^{a}	3.19 ^{b}	3.30 ^{b}	2.86 ^{c}	0.45	<0.001
IL-6, ng/mL	1.22	1.34	0.98	1.41	0.33	0.106
TNF-α, ng/mL	1.51	1.42	1.58	1.39	0.27	0.312
sIgA, μg/mL	41.13 ^{c}	47.56 ^{b}	50.26 ^{a}	49.75 ^{ab}	4.36	0.014

^{a,b,c} values in one row with no common superscripts are statistically different ($p \leq 0.05$). IgA: immunoglobulin A, IgM: immunoglobulin M, IgG: immunoglobulin G, IL-2: interleukin 2, IL-6: interleukin 6, TNF-α: tumor necrosis factor α, sIgA: secretory immunoglobulin A, SEM: standard error of means.

As shown in Table 8, compared with the control group, the addition of 0.5% calcium lactate to the diet reduced ($p \leq 0.05$) the maximal force of the shinbone of laying hens, did not affect ($p > 0.05$) the time of fracture stress, and increased ($p \leq 0.05$) the work required for shinbone rupture. The 0.25% addition level of calcium lactate in the diet showed no effects ($p > 0.05$) on the shinbone quality, but the 1.0% level decreased ($p \leq 0.05$) the work for bone rupture.

Table 8. Effects of dietary calcium lactate supplementation on shinbone quality of laying hens.

	Ctrl	Calcium Lactate, %			SEM	*p*-Value
		0.25	0.5	1.0		
Maximal force, N	118.78 ^{b}	101.42 ^{b}	141.42 ^{a}	119.92 ^{b}	18.59	0.080
Time, min	2.64	2.53	2.69	2.22	0.43	0.340
Work, N·mm	176.31 ^{b}	146.65 ^{bc}	236.62 ^{a}	129.65 ^{c}	51.53	0.048

^{a,b,c} values in one row with no common superscripts are statistically different ($p \leq 0.05$). SEM: standard error of means.

4. Discussion

The decline in eggshell quality is one of the biggest challenges in feeding old laying hens and causes large economic losses in egg production and processing. As a kind of organic calcium, calcium lactate showed a higher true digestibility coefficient of calcium than limestone, calcium phosphate, bone meal, and egg powder in pigs [23]. Lactic acid and calcium lactate are safe to add to animal feed [21], and the production cost of feed-grade calcium lactate is gradually reduced and widely used. Therefore, calcium lactate in feed is expected to improve the calcium utilization of elderly laying hens, enhance the eggshell quality, and prolong the effective laying period of hens.

Feed addition of 0.5% sodium butyrate and 0.5% calcium propionate reduced the feed cost of eggs without affecting the quality of the eggs [24]. The addition of benzoic acid products to free-range diets can reduce the negative impact of forage on nutrient utilization and improve the digestibility of ileal nutrients [25]. Compared with sodium selenite, organic selenium integrated with bacteria or yeast improved the performance, egg quality, and selenium contents of tissues and eggs and increased beneficial caecal bacterial proliferation and butyric acid content in laying hens [26,27]. The addition of organic sulfur and inorganic sulfur to the diet was beneficial to the ileal morphology and antioxidant capacity of laying hens [28]. It can be seen from the above that the addition of organic acids and organic elements to feed can effectively improve the production of laying hens, which was confirmed again in this study, which showed that the addition of calcium lactate to feed improved the egg-production performance of laying hens.

The continuous extension of the commercial production cycle has caused an imbalance in the utilization of calcium and phosphorus in older laying hens, often causing bone fractures and poor eggshell quality [29,30]. Compared with inorganic mineral elements, dietary supplementation with organic sources increased their bioavailability and improved egg quality during the late production stage of laying hens [10,14]. Organic acids can be used as antimicrobial molecules or health promoters in the feed of poultry and also increase the digestibility of nutrients [25,31]. In addition, dietary supplementation with organic acids in combination with phytase can increase the calcium–phosphorus utilization of laying hens [32]. In this study, the contents of calcium and phosphorus in serum were increased by the dietary addition of calcium lactate. Calcium and phosphorus homeostasis, which are directly related to the components required for eggshell calcification and bone mineralization, ultimately affect eggshell quality [29]. Therefore, it can be deduced that the dietary organic calcium lactate in the current study improved the shinbone, eggshell, and albumen quality of laying hens probably through increasing calcium bioavailability and intestinal nutrient absorbability.

The red blood cell count has proven to be one of the most commonly used blood tests and plays a vital role in determining overall health, which is valuable for the early diagnosis of some diseases [33]. Lower mean corpuscular hemoglobin concentration is associated with a poor surgical prognosis for several diseases [34,35]. Heterophils are the most abundant immune cell in the blood, and are mainly used to defend against pathogens [36]. Mononuclear leucocytes, the largest white blood cells, are an important component of the body's defense system. Basophilic granulocytes are mainly found in the blood, and will be induced by chemokines to migrate to tissues in the presence of inflammation [37]. In the current study, the dietary inclusion of calcium lactate increased the contents of red blood cells, corpuscular hemoglobin, mononuclear leucocytes, and basophilic granulocytes and decreased the number of heterophils. This indicated that calcium lactate could improve the aerobic capacity and hematogenic immunity of laying hens, which may be part of an explanation for improving the quality of eggshells and albumen. ALT is one of the most important indicators of liver function, and its elevated concentration in the blood indicates liver damage [38]. Serum creatine kinase is an enzyme that is commonly used in clinical practice to assess the diagnosis and monitoring of muscle injuries, myocardial injuries, and other diseases [39]. In this study, the concentrations of ALT and creatine kinase in

serum were decreased by experimental treatments, so it demonstrates the positive effects of calcium lactate on the liver and muscle health of animals.

Immunoglobulins play a fundamental role in the body's protection against internal and external threats [40]. Lysozyme is a 1,4-beta-*N*-acetylmuramidase with antimicrobial properties. Dietary lysozyme supplementation contributes to enhanced intestinal functions and gut microflora in piglets [41,42]. In the current study, the concentrations of IgA, IgG, lysozyme, and sIgA in the jejunal mucosa of laying hens were observably increased by experimental treatments, and that of IL-2, an important pro-inflammatory factor, was decreased. Diets with organic acids could maintain intestinal morphology and could promote digestion, absorption, and barrier function [43]. The weight index of the pancreas, the largest intestinal digestive enzyme-secreting organ, was increased in laying hens fed calcium lactate. Therefore, it shows that dietary calcium lactate could improve the intestinal digestible ability and jejunal mucosal immunity of laying hens, which is probably a potential reason for the improvement in laying performance.

5. Conclusions

The addition of calcium lactate in feed improved the egg-production performance and the shinbone, eggshell, and albumen quality of laying hens, which was probably realized through its positive effects on the liver and muscle health, intestinal digestible ability, calcium bioavailability, and jejunal mucosal immunity. From the comprehensive consideration of body health, egg-production performance, and egg quality, the optimal proportion of calcium lactate in the diet of laying hens is recommended to be 0.5%.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture14020256/s1, Table S1. The calcium sources of each treatment group. Table S2. The information of commercial kits used for chemical analysis.

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References

1. Bain, M.M.; Nys, Y.; Dunn, I.C. Increasing persistency in lay and stabilising egg quality in longer laying cycles. What are the challenges? *Br. Poult. Sci.* **2016**, *57*, 330–338. [CrossRef]
2. Whitehead, C.C. Overview of bone biology in the egg-laying hen. *Poult. Sci.* **2004**, *83*, 193–199. [CrossRef] [PubMed]
3. Whitehead, C.C.; Fleming, R.H. Osteoporosis in cage layers. *Poult. Sci.* **2000**, *79*, 1033–1041. [CrossRef]
4. Julian, R.J. Production and growth related disorders and other metabolic diseases of poultry—A review. *Vet. J.* **2005**, *169*, 350–369. [CrossRef] [PubMed]
5. Budgell, K.; Silversides, F. Bone breakage in three strains of end-of-lay hens. *Can. J. Anim. Sci.* **2004**, *84*, 745–747. [CrossRef]
6. Cransberg, P.; Parkinson, G.; Wilson, S.; Thorp, B. Sequential studies of skeletal calcium reserves and structural bone volume in a commercial layer flock. *Br. Poult. Sci.* **2001**, *42*, 260–265. [CrossRef]
7. Zhao, S.; Teng, X.; Xu, D.; Chi, X.; Ge, M.; Xu, S. Influences of low level of dietary calcium on bone characters in laying hens. *Poult. Sci.* **2020**, *99*, 7084–7091. [CrossRef] [PubMed]

8. Wang, J.; Wang, W.-W.; Qi, G.-H.; Cui, C.-F.; Wu, S.-G.; Zhang, H.-J.; Xu, L. Effects of dietary *Bacillus subtilis* supplementation and calcium levels on performance and eggshell quality of laying hens in the late phase of production. *Poult. Sci.* **2021**, *100*, 100970. [CrossRef]
9. Bar, A. Calcium homeostasis and vitamin D metabolism and expression in strongly calcifying laying birds. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **2008**, *151*, 477–490. [CrossRef]
10. Dong, Y.; Zhang, K.; Han, M.; Miao, Z.; Liu, C.; Li, J. Low level of dietary organic trace minerals improved egg quality and modulated the status of eggshell gland and intestinal microflora of laying hens during the late production stage. *Front. Vet. Sci.* **2022**, *9*, 920418. [CrossRef]
11. Stefanello, C.; Santos, T.; Murakami, A.; Martins, E.; Carneiro, T. Productive performance, eggshell quality, and eggshell ultrastructure of laying hens fed diets supplemented with organic trace minerals. *Poult. Sci.* **2014**, *93*, 104–113. [CrossRef]
12. Qiu, J.; Zhou, Q.; Zhu, J.; Lu, X.; Liu, B.; Yu, D.; Lin, G.; Ao, T.; Xu, J. Organic trace minerals improve eggshell quality by improving the eggshell ultrastructure of laying hens during the late laying period. *Poult. Sci.* **2020**, *99*, 1483–1490. [CrossRef]
13. Zhang, W.F.; Tian, M.; Song, J.S.; Chen, F.; Lin, G.; Zhang, S.H.; Guan, W.T. Effect of replacing inorganic trace minerals at lower organic levels on growth performance, blood parameters, antioxidant status, immune indexes, and fecal mineral excretion in weaned piglets. *Trop. Anim. Health Prod.* **2021**, *53*, 121. [CrossRef] [PubMed]
14. Yenice, E.; Mızrak, C.; Gültekin, M.; Atik, Z.; Tunca, M. Effects of organic and inorganic forms of manganese, zinc, copper, and chromium on bioavailability of these minerals and calcium in late-phase laying hens. *Biol. Trace Elem. Res.* **2015**, *167*, 300–307. [CrossRef] [PubMed]
15. Gheisari, A.A.; Sanei, A.; Samie, A.; Gheisari, M.M.; Toghyani, M. Effect of diets supplemented with different levels of manganese, zinc, and copper from their organic or inorganic sources on egg production and quality characteristics in laying hens. *Biol. Trace Elem. Res.* **2011**, *142*, 557–571. [CrossRef] [PubMed]
16. Min, Y.N.; Liu, F.X.; Qi, X.; Ji, S.; Cui, L.; Wang, Z.P.; Gao, Y.P. Effects of organic zinc on tibia quality, mineral deposit, and metallothionein expression level of aged hens. *Poult. Sci.* **2019**, *98*, 366–372. [CrossRef] [PubMed]
17. Sarlak, S.; Tabeidian, S.A.; Toghyani, M.; Shahraki, A.D.F.; Goli, M.; Habibian, M. Effects of replacing inorganic with organic iron on performance, egg quality, serum and egg yolk lipids, antioxidant status, and iron accumulation in eggs of laying hens. *Biol. Trace Elem. Res.* **2021**, *199*, 1986–1999. [CrossRef]
18. Cao, J.; Zhu, J.; Zhou, Q.; Zhao, L.; Zou, C.; Guo, Y.; Curtin, B.; Ji, F.; Liu, B.; Yu, D. Efficacy evaluation of novel organic iron complexes in laying hens: Effects on laying performance, egg quality, egg iron content, and blood biochemical parameters. *Anim. Biosci.* **2023**, *36*, 498–505. [CrossRef] [PubMed]
19. Song, M.; Jiao, H.; Zhao, J.; Wang, X.; Li, H.; Wang, P.; Ma, B.; Sun, S.; Lin, H. Dietary supplementation of calcium propionate and calcium butyrate improves eggshell quality of laying hens in the late phase of production. *Poult. Sci.* **2022**, *59*, 64–74. [CrossRef] [PubMed]
20. Hu, X.; Chen, Y.; Wu, X.; Liu, W.; Jing, X.; Liu, Y.; Yan, J.; Liu, S.; Qin, W. Combination of calcium lactate impregnation with UV-C irradiation maintains quality and improves antioxidant capacity of fresh-cut kiwifruit slices. *Food Chem. X* **2022**, *14*, 100329. [CrossRef]
21. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP); Bampidis, V.; Azimonti, G.; Bastos, M.d.L.; Christensen, H.; Dusemund, B.; Kouba, M.; Kos Durjava, M.; López-Alonso, M.; López Puente, S.; et al. Safety of lactic acid and calcium lactate when used as technological additives for all animal species. *EFSA J.* **2019**, *17*, e05914.
22. *NY/T 33-2004*; Chicken Feeding Standards. Ministry of Agriculture and Rural Affairs of the People's Republic of China: Beijing, China, 2004.
23. Salguero, S.C.; Rostagno, H.S.; Hannas, M.I.; Carvalho, T.A.; Maia, R.C.; Pessoa, G.B.S. Calcium digestibility from feedstuffs for pigs evaluated using two methods. *Arq. Bras. Med. Vet. Zoo.* **2014**, *66*, 1539–1546. [CrossRef]
24. Dahiya, R.; Berwal, R.S.; Sihag, S.; Patil, C.S. The effect of dietary supplementation of salts of organic acid on production performance of laying hens. *Vet. World* **2016**, *9*, 1478–1484. [CrossRef] [PubMed]
25. Iqbal, Z.; Metzger, F.; Singh, M.; Morgan, N.; Swick, R.A.; Perez-Maldonado, R.A.; M'Sadeq, S.A.; Zentek, J.; Ruhnke, I. Enzymes and/or combination of organic acid and essential oils supplementation in pasture-fed free-range laying hens increased the digestibility of nutrients and non-starch polysaccharides. *Poult. Sci.* **2019**, *98*, 1410–1424. [CrossRef] [PubMed]
26. Zainudin, N.N.; Hemly, N.I.M.; Muhammad, A.I.; Nayan, N.; Samsudin, A.A. Effects of supplementing organic- and inorganic-based selenium with vitamin E on intestinal histomorphology, caecal bacterial proliferation, and short-chain fatty acid profile in layer hens. *Trop. Anim. Health Prod.* **2023**, *55*, 90. [CrossRef] [PubMed]
27. Muhammad, A.I.; Mohamed, D.A.; Chwen, L.T.; Akit, H.; Samsudin, A.A. Effect of selenium sources on laying performance, egg quality characteristics, intestinal morphology, microbial population and digesta volatile fatty acids in laying hens. *Animals* **2021**, *11*, 1681. [CrossRef] [PubMed]
28. Kim, Y.B.; Lee, S.H.; Kim, D.H.; Lee, H.G.; Choi, Y.; Lee, S.D.; Lee, K.W. Effects of dietary organic and inorganic sulfur on laying performance, egg quality, ileal morphology, and antioxidant capacity in laying hens. *Animals* **2021**, *12*, 87. [CrossRef] [PubMed]
29. Sinclair-Black, M.; Garcia, R.A.; Ellestad, L.E. Physiological regulation of calcium and phosphorus utilization in laying hens. *Front. Physiol.* **2023**, *14*, 1112499. [CrossRef]
30. Sibanda, T.Z.; Flavel, R.; Kolakshyapati, M.; Welch, M.; Schneider, D.; Ruhnke, I. The association between range usage and tibial quality in commercial free-range laying hens. *Br. Poult. Sci.* **2020**, *61*, 493–501. [CrossRef]

31. Scicutella, F.; Mannelli, F.; Daghio, M.; Viti, C.; Buccioni, A. Polyphenols and organic acids as alternatives to antimicrobials in poultry rearing: A review. *Antibiotics* **2021**, *10*, 1010. [CrossRef]
32. Sari, M.; Onol, A.G.; Daskiran, M.; Cengiz, O. Egg production and calcium-phosphorus utilization of laying hens fed diets supplemented with phytase alone or in combination with organic acid. *Int. J. Poult. Sci.* **2012**, *11*, 181–189. [CrossRef]
33. Dai, X.; Zhou, G.; Xu, L. Associations between red blood cell count and metabolic dysfunction-associated fatty liver disease(MAFLD). *PLoS ONE* **2022**, *17*, e0279274. [CrossRef] [PubMed]
34. Huang, Y.L.; Hu, Z.D. Lower mean corpuscular hemoglobin concentration is associated with poorer outcomes in intensive care unit admitted patients with acute myocardial infarction. *Ann. Transl. Med.* **2016**, *4*, 190. [CrossRef]
35. Qu, X.; Zhang, T.; Ma, H.; Sui, P.; Du, J. Lower mean corpuscular hemoglobin concentration is associated with unfavorable prognosis of resected lung cancer. *Future Oncol.* **2014**, *10*, 2149–2159. [CrossRef]
36. Blanter, M.; Gouwy, M.; Struyf, S. Studying neutrophil function in vitro: Cell models and environmental factors. *J. Inflamm. Res.* **2021**, *14*, 141–162. [CrossRef]
37. Schwartz, C.; Eberle, J.U.; Voehringer, D. Basophils in inflammation. *Eur. J. Pharmacol.* **2016**, *778*, 90–95. [CrossRef] [PubMed]
38. Senior, J.R. Alanine aminotransferase: A clinical and regulatory tool for detecting liver injury-past, present, and future. *Clin. Pharmacol. Ther.* **2012**, *92*, 332–339. [CrossRef]
39. Ohno, K. Is the serum creatine kinase level elevated in congenital myasthenic syndrome? *J. Neurol. Neurosurg. Psychiatry* **2016**, *87*, 801. [CrossRef] [PubMed]
40. Almaghlouth, I.; Johnson, S.R.; Pullenayegum, E.; Gladman, D.; Urowitz, M. Immunoglobulin levels in systemic lupus erythematosus: A narrative review. *Lupus* **2021**, *30*, 867–875. [CrossRef]
41. Xiong, X.; Zhou, J.; Liu, H.; Tang, Y.; Tan, B.; Yin, Y. Dietary lysozyme supplementation contributes to enhanced intestinal functions and gut microflora of piglets. *Food Funct.* **2019**, *10*, 1696–1706. [CrossRef]
42. Oliver, W.T.; Wells, J.E. Lysozyme as an alternative to antibiotics improves growth performance and small intestinal morphology in nursery pigs. *J. Anim. Sci.* **2013**, *91*, 3129–3136. [CrossRef] [PubMed]
43. Wang, H.; Liang, S.; Li, X.; Yang, X.; Long, F.; Yang, X. Effects of encapsulated essential oils and organic acids on laying performance, egg quality, intestinal morphology, barrier function, and microflora count of hens during the early laying period. *Poult. Sci.* **2019**, *98*, 6751–6760. [CrossRef] [PubMed]