

# Effects of different inorganic selenium levels in laying quails (*Coturnix coturnix japonica*) diets on performance, egg quality, and serum biochemical parameters

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## Abstract

Inorganic selenium supplementation in poultry diet has been controversial. It has been linked that the excess and deficiency of this mineral can lead to health problems in these animals. However, this fact is not so evident in quails. In this research 120 female quails ( $220.6 \pm 8.2$  g) at 10 weeks of age were allocated to five treatment groups with six replicates of four quails in each. Experimental diets were formed by adding 0, 0.25, 0.50, 0.75 or 1.00 mg/kg of inorganic selenium (sodium-selenite) to the diet containing 0.12 mg/kg of selenium. We observed that performance parameters, mortality, egg external, and internal quality of quails were not affected by the supplementation of inorganic selenium to the diet. Serum glucose ( $P = 0.0020$ ) and creatinine ( $P = 0.0333$ ) levels were affected by inorganic selenium supplementation, but no differences were found for other parameters among those treatments. The addition of 0.50 mg/kg of inorganic selenium to the diet increased serum glucose levels of laying quails compared with the control group. While serum creatinine level was maximized with the addition of 0.25 mg/kg inorganic selenium to the diet, and it was minimized with the addition of 0.50 mg/kg inorganic selenium. Supplementation with inorganic selenium (0 to 1.00 mg/kg) in laying quail diets did not have any adverse effect on performance, mortality, and egg quality during the study. No abnormalities were found in the serum parameters that would lead to the suspicion of metabolic disease in the quails.

**Keywords:** egg production; egg quality; poultry nutrition; selenium; serum parameters.

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Additional information and declarations  
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## Study contribution

Selenium is an essential trace element for animal health and it plays an important role in the performance, reproductive system, and antioxidant defense system. However, excess selenium (mainly in the inorganic form) has been linked to health problems in poultry. The current study demonstrated that dietary sodium selenite, even at high doses, had no adverse effects on laying quail (in terms of productive performance, egg quality attributes, and serum parameters). Further research is needed to evaluate these findings over the course of quail's productive life.

## Introduction

Several factors such as environmental conditions, feed, or breed could affect the performance and egg quality in birds.<sup>(1)</sup> Among that, mineral composition, such as differences in selenium concentration, may affect a bird's performance and the chemical composition of its eggs.<sup>(2–5)</sup>

Selenium (Se) is an essential trace element for human and animal health, and it plays an important role in the performance,<sup>(2, 5–7)</sup> reproductive system,<sup>(8)</sup> and antioxidant defense system.<sup>(2, 3, 9)</sup> Bird performance may be improved by selenite reserves in muscles that support antioxidant defenses.<sup>(3, 8, 9)</sup>

There are two major selenium sources for poultry, namely, inorganic selenium (mainly sodium-selenite, Na<sub>2</sub> SeO<sub>3</sub>) and organic selenium.<sup>(5)</sup> Organic selenium is an integral part of many feed or food ingredients, while sodium-selenite, usually, added as a supplement to commercial diets and it is cheaper compared to organic selenium. The assimilation and absorption of organic and inorganic selenium differ. The absorption of organic selenium is carried out by the erythrocytes, similar to methionine. However, inorganic selenium is absorbed by simple diffusion. This is the reason why inorganic selenium is retained in lesser concentrations in the muscles and it is excreted in higher concentrations than organic selenium.<sup>(4)</sup>

The National Research Council<sup>(10)</sup> proposed that diets for Japanese quails must contain a minimum 0.15 mg/kg of selenium; if it is not met, the antioxidant system can be compromised and have serious consequences for their metabolism.<sup>(11)</sup> Excess selenium (mainly in the inorganic form) has been linked to health problems in poultry. In Europe and the United States, supplementation with inorganic forms of selenium is permitted to a maximum level of 0.50 mg/kg while the organic forms are allowed up to 0.30 mg/kg.<sup>(12)</sup> Previous studies suggested that the use of inorganic selenium is more limited considering the possible toxic effects.<sup>(13)</sup> Although this fact appears obvious in broilers and laying hens, it is not so clear in quail.

To improved antioxidant and immune responses, selenium in poultry feed needs to be supplemented at a suitable dose.<sup>(14)</sup> Previous studies have compared the effects of different selenium sources (organic or inorganic) on different poultry productivity parameters.<sup>(2, 15–18)</sup> However, the optimal level of organic selenium which can be included in the diet of laying quails without any harmful effects has not been determined. Hence, the current study investigated the effects of diets containing different levels of sodium selenite on performance, egg quality, and serum biochemical parameters of laying quails.

**Table 1.** Ingredients of basal diet and its chemical composition (as fed)

Ingredients	g/100 g	Chemical composition	g/100 g
Corn	54.0	Metabolizable energy, kcal/kg	2902
Soybean meal (46 % CP)	27.0	Crude protein	20.1
Sunflower meal (30 % CP)	7.0	Crude fat	7.3
Soybean oil	4.3	Crude fiber	4.1
Calcium carbonate	5.6	Moisture	12.7
Dicalcium phosphate	1.2	Calcium	2.5
Salt	0.4	Available phosphorus	0.4
Premix <sup>1</sup>	0.3	Lysine	1.0
DL methionine	0.2	Methionine	0.5
		Cystine	0.4
		Methionine + cystine	0.8
		Selenium, mg/kg	0.1
Total	100.00		

<sup>1</sup> Premix provided the following per kilogram of diet; manganese (manganese oxide): 80 mg, iron (ferrous carbonate): 60 mg, copper (cupric sulfate pentahydrate): 5 mg, iodine: 1 mg, vitamin A (trans-retinyl acetate): 8 800 IU, vitamin D<sub>3</sub> (cholecalciferol): 2 200 IU, vitamin B<sub>2</sub> (Riboflavin): 4.4 mg, thiamin: 2.5 mg, vitamin B<sub>12</sub> (cyanocobalamin): 6.6 mg, folic acid: 1 mg, biotin: 0.11 mg, choline: 220 mg.

## Materials and methods

### Ethical statement

This research was reviewed and approved by the Committee for Experimental Animal Care, Faculty of Agriculture, University of Selçuklu (Konya, Turkey). Criteria specified by European policy for protecting animals<sup>(19)</sup> were followed during the experimental period.

### Experimental birds, rearing and feeding

The experiment was conducted among 120 female Japanese quails (*Coturnix coturnix japonica*) of similar body weight ( $220.61 \pm 8.22$  g) and 10 weeks of age obtained from a commercial company at Selçuklu, Konya, Turkey (38°1'36, 32°30'45). A completely randomized design was used for this experiment. Animals were randomly allocated to 5 identical cages that had the same environmental conditions. The study was conducted in 5 experimental groups consisting of 6 replicates, each containing 4 female quails.

Quails were housed in clean and disinfected battery cages (30 cm wide 45 cm long). The quails were maintained in a well-ventilated room with a lighting program of 16 h. A temperature of  $20 \pm 2$  °C was arranged in each pen. Each pen was provided with an individual feeder and drinker to allow *ad libitum* intake.

Quails were fed for 70 days with 5 treatment diets that added 0, 0.25, 0.50, 0.75, or 1.0 mg/kg sodium-selenite (Na<sub>2</sub> SeO<sub>3</sub>) also called inorganic selenium, to the basal diet. The basal diet was developed according to the National Research Council<sup>(10)</sup> to supply requirements layer quails. The chemical composition of the basal diet was analyzed according to the AOAC<sup>(20)</sup> proceedings. Table 1 shows the ingredients of the basal diet and its chemical composition.

### Determination of performance parameters

At the beginning of the experiment, the quails were randomly allotted to the five dietary treatments, and they were weighed at the beginning and at the end of the experiment with a precision weighing balance ( $\pm 0.01$  g) to determine changes in body weight.

Experimental diets were given by weighing to each subgroup, and subsequently, feed intake (FI) was calculated as the daily feed intake (g) per quail. Simultaneously (at 10:00 am), eggs were collected and recorded. Egg production was determined by dividing the number of eggs obtained in a day by the number of quails and multiplying by 100 and it was given as a percentage (%).

Egg weight (EW) was determined by weighing one the eggs collected on the last three days of the experiment with a precision weighing balance ( $\pm 0.01$  g). From these data, egg mass (EM) was calculated as daily egg weight (g) per quail according to the following equation (1):

$$\text{Egg mass} = \frac{(\text{Egg production} \times \text{Egg weight})}{100} \quad (1)$$

Finally, feed conversion ratio (FCR) was determined according to next equation (2):

$$\text{Feed conversion ratio} = \frac{\text{Feed intake (g)}}{\text{Egg mass}} \quad (2)$$

### Determination of egg quality parameters

During the experiment, broken, cracked, and damaged eggs were recorded and calculated as a percentage of the number of eggs. Egg internal and external quality parameters were determined at room temperature and at Selcuk University, Faculty of Agriculture, Egg Quality Laboratory from all eggs collected in the last three days of the trial. Eggshell breaking strength was assessed by applying supported-systematic pressure to the blunt of the eggs (Egg Force Reader, Orka Food Technology, Israel).

Immediately after the determination of the eggshell breaking strength, the eggs were broken on a clean, glass surface, and after the residues in the eggshell were cleaned, the shells were dried at room temperature for three days and weighed, and relative weights were calculated as a ratio (%) of the egg weight. Eggshell thickness was calculated by averaging the measurements obtained from three sections (equator, blunt, and pointed parts) of the eggshell using a micrometre (Mitutoyo, 0.01 mm, Japan). Eggs, whose external quality characteristics were determined, were broken on a surface and their albumen and yolk heights were measured with a height gauge and their length and width were measured using a 0.01 mm digital caliper.

The parameters calculated from these data and the equations used are as follows. Albumen index was calculated using equation 3:

$$\text{Albumen index} = \frac{\text{Albumen height}}{\frac{\text{Albumen width} + \text{Albumen length}}{2}} \times 100 \quad (3)$$

To determinate yolk index equation 4 was used:

$$Yolk\ index = \frac{Yolk\ height}{Yolk\ diameter} \times 100 \quad (4)$$

Finally, Haugh unit for each egg was calculated using data of egg weight and albumen height according to equation 5 proposed by Stadelman and Cotterill.<sup>(21)</sup>

$$Haugh\ unit = 100 \times \log(albumen\ height + 7.57 - 1.7 \times EW^{0.37}) \quad (5)$$

### Serum biochemical analysis

At the end of the experimental period (10th week), two quails were randomly selected from each subgroup ( $n = 60$ ) and 3 mL blood samples were collected from the neck vein to determine serum parameters. After, blood samples were centrifuged at 4 000 rpm for 10 min at 20 °C and serum was extracted. The serum was stored at -20 °C until analysis, and the concentrations of glucose, triglyceride, cholesterol, total protein, albumin, globulin, urea, creatinine, calcium, and phosphorus in the serum were determined according to Tietz<sup>(22)</sup> in an auto-analyser device employing commercially available kits (DDS® Spectrophotometric Kits, Dysis Diagnostic Systems Co., Istanbul, Turkey).

### Statistical analysis

A one-way ANOVA was used to test the effect of the experimental diets on performance, egg quality, and serum parameters in the quails. If ANOVA showed significant differences among means (main effects), a planned multiple comparison of means was examined by Duncan's multiple range test. The statistical differences were defined as  $P < 0.05$  and trends as  $P < 0.10$ . All statistical analyses were carried out using the SPSS Package 23 (IBM SPSS Statistic, 2017).

## Results

### Performance parameters

No mortality or illness symptoms were observed during the entire trial among the diets, and all quails were alive at the end of the experiment. Table 2 demonstrates the influence of selenium dietary supplementation on the performance parameters in layer quails. The final body weight and body weight change were determined as 243.6 g and 23.0 g, respectively, feed intake was 28.3 g/day/quail, and the feed conversion ratio was 2.5. Additionally, the averages of egg production, egg weight, and egg mass were 92.0 %, 12.4 g, and 11.4 g/day/quail, respectively. According to the data in Table 2, the administration of inorganic selenium to the diet at different levels did not statistically affect performance parameters (in terms of final body weight, body weight change, feed intake, egg production, egg weight, egg mass, and feed conversion ratio).

**Table 2.** Effects of dietary inorganic selenium levels on quails' performance parameters

	Supplemented inorganic selenium (mg/kg)					SE±	P Value
	0	0.25	0.50	0.75	1.00		
Initial body weight (g)	220.8	221.3	219.6	221.9	219.6	2.5	0.9511
Final body weight (g)	243.8	246.3	243.6	241.8	242.6	6.1	0.9893
Body weight change (g)	23.1	25.0	24.0	19.9	23.0	5.2	0.9689
Feed intake (g/day/quail)	27.7	28.6	28.4	28.5	28.3	0.8	0.9468
Egg production (%)	91.7	92.6	93.2	91.4	90.7	1.1	0.5341
Egg weight (g)	12.6	12.5	12.1	12.3	12.7	0.3	0.6383
Egg mass (g)	11.5	11.6	11.3	11.2	11.5	0.3	0.8782
Feed conversion ratio	2.4	2.5	2.5	2.5	2.5	0.1	0.7157

SE ± : standard error of the estimation

**Table 3.** Effect of the addition of inorganic selenium to laying quail diets on egg quality parameters

	Supplemented inorganic selenium (mg/kg)					SE±	P value
	0	0.25	0.50	0.75	1.00		
Eggshell breaking strength (kg)	1.5	1.4	1.5	1.5	1.5	0.1	0.2040
Albumen index	19.6	19.9	19.7	21.7	19.1	0.7	0.3184
Yolk index	61.8	61.8	61.0	62.2	62.1	0.8	0.8586
Haugh unit	105.9	107.1	106.0	108.0	106.3	0.9	0.4239
Eggshell weight (%)	8.0	8.0	8.1	8.1	8.1	0.2	0.9410
Eggshell thickness (mm)	0.2	0.2	0.2	0.2	0.2	0.0	0.9402
Damaged egg rate (%)	0.3	0.2	0.2	0.0	0.2	0.2	0.8344

SE ± : standard error of the estimation

### Egg quality parameters

The effects of the dietary supplementation of inorganic selenium on the egg quality are given in Table 3. The examined egg internal and external quality parameters were not significantly affected by the treatments. The minimum and maximum values were as follows: eggshell breaking strength (1.4 – 1.5 kg), albumen index (19.1 – 21.7), yolk index (61 – 62.2), Haugh unit (105.9 – 108), eggshell weight (8.0 – 8.1 %), eggshell thickness (0.20 mm), and damaged egg rate (0 – 0.3 %).

### Serum biochemical parameters

The overall mean values for the serum biochemistry parameters of layer quails are shown in Table 4. The minimum and maximum values in parentheses were as follows: glucose (307.2 – 342.3 mg/dL), urea (10.00 – 12.60 mg/dL), creatinine (0.3 – 0.4 mg/dL), albumin (1.5 – 1.7 g/dL), globulin (2.4 – 2.7 g/dL), total protein (3.9 – 4.4 g/dL), cholesterol (165.8 – 229.2 mg/dL) calcium (21.4 – 23.4 mg/dL), and phosphorus (6.6 – 7.4 mg/dL).

**Table 4.** Effect of the addition of inorganic selenium to laying quail diets on serum biochemical parameters

	Supplemented inorganic selenium (mg/kg)					SE±	P value
	0	0.25	0.50	0.75	1.00		
Glucose (mg/dL)	311.4 <sup>bc</sup>	328.5 <sup>ab</sup>	342.3 <sup>a</sup>	307.2 <sup>c</sup>	312.0 <sup>bc</sup>	6.2	0.0020
Urea (mg/dL)	10.6	10.0	11.6	10.4	12.6	1.5	0.7451
Creatinine (mg/dL)	0.3 <sup>ab</sup>	0.3 <sup>a</sup>	0.3 <sup>ab</sup>	0.3 <sup>b</sup>	0.3 <sup>ab</sup>	0.0	0.0333
Albumin (g/dL)	1.6	1.6	1.6	1.6	1.7	0.1	0.8071
Globulin (g/dL)	2.7	2.4	2.7	2.5	2.7	0.2	0.4932
Total protein (g/dL)	4.2	3.9	4.3	4.0	4.4	0.3	0.6710
Cholesterol (mg/dL)	184.4	176.0	222.8	165.8	229.2	22.0	0.1843
Calcium (mg/dL)	21.4	22.9	22.4	23.4	21.9	1.1	0.7077
Phosphorus (mg/dL)	6.6	7.1	7.4	7.1	6.7	0.4	0.6101

SE ± : standard error of the estimation

<sup>a, b, c</sup> Means with different superscripts in the same row were significantly different ( $P < 0.05$ ).

According to Table 4, the addition of different levels of inorganic selenium to the diet did not statistically affect serum biochemical parameters except for glucose ( $P = 0.0020$ ) and creatinine ( $P = 0.0333$ ). The serum glucose concentration of the quails fed with the diet supplemented with 0.50 mg/kg inorganic selenium was found to be higher (342.3 mg/dL) than the group fed with the diet supplemented with 0.75 mg/kg selenium-selenite (307.2 mg/dL). Also, serum creatinine levels were higher in quails that received 0.25 mg/kg of sodium-selenite than in those that received 0.75 mg/kg.

## Discussion

Selenium is trace mineral to improve human and animal health. Thus, to maintain bird health and prevent diseases, such as muscular dystrophy and myopathies of the gizzard, it is important to formulate a diet with an adequate level of selenium.<sup>(23)</sup>

The effect of sodium selenite supplementation on performance, including final body weight, body weight change, feed intake and feed conversion ratio, were similar for all experimental groups. Supplementation with sodium selenite did not affect any of these parameters. Results showed productivity of quails up to 20 weeks of age did not change, regardless of the selenite supplementation level. Our results are in accordance with most of previous studies that report no influence of sodium selenite on body weight gain and body weight change.<sup>(14, 23-25)</sup> Tsekhmistrenko et al.<sup>(13)</sup> observed that dietary sodium selenite increased body weight gains of quails at the beginning of the study. However, this trend disappeared after five weeks. Conversely, Yang et al.<sup>(15)</sup> indicated that inorganic selenium decreased the vitality and feed efficiency of broilers by 0.93 and 4.84 %. Mozin et al.<sup>(26)</sup> proposed that supplementation of diets with 0.4 ppm sodium selenite reduces feed intake affecting turkey production performance. Accordingly, these authors proposed that when the birds were offered the highly toxic selenium diet (above 0.3 ppm), the birds would discriminate the diets by reducing feed intake. However, Taylor et al.<sup>(27)</sup> have shown no effects on turkey performance when 5 µg/g of sodium selenite was



added. Based on the mentioned literature, it appears that broilers could be more sensitive to higher sodium selenite doses than quails or turkeys.

Apparently, egg production, egg weight and egg mass were not affected by sodium-selenite supplementation, as there was no difference in productive performance. Results showed that supplementary dietary selenium did not improve productive performance or egg production of laying quails. As observed here, results for animals on the basal diet were comparable to those on the experimental diets. Therefore, selenium-enriched diets (up to 1 mg/kg) for laying quails (for 10 weeks) do not seem to affect egg production.

These findings agree with those of previous authors<sup>(28-30)</sup> who reported that supplemental dietary sodium selenite did not affect egg production, egg weight or egg mass in laying hens. Nassef et al.<sup>(24)</sup> found similar results for performance parameters, except for egg weight. According to the report of these authors, egg weight was higher in quails fed a diet supplemented with 0.40 mg/kg sodium-selenite compared to birds fed 0.20 mg/kg of dietary selenium. These findings contrast with those from previous experiments documenting dietary supplementation with different levels of sodium selenite improved egg production and egg weight.<sup>(5, 31, 32)</sup> According to Meng et al.,<sup>(31)</sup> a potential reason for such discrepancies may be differences in the duration of the experiments.

As observed for productive variables, dietary selenite did not impact egg quality attributes, even at high (1 mg/kg) or low (0.1 mg/kg) levels of sodium selenite supplementation. This trend remained unchanged during the first 20 weeks of quails' life. Therefore, farmers could lower production costs by avoiding sodium selenite supplementation, at least during the first 20 weeks.

The results of the current experiment agree with the findings of previous authors<sup>(33, 34)</sup> who did not find differences in egg quality when sodium-selenite was added to quail diets. Meng et al.<sup>(31)</sup> revealed that sodium selenite has a positive impact on enhancing the yolk index, Haugh unit and shell thickness compared with those fed control ration in Aseel varieties. Nemati et al.<sup>(30)</sup> stated that eggshell weight in quails tended to improve with the addition of different levels of sodium-selenite to the diet. Chinrasri et al.<sup>(33)</sup> pointed out that the differences found in terms of Haugh unit and egg weight depend on the selenium source used and not on the dose of inorganic selenium.

Previous authors considered that the use of high inorganic selenium doses in the diet of quails be limited due to the possible toxic effects.<sup>(13)</sup> However, our results showed that an amount of sodium-selenite above 0.50 mg/kg did not negatively influence egg production and quail performance during the study time (10–20 weeks of age). Additionally, there was no adverse effect of sodium-selenite up to 1 mg/kg diet on the appetite, as indicated by similar feed intake among all groups.

The National Research Council<sup>(10)</sup> proposed that diets for Japanese quails must contain a minimum of 0.15 mg/kg of selenium. Lower levels have been associated with the development of cardiovascular disease, impaired growth and development, poor feathering, reduce egg production, susceptibility to viral infections, and an increased risk of mortality.<sup>(13)</sup> However, all our experimental animals were alive and apparently healthy at the end of the study, even quails fed the control diet without supplementation (0.1 mg/kg of selenium), as shown in Table 1. Surai et al.<sup>(8)</sup> proposed that the ingredients of poultry diets have variable selenium content, and perhaps this content is adequate to satisfy the requirements of laying quails.



In this study, quails fed diets supplemented with sodium-selenite at 0.5 mg/kg had the highest serum glucose level. It could be assumed that if the amount of glucose in the blood is increased, the production of eggs and the development of the quail should be improved.<sup>(24)</sup> However, serum glucose levels had no effect ( $P = 0.5341$ ) on production parameters. Hence, further long-term studies are needed to better understand the effects of selenium supplementation in quails' productive performance and health.

The highest serum creatinine value was observed in quails fed a diet containing 0.25 mg/kg sodium-selenite. Serum urea and creatinine concentrations increase when renal tubules are severely injured.<sup>(34)</sup> However, there were no differences in the urea concentration between the experimental groups. Thus, we believe it is unlikely that there was some kidney damage, although we did not perform histopathological examination of organs.

The results of this experiment are partially in agreement with those of previous studies. Liu et al.<sup>(35)</sup> found that the addition of sodium selenite to the diet had no effect on serum albumin, total protein, and urea nitrogen. Yang et al.<sup>(15)</sup> reported that selenium does not affect the total protein, globulin, and glucose concentration. Ibrahim et al.<sup>(14)</sup> reported that albumin, total protein, and urea nitrogen levels were not altered by sodium selenite, while a lower concentration of creatinine was observed.

Determination of biochemical parameters in the blood is a tool that assists in the diagnosis of metabolic diseases.<sup>(12)</sup> Tsekhmistrenko et al.<sup>(13)</sup> proposed that high concentrations of sodium-selenite in the diet would lead to greater accumulation of selenium in the brain, heart, and liver, causing disease. Despite differences in serum creatinine and glucose levels between the study groups, the rest of the serum parameters did not differ. These findings suggest the nonexistence of metabolic diseases among the experimental groups.

Given these facts, it is reasonable to consider that sodium selenite supplementation may be avoided without affecting quail's production performance, egg quality and serum biochemical parameters for up to 20 weeks. However, in the current experiment, sodium selenite in the diet of quail was evaluated only for a limited period of time (20 weeks). According to Royter et al.,<sup>(36)</sup> the economically productive life of a laying quail is 48 weeks. In order to verify that there are no adverse effects on the animal's development or health status, these doses would need to be evaluated again throughout the animal's life.

## Conclusion

This study showed that supplementation of laying quail diets with sodium selenite up to 1 ppm for 20 weeks had no effect on production performance, egg quality, or serum biochemical parameters. During the study period, quails showed a wide margin of tolerance to both low (0.1 mg/kg) and high dietary sodium selenite (1 mg/kg). Further research would need to be done in the future to determine whether this trend remains stable across the quails' productive lives.

## Data availability

All relevant data are within the manuscript. Datasets used in this experiment are available from the corresponding author on reasonable request.

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## Conflicts of interest

The authors have no conflict of interest to declare in regard to this publication.

## Author contributions

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