Dynamic Alterations on Synthesis and Transportation of Vitamin C and Deposition Status in Produced Eggs Induced by Dietary Vitamin C Supplementation in Hy-Line Brown Layer Model

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Research

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Abstract

Background: Some previous studies have indicated that in ovo feeding (IOF) of vitamin C (VC) had positive effects on the performance in poultry. In order to realize embryonic VC supplementation, an idea about hen's dietary VC supplementation to achieve VC enrichment in produced eggs was proposed. And this study was executed to investigate the effects of dietary VC supplementation on synthesis and transportation of VC in layers and VC deposition status in produced eggs.

Results: Compared with Arbor Acres breeder eggs, egg VC content was lower in Isa Brown breeder eggs and Hy-Line Brown layer eggs ($P < 0.05$). Sodium-dependent vitamin C transporter 1 (SVCT1) and SVCT2 expression was higher in ileum than in duodenum and jejunum ($P < 0.05$). SVCT1 expression was extremely higher in magnum than in ovary, while SVCT2 expression was lower ($P < 0.05$). L-gulonolactone oxidase (GLO) expression was extremely higher and SVCT1 expression was higher in kidney than in liver, while SVCT2 was lower ($P < 0.05$). 400 mg/kg VC supplementation increased SVCT1 expression in duodenum, ovary and magnum, while decreased GLO and SVCT1 expression in liver ($P < 0.05$). 200 and 400 mg/kg VC supplementation increased SVCT2 expression in duodenum, while decreased GLO and SVCT1 expression in kidney and SVCT2 expression in liver ($P < 0.05$).

Conclusions: Hy-Line Brown layer was a useful model for investigating effects of dietary VC supplementation on VC deposition in produced eggs. Dietary VC supplementation promoted VC absorption in duodenum and jejunum, but reduced endogenous VC synthesis in liver and kidney. Although dietary VC supplementation enhanced VC transportation in ovary and magnum, it finally failed to increase VC deposition in produced eggs.

Background

Nutritional supplementation at embryonic stage is expected to become a great potential way to improve the performance in poultry [1, 2]. Some previous studies have indicated that in ovo feeding (IOF) of vitamin C (VC) had positive effects on the performance in poultry [3, 4, 5, 6]. In humans, more and more studies also have proved that the epigenetics diet (including vitamin C in fruits), when offered for pregnant mothers, leaded to beneficial health outcomes in newborn baby by nutri-epigenetics [7]. Unlike viviparous animals, birds lack a placental structure and their embryonic development is separated from the mother, so that their VC sources theoretically rely on egg deposition from mother, self-synthesis by embryo or external supplementation via in ovo injection. Although VC can be synthesized via the glucuronatexylulose-xylulose cycle in the presence of L-gulonolactone oxidase (GLO) by developing embryo [8], its synthetic amount may be insufficient [9], which is why hatched chicks usually need to be supplemented with VC in farm or hatchery. In order to realize embryonic VC supplementation, an idea about hen's dietary VC supplementation to achieve VC enrichment in produced eggs was proposed.

Vitamins are deposited in both egg yolk and albumen, but fat-soluble vitamins are merely deposited in egg yolk, while water-soluble vitamins are deposited in both egg yolk and albumen [10]. The researches

have confirmed that it is possible to achieve enrichment of some vitamins in eggs by hen's dietary supplementation, in particular all fat-soluble vitamins, but also some water-soluble vitamins (vitamin B_{12} and folate) [11]. As for other water-soluble vitamins, the literatures are scare. Although there were reports in the literature that eggs do not contain detectable doses of VC [12], it has not been reported whether hen's dietary VC supplementation could enrich VC in produced eggs, because VC is not a vitamin that must be added in vitamin premix of poultry according to NRC.

Egg yolk and albumen are formed at hen's ovary and magnum, respectively [13, 14]. So, if VC could be deposited in yolk and albumen of produced eggs, it would be inseparable from the transportation and secretion function in hen's ovary and magnum. The source of hen's VC pool is mainly from intestinal absorption by sodium-dependent VC transporter 1 and 2 (SVCT1 and SVCT2) and liver's and kidney's synthesis by GLO [15, 16, 17, 18, 19]. Although dehydroascorbic acid, the oxidized form of VC, can be transported by glucose transporters, plasma dehydroascorbic acid content was estimated as <1-2% of that of VC [20]. Therefore, the transportation and distribution of VC in various tissues (including ovary and magnum) mainly depends on blood circulation and VC transporters.

The embryonic nutrition is expected to become a new direction to improve the performance in poultry, which was proved by many researches involved in IOF of VC. In this study, we want to confirm whether hen's dietary VC supplementation could also achieve the enrichment of VC in produced eggs. We hypothesized that Arbor Acres breeder hens, Isa Brown breeder hens and Hy-Line Brown layers all have capacity of VC deposition in produced eggs and Hy-Line Brown layers can be regarded as a useful animal model, and dietary VC supplementation can promote absorption in intestine and transportation in reproductive tract (ovary and magnum) and may reduce synthesis in kidney and liver in Hy-Line Brown layers model, finally achieving VC deposition in produced eggs.

Materials And Methods

Experiment 1

Experimental procedures

Experiment 1 was conducted to demonstrate the capacity of VC deposition in produced eggs among Arbor Acres breeder hens, Isa Brown breeder hens and Hy-Line Brown layers. On the same day, 15 fertilized eggs were selected from 52-week-old Arbor Acres breeder hens, 19 fertilized eggs were selected from 39-week-old Isa Brown breeder hens and 15 commercial eggs were selected from 47-week-old Hy-Line Brown layers. Every egg was weighed, egg albumen and yolk were separated and weighed, and then egg albumen and yolk were packed into 50 mL cryotube, respectively. After placed at -80°C for 24 h, the egg albumen and yolk samples were immediately put into the pre-cooled Siemens vacuum freeze dryer (Siemens, Berlin, Germany) for preparation of lyophilized powders. Lyophilized powders of egg albumen and yolk were weighed and stored at -20°C for further analysis.

Egg parameters

Egg parameters include egg weight (EW), the ratio of yolk fresh weight to albumen fresh weight (YFW/AFW), the ratio of yolk dry weight to albumen dry weight (YDW/ADW), yolk moisture content (YMC) and albumen moisture content (AMC). The formula employed was as follows: YFW/AFW (%) = (the yolk fresh weight (YFW) / the albumen fresh weight (AFW)) × 100; YDW/ADW (%) = (the yolk dry weight (YDW) / the albumen dry weight (ADW)) × 100; YMC (%) = ((YFW-YDW) / YFW) × 100; AMC (%) = ((AFW-ADW) / AFW) × 100.

Measurement of vitamin C content in the eggs

5 mL pre-cooled physiological saline was added into a 10 mL centrifuge tube and mixed with 1 g albumen or yolk lyophilized powder by violently shaking and the mixture was centrifuged at 3500 rpm for 15 min. Then, the VC content in the supernatant was measured by commercially available kits (A009, Nanjing Jiancheng Bioengineering Institute, Nanjing, China) based on kits instructions. According to the kits specification, the sample quality control and testing parameter settings were strictly executed. The VC content in the eggs was calculated as follows: yolk VC content (YVCC, μg/g YDM) = (the VC content in the supernatant (μg/mL) × 5 mL) / 1 g YDM; albumen VC content (AVCC, μg/g ADM) = (the VC content in the supernatant (μg/mL) × 5 mL) / 1 g ADM.

Experiment 2

Experimental procedures

A total of 24 Hy-Line Brown layers (42-week-old) were randomly divided into 3 treatments with 8 replicates. The basal diet was a corn-soybean meal with no VC supplementation (Table 1) and 3 experimental diets were supplemented with 0, 200 and 400 mg/kg VC on the basal diet, respectively. In order to maintain the consistency of VC intake in the same treatment, the limited feed supply was conducted according to the previous feeding test in Hy-line Brown layers with the same basic diet formula. 120 g feed per laying hen was supplied daily with adequate drinking water and feeding time was at 9:00 and 16:00, respectively. The feeding trial lasted for 42 days: a pre-feeding period of 14 days with fed basal diet and a formal feeding period of 28 days with fed experimental diets. The formal feeding period of 28 days is named sequentially from day 1 to 28. The produced eggs of day 23 and 28 were collected for egg quality analysis and the preparation of lyophilized powder, respectively. At the end of feeding trial, all laying hens were fasting for 12 h. Then, blood samples were taken from the jugular vein, and all birds were euthanized by cervical dislocation. The tissue samples (liver, kidney, ovary and magnum) and the intestinal mucosa samples (duodenum, jejunum and ileum) were collected, snap-frozen in liquid N_2 and then stored at -80°C for further analysis.

Laying performance and egg quality

The daily produced eggs were weighed and its number was counted. Egg production rate, EW and feed-to-egg ratio were calculated. The produced eggs of day 23 were analyzed for egg quality parameters, which

was measured as yolk color, haugh unit, albumen height, shell strength, and shell thickness, on the day of laying.

Egg parameters and measurement of vitamin C content in produced eggs

The produced eggs of day 28 were prepared for lyophilized powders and the procedures were the same with Experiment 1. Egg parameters include EW, YFW, AFW, YFW/AFW, YDW, ADW, YDW/ADW, YMC and AMC. The lyophilized powders of albumen and yolk were used for measurement of VC content in produced eggs and the detection method was the same with Experiment 1.

Measurement of vitamin C content in serum

The blood samples were centrifuged at 3000 rpm for 10 min to obtain serum samples and the serum samples were stored at -20°C for further measurement of VC content. Then, the VC content in the serum was measured by commercially available kits (A009, Nanjing Jiancheng Bioengineering Institute, Nanjing, China) based on kits instructions. According to the kits specification, the sample quality control and testing parameter settings were strictly executed.

Quantitative real-time PCR

Total RNA, from liver, kidney, intestinal mucosa (duodenum, jejunum and ileum) and reproductive tract (ovary and magnum), was extracted following TRIzol Reagent protocol (Invitrogen, Carlsbad, CA, USA). The concentration, purity and integrity of RNA samples were verified and cDNA was synthesized with a Primer Script RT Reagent Kit (TaKaRa, Dalian, China). The mRNA expression of SVCT1 and SVCT2 in liver, kidney, intestine (duodenum, jejunum and ileum) and reproductive tract (ovary and magnum) and the mRNA expression of GLO in liver and kidney were analyzed with SYBR Premix Ex Taq kit (TaKaRa, Dalian, China) on the iCycler IQ5 (Bio-Rad, Hercules, CA, USA). Detailed reaction system was referred to our previous description [6]. The primers are listed in Table 2. All samples were run in triplicate and the average cycle threshold (Ct) values were normalized to β-actin and quantified by the $2^{-\Delta\Delta Ct}$ method [21].

Statistical analysis

The data, comparison of the relative mRNA expression of SVCT1 and SVCT2 between different tissues (ovary vs magnum and liver vs kidney) and GLO (liver vs kidney), were analyzed by independent sample t test and all other data were analyzed by one-way ANOVA using the SPSS 21.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was considered at $P < 0.05$ and trends at $P < 0.1$.

Results

Experiment 1

Comparison of egg parameters and vitamin C content among 3 different eggs

As shown in Table 3, EW was heaviest in Arbor Acres breeder hen and was lightest in Hy-Line Brown layer ($P < 0.05$). Compared with Isa Brown breeder hen and Hy-Line Brown layer, YFW/AFW, YDW/ADW, AMC, YVCC and AVCC were all significantly higher in Arbor Acres breeder hen ($P < 0.05$). Compared with Arbor Acres breeder hen and Isa Brown breeder hen, YMC was significantly higher in Hy-Line Brown layer ($P < 0.05$).

Experiment 2

Laying performance, egg quality, egg parameters, and vitamin C content in serum and produced eggs

As shown in Table 4, dietary VC supplementation had no significant effects on egg production rate, EW and feed-to-egg ratio ($P > 0.05$). The limited feed supply was 120 g daily among three groups. As shown in Table 5, dietary VC supplementation had no significant effects on EW, yolk color, haugh unit, albumen height, shell strength and shell thickness on day 23 ($P > 0.05$). As shown in Table 6, dietary VC supplementation had no significant effects on egg parameters (EW, YFW, AFW, YFW/AFW, YDW, ADW, YDW/ADW, YMC and AMC) and egg VC content (YVCC and AVCC) on day 28 and serum VC content (SVCC) ($P > 0.05$).

Tissues mRNA expression profile of SVCT1, SVCT2 and GLO

As shown in Table 7, compared with duodenum and jejunum, the mRNA expression of SVCT1 and SVCT2 were both significantly higher in ileum ($P < 0.05$). Compared with ovary, the mRNA expression of SVCT1 was significantly higher in magnum, while the mRNA expression of SVCT2 was significantly lower ($P < 0.05$). Compared with liver, the mRNA expression of GLO and SVCT1 were both significantly higher in kidney, while the mRNA expression of SVCT2 was significantly lower ($P < 0.05$).

Vitamin C absorption: mRNA expressions of SVCT1 and SVCT2 in intestine

400 mg/kg VC supplementation increased the mRNA expression of SVCT1 in duodenum (Figure 1A, $P < 0.05$) and had an increasing trend in jejunum (Figure 1B, $P = 0.067$). 200 and 400 mg/kg VC supplementation increased the mRNA expression of SVCT2 in duodenum (Figure 1B), but decreased the mRNA expression of SVCT2 in ileum (Figure 1C, $P < 0.05$). However, VC supplementation had no significant effect on SVCT1 in ileum (Figure 1C) and SVCT2 in jejunum (Figure 1B, $P > 0.05$).

Vitamin C deposition: mRNA expressions of SVCT1 and SVCT2 in ovary and magnum

400 mg/kg VC supplementation increased the mRNA expression of SVCT1 in ovary (Figure 2A) and magnum (Figure 2B, $P < 0.05$). However, VC supplementation had no significant effect on SVCT2 in ovary (Figure 2A) and magnum (Figure 2B, $P > 0.05$).

Vitamin C synthesis: mRNA expressions of GLO, SVCT1 and SVCT2 in liver and kidney

400 mg/kg VC supplementation decreased the mRNA expression of GLO and SVCT1 in liver (Figure 3A, $P < 0.05$). 200 and 400 mg/kg VC supplementation decreased the mRNA expression of GLO and SVCT1 in

kidney (Figure 3B) and SVCT2 in liver (Figure 3A, $P < 0.05$). However, VC supplementation had no significant effect on SVCT2 in kidney (Figure 3B, $P > 0.05$).

Discussion

Arbor Acres breeder hen, Isa Brown breeder hen and Hy-Line Brown layer all have the ability to lay eggs and have the similar reproductive tract structure for yolk and albumen synthesis in ovary and magnum, respectively. Although their egg parameters were both significantly different, egg VC deposition was observed and egg VC content was lower in Isa Brown breeder and Hy-Line Brown layer than in Arbor Acres breeder. Researches about VC intervention showed that the potential for demonstrating benefit or harm from VC supplementation was greatly increased in animals with low plasma or tissue VC content at baseline [22, 23, 24]. Therefore, Hy-Line Brown layer was selected as a useful model for studying the effects of dietary VC supplementation on synthesis and transportation of VC and deposition status in produced eggs.

In this study, no significant impact of dietary VC supplementation on laying performance and egg quality was observed, which was similar with Wang's results [25] and was different from Ahmed's results [26]. Such inconsistent results from VC supplementation was related to different oxidative stress status in laying hens, which was stronger in old layers at 75 weeks old than in young layers at 35 weeks old [8], and Hy-Line Brown layers in this study was at 40 weeks old.

In order to explore VC deposition in produced eggs, induced by hen's dietary VC supplementation, three aspects of detection were performed, including VC absorption in intestine, VC synthesis in liver and kidney, and egg VC deposition in ovary and magnum. GLO is a key enzyme for VC synthesis and gene mutations in GLO leads some species (like humans) to loss VC synthesis ability [27]. However, Hy-line Brown layer has the ability of VC synthesis, which is much higher in kidney than in liver [8, 28]. VC absorption, transportation and distribution are regulated primarily by SVCTs [15].

Tissue mRNA expression profile showed that ileum was the main site for VC absorption with higher mRNA expression of SVCTs. The mRNA expression of SVCT1 and SVCT2 showed inconsistent results (ovary vs magnum and liver vs kidney), thus the final absorption in intestine needed to be reflected by the serum VC content and egg VC deposition. The mRNA expression of GLO was extremely higher in kidney than in liver, indicating that kidney is main tissue for VC synthesis in layers, which was also supported by the results of GLO enzyme activity in liver accounting for only 0.5-0.7% of that in kidney [8].

SVCT1 is the absorptive VC transporter with low affinity and high capacity, which is mainly expressed in endothelial system (including intestine and kidney) and is responsible for whole-body homeostasis of VC, while SVCT2 is the predominant tissue transporter with high affinity and low capacity, which is widely expressed in various tissues and is responsible for tissue transporter for vitamin C [29]. Therefore, higher mRNA expression of SVCTs in duodenum and jejunum (especially SVCT1) suggested that dietary VC supplementation promoted VC absorption in duodenum and jejunum. The activation of VC absorption capacity in duodenum and jejunum may lead to a slight reduction of VC absorption in ileum, but only the

mRNA expression of SVCT2, not a major transporter and low capacity, was reduced. In addition, compared with VC in plant-derived feed ingredients, VC in the form of additives could contact the intestinal wall faster and earlier in intestinal lumen without digesting plant cells to release VC [30], thereby promoting VC absorption in duodenum and jejunum.

Dynamic changes of mRNA expression of SVCTs in intestine suggested that dietary VC supplementation enhanced VC absorption in duodenum and jejunum, but surprisingly, no serum VC content changes were observed among the three groups. Some researches indicated that peak plasma VC content arrived at plateau with increasing oral dose and intravenous doses produced plasma content extremely higher (30-70 fold) than the maximum tolerated oral doses [31]. So, dietary VC supplementation is not feasible to achieve high plasma VC content. Unlike fat-soluble vitamins, VC was rapidly metabolized and excreted within 3 h after oral high-dose VC (the time was shorter after intravenous injection) for the stability of plasma VC content [32]. In this study, serum samples were from Hy-Line Brown layer after 12 h fasting and this is why no increase in serum VC content was observed. In addition, the lower mRNA expression of GLO in liver and kidney and the higher mRNA expression of SVCTs in ovary and magnum, induced by dietary VC supplementation, together verified that serum VC content did rise before and intestinal absorption did enhance.

Dietary VC supplementation significantly reduced VC synthesis with decreasing mRNA expression of GLO in liver and kidney. Although dietary VC supplementation increased GLO enzyme activity in liver in Gan's research, its activity was reduced in kidney and VC synthesis was primarily in kidney, as evidenced by GLO enzyme activity in liver accounting for only 0.5-0.7% of that in kidney [8, 33]. Dynamic changes on SVCTs were consistent with GLO in liver and kidney together proved that dietary VC supplementation leaded a reduction of endogenous VC synthesis.

Yolk and albumen are formed at ovary and magnum, respectively. In this study, VC content in albumen was higher in yolk, which may be related with 40 fold higher mRNA expression of SVCT1 in magnum than in ovary, but its mechanism of VC secretion into egg albumen and yolk was unknown [20]. Compared with high moisture content (82%) and no fat in albumen, there is lower moisture content (53%) and higher fat content (28%) in yolk [34]. And in this study, the moisture content was 49% in yolk and 88% in albumen, respectively. According to the mRNA expression of SVCT1 in ovary and magnum and the characteristics of nutrient composition in yolk and albumen, VC, a water-soluble vitamin, may be easier deposited in egg albumen. However, dietary VC supplementation had no effect on VC deposition in egg yolk and albumen, which was contrary with higher mRNA expression of SVCT1 in ovary and magnum. Whether VC was taken orally or intravenously, the plasma VC content could be quickly returned stability by metabolism and excretion [31]. Especially, it was difficult to achieve a high dose of plasma VC content through dietary VC supplementation [15, 31]. The follicle maturation process is accompanied by the deposition of lipids [35], which is not benefit for VC deposition, and it only takes 1.5 h to complete albumen formation in magnum [13], which leads a very short window time provided for VC deposition. In addition, limited increase in SVCT1 expression induced by dietary VC supplementation may make it difficult to activate the

secretion of ovary and magnum, so dietary VC supplementation failed to increase VC deposition in produced eggs.

Obviously, there are some limitations in this study. According to industry routine dosage (dietary 200 mg/kg VC supplementation during hot season), 0, 200 and 400 mg/kg VC supplementation were selected. However, the high-dose vitamin C group should be set a higher dosage, which may be more convincing to demonstrate the effects of dietary VC supplementation on the results of VC deposition in produced eggs. In addition, egg VC deposition was affected by VC transport and secretion in ovary and magnum, but there was no research basis for the mechanism of VC secretion and VC secretion was not detected in this study.

Conclusion

In summary, based on egg VC content at baseline, Hy-Line Brown layer is a useful model for investigating effects of dietary VC supplementation on VC deposition in produced eggs. Dietary VC supplementation had no significant impact on laying performance and egg quality in Hy-line Brown layers at 40 weeks old. The mRNA expression of GLO indicated that the ability of VC synthesis is extremely stronger in kidney than in liver, while higher VC content in albumen may be related to higher mRNA expression of SVCT1 in magnum. Although the mRNA expression of SVCTs indicated that ileum was the main site for VC absorption, dietary VC supplementation enhanced intestinal VC absorption mainly due to the absorption of duodenum and jejunum, which reduced the ability of endogenous VC synthesis in liver and kidney and had no significant effect on VC deposition in produced eggs, even with increased mRNA expression of SVCT1 (Figure 4).

List Of Abbreviations

IOF: in ovo feeding;**VC**: vitamin C; **GLO**: L-gulonolactone oxidase; **SVCT1**: sodium-dependent vitamin C transporter 1; **SVCT2**: sodium-dependent vitamin C transporter 2;**EW**: egg weight; **YFW**: yolk fresh weight; **AFW**: albumen fresh weight; **YDW**: yolk dry weight; **ADW**: albumen dry weight; **YMC**: yolk moisture content; **AMC**: albumen moisture content; **YVCC**: yolk vitamin C content; **AVCC**: albumen vitamin C content; **SVCC**: serum vitamin C content.

Declarations

Ethics approval and consent to participate

The use of animals and all experimental protocols were authorized by the Institutional Animal Care and Use Committee of Northwest A&F University (Yangling, Shaanxi, China).

Consent for publication

Not applicable.

Availability of data and material

All data generated or analyzed during this study are available from the corresponding author by request. The datasets supporting the conclusions of this article are included in the article.

Competing interests

The authors declare that no competing interests exist. The manuscript has not been published previously.

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Authors' contributions

YFZ, JFZ and XJY conceived and designed the experiments; YFZ, JFZ, WG, KLQ and JKY mainly performed the experiments; YFZ analyzed the data and wrote the manuscript; JFZ, ZZR, XY and XJY participated in the revision of the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1. Composition and nutrient levels of experimental basal diet

Items (%, unless noted)	Regular phosphorus
Ingredient	
Corn	59.76
Soybean meal	23.00
Distillers dried grains with solubles	4.00
Calcium carbonate	8.65
Dicalcium phosphate	1.05
Soybean oil	1.25
Sodium chloride	0.25
L-Lysine-H_2SO_4	0.13
L-Threonine	0.31
DL-Methionine	0.25
Choline chloride	0.10
Sand	0.25
Premix*	1.00
In total	100.00
Nutrient levels (calculated)	
Metabolizable energy (kcal/kg)	2700
Crude protein	16.5
Total phosphorus	0.51
Non-phytate phosphorus	0.32
Calcium	3.50

* Provided per kg of diet: manganese 60 mg, copper 8 mg, zinc 80 mg, iodine 0.35 mg, selenium 0.3 mg, vitamin A 8000 IU, vitamin D_3 1600 IU, vitamin E 30 mg, vitamin K_3 1.5 mg, thiamine 4 mg, riboflavin 13 mg, pantothenic acid 15 mg, nicotinamide 20 mg, pyridoxine 6 mg, biotin 0.15 mg, folic acid 1.5 mg, and cobalamin 0.02 mg.

Table 2 Primer sequence of target genes

Gene	Accession number	Primer sequences	Product Size (bp)
β-actin	NM_205518.1	F: ATTGTCCACCGCAAATGCTTC R: AAATAAAGCCATGCCAATCTCGTC	113
SVCT1	XM_004944768.3	F: GCTGTACCAGATCGAGGACG R: AGGTGAAGATGGTGCCGATG	173
SVCT2	XM_025142777.1	F: AGGCAAACACTGGGGTATCG R: GCGAGCATAGAAGCCGTACT	247
GLO	XM_015285218.2	F: GCCAAGGAGGATTCAAGTT R: GATGTCAGAGGGCGAGTG	167

SVCT1, sodium-dependent vitamin C transporter 1; SVCT2, sodium-dependent vitamin C transporter 2; GLO, L-gulonolactone oxidase.

Table 3. Comparison of egg parameters and vitamin C content among Arbor Acres breeder eggs, Isa Brown breeder eggs and Hy-Line Brown layer eggs

Items	Varieties of egg			SEM	*P*-value
	Arbor Acres	Isa Brown	Hy-Line Brown		
Egg parameters					
EW (g)	72.08[c]	60.94[b]	58.79[a]	0.888	< 0.001
YFW/AFW	0.62[b]	0.47[a]	0.47[a]	0.014	< 0.001
YDW/ADW	2.57[b]	1.80[a]	1.65[a]	0.067	< 0.001
YMC (%)	50.75[a]	50.05[a]	54.09[b]	0.427	< 0.001
AMC (%)	88.11[b]	86.81[a]	86.89[a]	0.119	< 0.001
Egg vitamin C content					
YVCC (μg/g YDM)	42.07[b]	15.29[a]	19.91[a]	2.455	< 0.001
AVCC (μg/g ADM)	185.25[b]	126.03[a]	114.73[a]	5.139	< 0.001

EW, egg weight; YFW/AFW, the ratio of yolk fresh weight to albumen fresh weight; YDW/ADW, the ratio of yolk dry weight to albumen dry weight; YMC, yolk moisture content; AMC, albumen moisture content; YVCC, yolk vitamin C content; AVCC, albumen vitamin C content. [a, b] Means within a row with different superscript letters are different at $P < 0.05$.

Table 4. Effects of dietary vitamin C supplementation on laying performance in Hy-Line Brown layers

Items	Vitamin C treatments (mg/kg)			SEM	*P*-value
	0	200	400		
EW (g)	60.86	60.61	60.76	0.100	0.605
Egg production rate (%)	95.71	92.46	95.36	0.876	0.253
Daily feed intake (g)	120	120	120	-	-
Feed-to-egg ratio	2.13	2.21	2.09	0.024	0.123

EW, egg weight.

Table 5. Effects of dietary vitamin C supplementation on egg quality of Hy-Line Brown layers on day 23

Items	Vitamin C treatments (mg/kg)			SEM	*P*-value
	0	200	400		
EW (g)	61.27	59.94	61.21	0.787	0.798
Yolk color	6.87	6.47	6.58	0.119	0.389
Haugh unit	99.48	99.68	100.26	0.925	0.938
Albumen height (mm)	10.08	10.07	10.32	0.203	0.851
Shell strength (kg/cm^2)	43.92	40.86	48.50	1.457	0.119
Shell thickness (mm)	0.36	0.35	0.37	0.006	0.297

EW, egg weight. Shell strength, measurement with big end of the egg facing up. Shell thickness, mean shell thickness of small end, middle and big end of the egg.

Table 6. Effects of dietary vitamin C supplementation on egg parameters and egg vitamin C content on day 28 and serum vitamin C content of Hy-Line Brown layers

Items	Vitamin C treatments (mg/kg)			SEM	*P*-value
	0	200	400		
Egg parameters					
EW (g)	59.34	57.80	61.12	0.928	0.369
YFW (g)	15.35	15.25	15.57	0.275	0.901
AFW (g)	36.74	35.89	37.66	0.626	0.541
YFW/AFW	0.418	0.426	0.415	0.008	0.872
YDW (g)	7.82	7.73	7.84	0.146	0.951
AWMW (g)	4.56	4.47	4.70	0.086	0.574
YDW/AWMW	1.73	1.74	1.68	0.043	0.864
YMC (%)	49.10	49.37	49.63	0.144	0.321
AMC (%)	87.58	87.53	87.51	0.146	0.981
Egg vitamin C content					
YVCC (μg/g YDM)	13.36	12.37	15.54	1.175	0.558
AVCC (μg/g ADM)	114.57	98.22	107.97	3.958	0.259
YVCC (μg/egg)	102.15	95.78	118.81	7.911	0.497
AVCC (μg/egg)	523.81	438.33	507.77	21.004	0.233
Serum vitamin C content					
SVCC (μg/mL)	21.42	22.06	22.73	0.688	0.760

EW, egg weight; YFW, yolk fresh weight; AFW, albumen fresh weight; YFW/AFW, the ratio of yolk fresh weight to albumen fresh weight; YDW, yolk dry weight; ADW, albumen dry weight; YDW/ADW, the ratio of yolk dry weight to albumen dry weight; YMC, yolk moisture content; AMC, albumen moisture content; YVCC, yolk vitamin C content; AVCC, albumen vitamin C content; SVCC, serum vitamin C content.

Table 7. The relative mRNA expression of sodium-dependent SVCT1 and SVCT2 in intestine (duodenum, jejunum and ileum) and reproductive tract (ovary and magnum), and SVCT1, SVCT2 and GLO in liver and kidney of Hy-Line Brown layers

Items	Sites			SEM	P-value
	Duodenum	Jejunum	Ileum		
SVCT1	1.03^{a}	8.03^{a}	18.94^{b}	2.268	< 0.001
SVCT2	1.03^{a}	1.16^{a}	2.78^{b}	0.223	< 0.001
	Ovary	Magnum			
SVCT1	1.11	40.65		1.928	< 0.001
SVCT2	1.20	0.58		0.267	0.047
	Liver	Kidney			
GLO	1.05	881.18		84.656	< 0.001
SVCT1	1.03	6.18		0.451	< 0.001
SVCT2	1.04	0.66		0.374	0.010

SVCT1, sodium-dependent vitamin C transporter 1; SVCT2, sodium-dependent vitamin C transporter 2; GLO, L-gulonolactone oxidase.

[a, b] Means within a row with different superscript letters are different at $P < 0.05$.

Figures

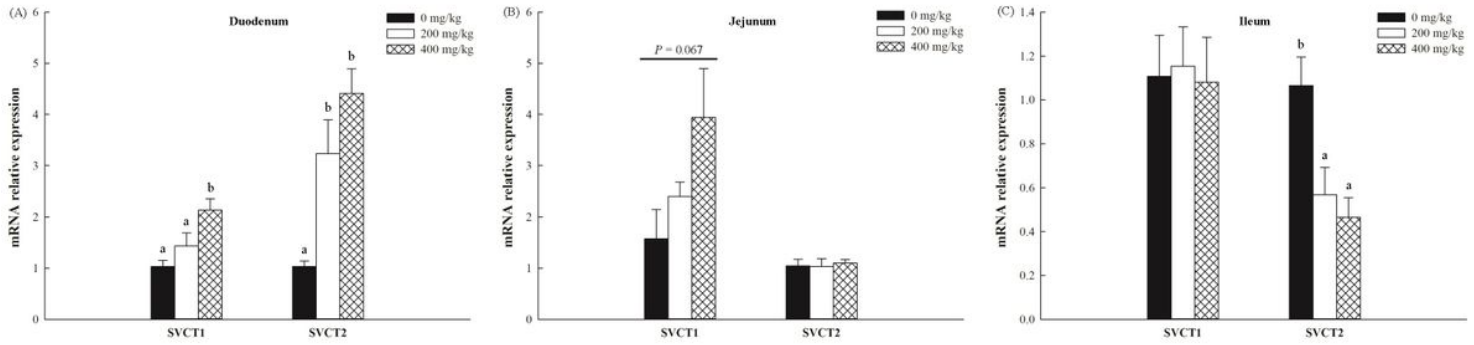

Figure 1

Effects of dietary vitamin C supplementation on the mRNA expression of SVCT1 and SVCT2 in duodenum (A), jejunum (B) and ileum (C) of Hy-Line Brown layers. 0, 200 and 400 mg/kg stand for the experimental diets supplemented with 0, 200 and 400 mg/kg VC on the basal diet, respectively. Values are means ± SEM.a-bValues with different superscript letters are different (P< 0.05, n=8). SVCT1, sodium-dependent vitamin C transporter 1; SVCT2, sodium-dependent vitamin C transporter 2.

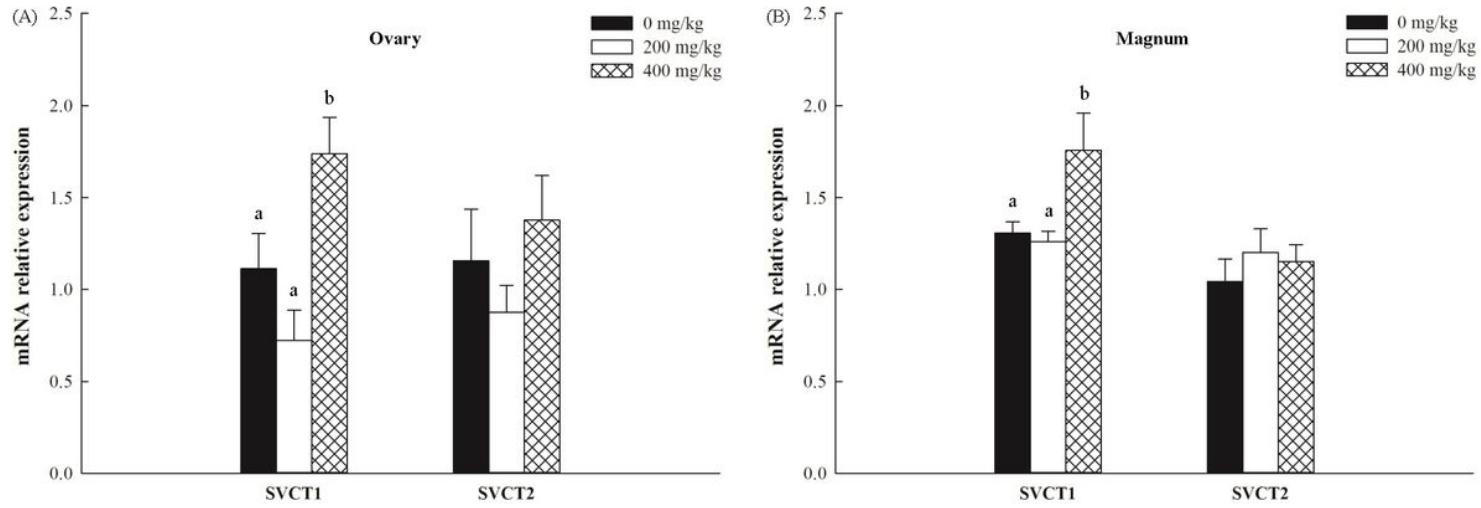

Figure 2

Effects of dietary vitamin C supplementation on the mRNA expression of SVCT1 and SVCT2 in ovary(A) andmagnum(B) of Hy-Line Brown layers. 0, 200 and 400 mg/kg stand for the experimental diets supplemented with 0, 200 and 400 mg/kg VC on the basal diet, respectively. Values are means ± SEM.a-bValues with different superscript letters are different (P< 0.05, n=8). SVCT1, sodium-dependent vitamin C transporter 1; SVCT2, sodium-dependent vitamin C transporter 2.

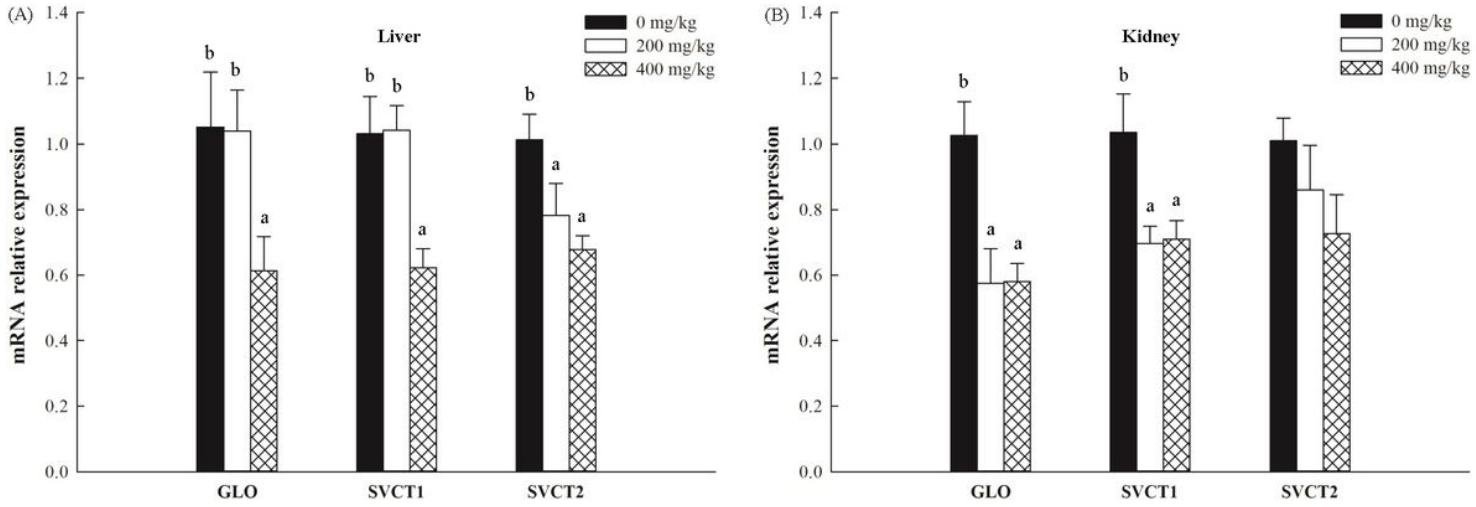

Figure 3

Effects of dietary vitamin C supplementation on the mRNA expression of GLO, SVCT1 and SVCT2 in liver(A) andkidney(B) of Hy-Line Brown layers. 0, 200 and 400 mg/kg stand for the experimental diets supplemented with 0, 200 and 400 mg/kg VC on the basal diet, respectively. Values are means ± SEM.a-bValues with different superscript letters are different (P< 0.05, n=8). GLO, L-gulonolactone oxidase;SVCT1, sodium-dependent vitamin C transporter 1; SVCT2, sodium-dependent vitamin C transporter 2.

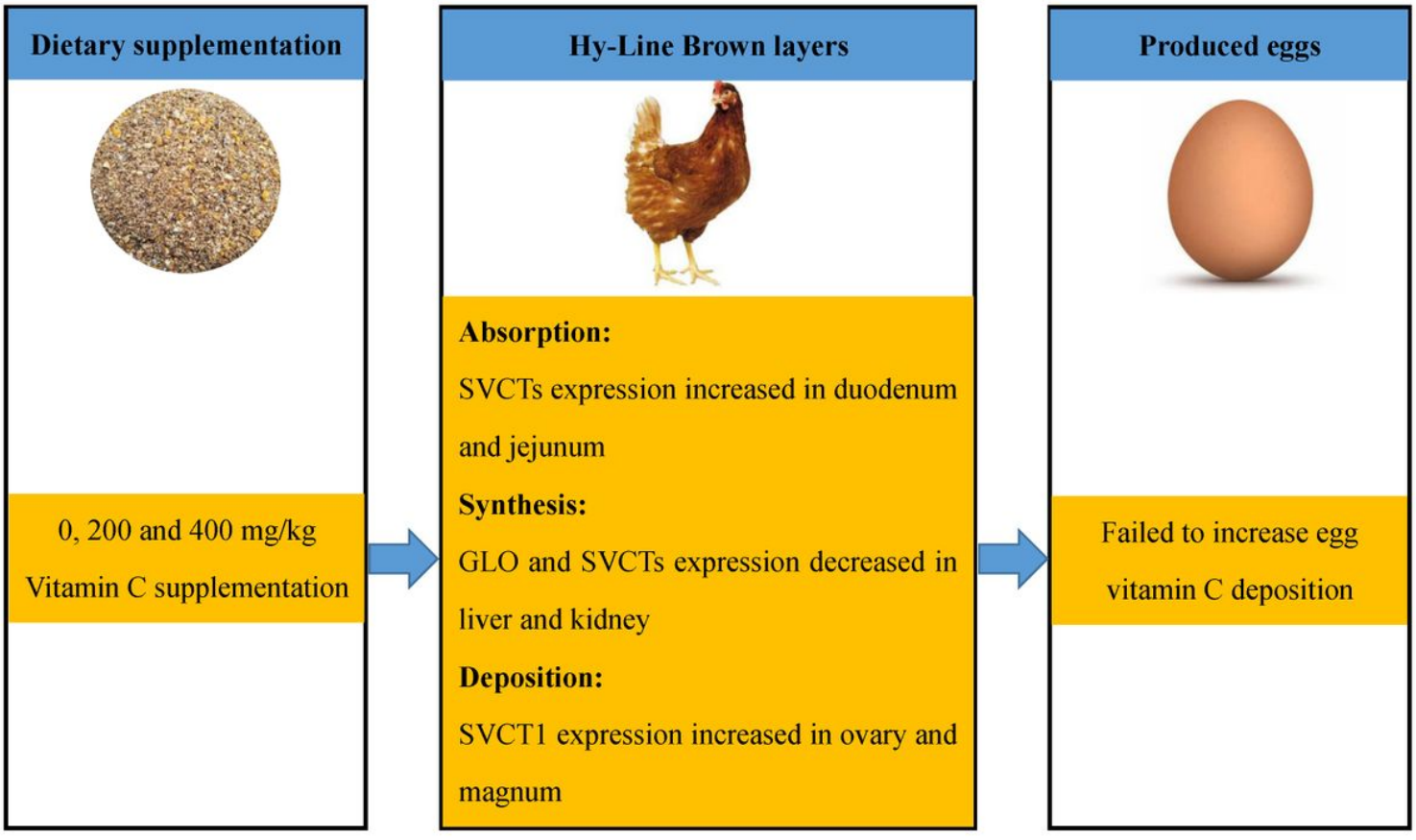

Figure 4

Graphical illustration of the core findings in this study.SVCT1, sodium-dependent vitamin C transporter 1; SVCTs, sodium-dependent vitamin C transporters (including SVCT1 and SVCT2); GLO, L-gulonolactone oxidase.