Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X 2022 / v.24 / n.1 / 001-008

http://dx.doi.org/10.1590/1806-9061-2019-1218

Original Article

Calcium Requirement and Vitamin D Supplementation in Meat-Type Quail at First Stage of Growth

■Author(s)

Perine TP[I] https://orcid.org/0000-0001-8173-4134
Grieser DO[IV] https://orcid.org/0000-0002-3505-0871
Furlan AC[I] https://orcid.org/0000-0002-0453-2018
Ton APS[II] https://orcid.org/0000-0002-5826-1874
Benites M[I] https://orcid.org/0000-0000-0000-0000
Zancanela VT[III] https://orcid.org/0000-0001-7340-109X
Stanquevis CE[I] https://orcid.org/0000-0003-4361-5307
Marcato SM[I] https://orcid.org/0000-0003-4559-4183

[I] State University of Maringá. Department of Animal Science. Maringá, Parana-Brazil.
[II] Federal University of Mato Grosso. Institute of Agrarian and Environmental Sciences. Sinop, Mato Grosso, Brazil.
[III] Federal University of Sergipe. Department of Animal Science. Nossa Senhora da Glória, Sergipe, Brazil.
[IV] Federal University of South and Southeast of Para. Institute of Studies of the Humid Tropics. Xinguara, Para, Brazil.

■Mail Address

Corresponding author e-mail address
Daiane de Oliveira Grieser
Universidade Estadual de Maringa - Departamento de Zootecnia , Rua Doutor Mário Clapplier Urbinatti, 724 E - Maringá/PR, Brasil - 87020-900.
Phone: +55 44 97204269
Email: daianegrieser@gmail.com

■Keywords

Bone parameters, nutritional requirement, performance, weight gain.

Submitted: 04/August/2020
Approved: 04/January/2021

ABSTRACT

Currently, feed formulations for quail are based on foreign data or data from other species; this undermines productivity with the use of excessive or deficient levels of some nutrients. Therefore, this study aimed to determine the nutritional requirement levels of calcium (Ca) and supplementation vitamin D in meat-type quail from 1 to 14 d of age. The experiment was a 4 × 4 factorial arrangement of treatments (levels of Ca: 0.42, 0.58, 0.74 and 0.90% and levels of vitamin D: 1,000, 2,000, 3,000 and 4,000 IU) with 3 replicate pens and 43 quails per pen. Weight gain increased quadratically with increased levels of Ca and vitamin D; this variable yielded estimated levels of 0.73% Ca and 2,883 IU vitamin D. There was no Ca x vitamin D interaction effect on bone variables. Bone Ca, bone phosphorus, bone ash, femoral bone strength, tibial bone strength and the femoral Seedor index increased quadratically with an increase in Ca levels. These variables yielded estimated values of 0.78, 0.71, 0.78, 0.77, 0.83, and 0.71% Ca, respectively. It is concluded that the requirements of Ca and vitamin D for the maximum weight gain of meat-type quails, in the period from one to 14 days of age, were 0.73% Ca (p=0.001) and 2,883 IU vitamin D (p=0.02), respectively.

INTRODUCTION

Currently, Brazil is the second largest worldwide producer of quail eggs and one of the foremost meat producers after the introduction and expansion of the European species population. This may be due to the low initial investment and the fast return on investment associated with quail production (Silva *et al.*, 2009).

Despite these positive aspects of the industry, quail production has some challenges to face before it can meet producers' needs. In particular, there is a lack of scientific results regarding the production and especially the nutrition of these birds. The lack of nutrition researches for this species means that the diets are formulated based on the requirements for other species with physiological similarities, such as broiler chickens, for example.

The most important portion of the studies that evaluate calcium and vitamin D levels target the egg production phases, as these have a huge influence on the nutrients of egg formation. Thus, there is a current lack of information that can be used to estimate the optimum supplementation levels during the initial stage, when these nutrients play a fundamental role in bone metabolism and production.

Calcium deposition in the skeleton is higher in the early stages of life, so that by the end of the first month of life, chicks have 80% of the total calcium of an adult bird (Edwards, 2000). On the other hand, vitamins, according to Combs (2008), are not synthesized in sufficient quantities to meet the nutritional requirement of the birds, being necessary their supply in the diets, usually in minimal quantities, for the development, maintenance, production and reproduction of the animals. In the case of vitamin D, its most important function is in the homeostasis Ca^{2+} and phosphate. This is regulated by a multi-hormonal system that occurs at the points of intestinal absorption, bone augmentation and mobilization, and renal excretion, involving the production of 1,25 $(OH)_2D_3$ as needed, which works according to PTH and calcitonin (CT).

Therefore, this study aimed to determine the nutritional requirements levels of calcium (Ca) and supplementation vitamin D and in meat-type quail from 1 to 14 d of age, to maximize growth performance and to verify their effects on the bone development of these birds.

MATERIALS AND METHODS

The experimental procedure was approved by the Ethics Committee on the Use of Animals at the State University of Maringa (Protocol number 091/2012). This research was conducted in Maringa (latitude: 23°25′ S, longitude: 51°57′ W, and at an altitude of 596 m), located in the northwest of Paraná, Brazil, with a subtropical and tropical climate.

The study used 2,064 meat-type quail (*Coturnix coturnix sp*) between 1 to 14 d of age, which were kept in a conventional hangar divided into 48 pens of 2.5 m^2. The experimental design was a completely randomized, 4 × 4 factorial scheme (4 calcium (Ca) levels: 0.42, 0.58, 0.74, and 0.90% and 4 vitamin D levels: 1,000, 2,000, 3,000, and 4,000 IU), with a total of 16 treatments, 3 replications and 43 quail per experimental unit.

The temperature and humidity were recorded early in the morning and late afternoon during all experimental periods using a thermohygrometer. The experimental diets were formulated based on corn and soybean meal. The different Ca level contents in the diets were obtained by varying the amount of inert limestone, Ca phosphate and kaolin (Table 1).

The nutritional requirements of phosphorus were performed according to the recommendation of Silva *et al.*, (2009). The experimental diets were formulated to meet the requirements proposed by Rostagno *et al.*, (2011) for broilers in the initial phase from 1 to 21 d of age, with the exception of Ca and vitamin D. The chemical composition and energy values of the feeds were obtained from Rostagno *et al.* (2011).

Table 1 – Composition of the basal diet fed to quail during the first stage of growth[1].

Ingredient	Quantity (g/kg)
Corn	361.4
Soybean meal (45%)	532.9
Soybean oil	61.4
Monocalcium phosphate	13.5
Vitamin D_3[1]	1.0
Limestone + inert substance[2]	14.0
Vitamin and mineral premix[3]	4.0
Salt	4.0
DL-Met	4.5
L-Lys-HCl	2.0
L-Thr	1.2
Antioxidant[4]	0.1
Calculated values	
Metabolizable energy (MJ/kg)	12.55
Crude protein (g/kg)	275
Available P (g/kg)	4.1
Digestible Lys (g/kg)	16
Digestible Met + Cys (g/kg)	11.5
Digestible Thr (g/kg)	10.4
Digestible Trp (g/kg)	3.3
Cl (g/kg)	2.8
Na (g/kg)	1.8
K (g/kg)	10.8

[1]Vitamin D_3 (500,000 IU/g) was diluted with rice straw to achieve desired levels (1,000, 2,000, 3,000, or 4,000 IU/kg diet).

[2]Used 14.0 g inert substance (Kaolin, Nucleopar, Mandaguari, Brazil) + 0 g limestone, 9.8 g inert substance + 4.2 g limestone, 5.6 g inert substance + 8.4 g limestone, and 1.4 g inert substance + 12.6 g limestone to achieve dietary Ca concentrations of 0.42, 0.58, 0.74, and 0.90% Ca, respectively.

[3]Provided per kilogram of diet: retinol acetate, 2,800 IU; dl-α-tocopheryl acetate, 25 IU; thiamine hydrochloride, 1.4 mg; riboflavin, 5 mg; pyridoxine hydrochloride, 2.4 mg; cyanocobalamin, 12 mg; menadione nicotinamide bisulphite, 2.4 mg; D-calcium pantothenate, 12 mg; niacin acid, 35 mg; folic acid, 0.7 mg; biotin, 0.07 mg; choline chloride, 0.3 mg; butylated hydroxytoluene, 4 mg; zinc oxide, 0.048 g; ferrous sulphate, 0.05 g; manganese sulphate, 0.058 g; copper sulphate, 4 mg; potassium iodate, 1 mg; cobaltous sulfate heptahydrate, 0.2 mg; sodium selenite, 0.25 mg; and carrier q.s.p., 4 g.

[4]Butylated hydroxytoluene.

Quails, feed, and leftovers were weighed weekly to obtain the values of body weight (BW), weight gain (g / bird) (WG), feed intake (g / bird) (FI) and feed conversion (FC) of birds. Two quail per experimental unit were chosen for an evaluation of bone variables. They were stunned by electroshock and killed by cervical dislocation at the end of the experiment (14 d of age), according to the standards proposed by the ethics committee.

Seedor index

The bones of the selected quails (femur and tibia) were weighed on a precision scale and their length measured using a digital caliper to subsequently calculate the Seedor index (bone weight (mg) / length (mm)) (Seedor *et al.*, 1996).

The femur and tibia, after being measured the Seedor index, were immersed in petroleum ether for 24 hours to be degreased. Then they were dried in a forced air oven at 55°C for 72 hours for mineral density, bone strength and quantification of Ca and phosphorus concentration in the bone.

Optical density

Radiographic optical density was analyzed at the Dental Clinic in the University Hospital of Maringa. First, the bones were placed in the same position under a film (mark Kodak Intraoral E-Speed Film, size 2, periapical type) and radiographed with an X-ray dental appliance (model Spectro 70X electronic; DabiAtlante, Ribeirão Preto, Brazil). It was operated at 70 kVp, 8 mA, with a 0.2 s exposure time, based in a pilot test, which focused the X-ray beam perpendicular to the film at a focus-film distance of 10 cm.

After that, radiographic films were processed using an automatic processing machine (Revel Industry and Equipment Trade Ltd.) with a time of 150 s and operating with Kodak RP X-Omat solutions. Then, they were scanned into the Image Tool program (version 3.0) and recorded in files with a progressive JPG extension. Afterwards, using Adobe Photoshop CS6 software the readings and determination of the bone density were attained with the tool Histogram (which analyzes the radiographic density of a selected area that is distributed in a color scale). Areas that are distributed in 256 shades of gray were selected (0 represents the black and 256 the white). Three central points with a fixed size (10 px x 10 px) were selected from the bone and an average value was obtained.

An aluminum scale of 10 degrees (1 mm of thickness between degrees), was used as a radiographic reference. The data obtained in gray values was converted to relative values based on the third degree aluminum scale.

Bone resistance

Bone resistance was determined in a pressure and the bones were positioned supporting the epiphyseal regions, leaving the central region free (where the force was applied). The descent probe speed was 5 mm/s, used a load of 500 N (Newton) for all samples. After the bone resistance, the left femur was ground and dried in a forced air oven (105°C for 12 hours) to determine the Ca and phosphorus in the bone, using the methodology described by Silva & Queiroz (2002).

Statistical analysis

The data was analysed using the Statistical Analysis and Genetic System program – SAEG (Federal University of Viçosa, 1997). The following model was used:

$$Y_{ijkl} = b_0 + b_1C_i + b_2V_j + b_3C_i^2 + b_4V_j^2 + b_5CV_{ij} + FA + e_{ijkl}$$

Where:

Y_{ijkl} = observed value of the studied variables relative to experimental unit k,

fed a diet containing a level of calcium and a level of vitamin D;

C_i = calcium level, where: $C^1 = 0.42$; $C^2 = 0.58$; $C^3 = 0.74$; and $C^4 = 0.90\%$;

V_j = vitamin D level, where: $V^1 = 1000$; $V^2 = 2000$; $V^3 = 3000$; and $V^4 = 4000$ IU;

b_0 = general constant;

b_1 = coefficient of linear regression as a function of calcium level;

b_2 = coefficient of linear regression as a function of vitamin D level;

b_3 = coefficient of quadratic regression as a function of calcium level;

b_4 = coefficient of quadratic regression as a function of vitamin D level;

b_5 = coefficient of linear regression as a function of the interaction between calcium level and D vitamin;

FA = lack of adjustment of the regression model;

e_{ijkl} = random error associated with each observation.

The nutritional requirement for Ca and vitamin D were estimated using a quadratic model or a discontinuous Linear Response Plateau (LRP), determined by the data fit for each variable analyzed.

Results were obtained from the response surface analysis, which is a collection of mathematical and statistical techniques used to analyze problems pertaining to the influence of independent variables on response dependent variables, with the ultimate goal of optimizing responses (Montgomery, 2009).

RESULTS

There was no interaction due to the effects of Ca and vitamin D levels on quail from 1 to 14 d of age (Table 2). Ca (p=0.001) and vitamin D (p=0.02) had a positive quadratic effect on WG. The estimates for maximum WG (74.83 g/d) and FI (144.06 g/quail) were based on diets that contained 0.73 and 0.72% Ca and 2,883 IU vitamin D, respectively.

Table 2 – Effect of Ca and vitamin D on growth performance of quails during 1 to 14 d of age[1].

Levels	FI (g/quail)	WG (g/d)	FC (g/g)
Ca (%)			
0.42	135.1	68.2	1.98
0.58	142.3	72.5	1.97
0.74	143.8	73.3	1.96
0.90	140.8	72.0	1.95
Vitamin D (IU/kg)			
1,000	139.6	69.5	2.01
2,000	139.8	72.1	1.93
3,000	143.0	72.8	1.97
4,000	139.8	71.7	1.95
SEM	1.0	0.5	0.01
p-value			
Ca			
Ln	0.029	0.001	> 0.05
Qd	0.008	0.001	> 0.05
Vitamin D			
Ln	> 0.05	0.044	> 0.05
Qd	> 0.05	0.020	> 0.05
Ca x vitamin D			
	> 0.05	> 0.05	> 0.05

[1]FI = feed intake; WG = weight gain; FC = feed conversion (FI/WG); SEM = pooled standard error of the mean; Ln = linear; and Qd = quadratic.

The response surface model is a powerful model compared to other experimental models of nutritional requirements. The quadratic model may overestimate the requirements in some cases. The data adjustments with response surface demonstrated a higher R^2 (WG = 0.99%) and lower RSD (WG = 2.75), in comparison with the quadratic regression (Figure 1).

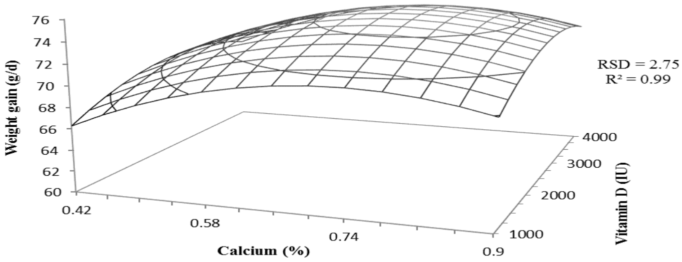

Figure 1 – Response surface of the effects of Ca and vitamin D on weight gain (WG). RSD = residual standard deviation.

FI decreased (p=0.008) when the diet contained Ca levels greater than 0.72% (Tab. 2). There was no effect of Ca and vitamin D on FC in quail from 1 to 14 d of age. There was no interaction between Ca and vitamin D levels on bone variables evaluated at 14 d of age, which indicates that the studied nutrients acted independently during this first production phase (Table 3).

Table 3 – Effect of Ca and vitamin D on bone and blood variables of quails during 1 to 14 d of age1.

Levels	BC (%)	BPH (%)	BA (%)	FBR (kgf)	TBR (kgf)	FSI (mg/mm)	TSI (mg/mm)	BD (mm Eq/Al)	BLC (%)
Ca (%)									
0.42	9.8	7.8	34.5	4.9	4.8	5.2	7.6	1.6	4.0
0.58	11.3	7.3	39.4	5.1	4.9	5.7	8.2	1.5	3.5
0.74	11.7	8.5	40.8	5.3	5.2	5.8	7.6	1.7	3.3
0.90	11.7	7.1	40.3	5.2	5.1	5.5	8.0	1.6	4.4
Vitamin D (IU/kg)									
1,000	11.2	6.9	37.7	5.0	4.9	5.4	7.5	1.4	3.7
2,000	11.3	7.4	40.2	5.3	5.1	5.6	7.9	1.6	4.1
3,000	10.9	7.2	38.2	5.0	4.9	5.5	8.3	1.7	3.9
4,000	11.2	9.1	38.9	5.1	5.1	5.6	7.8	1.8	3.5
SEM	0.16	0.35	0.50	0.05	0.04	0.07	0.15	0.04	0.15
p-value									
Ca									
Ln	< 0.001	> 0.05	< 0.001	0.001	< 0.001	> 0.05	> 0.05	> 0.05	> 0.05
Qd	0.002	0.009	< 0.001	0.043	0.058	0.009	> 0.05	> 0.05	0.004
Vitamin D									
Ln	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	0.015	> 0.05
Qd	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
Ca x Vitamin D									
	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

[1] BC = Bone Ca; BPH = Bone phosphorus; BA = Bone ash; FBR = Femoral bone resistance; TBR = Tibial bone resistance; FSI = Femoral Seedor index; TSI = Tibial Seedor index; BD = Bone densitometry; BLC = Blood Ca; SEM = pooled standard error of the mean; Ln = Linear (Ln); and Qd = Quadratic

Ca levels had a quadratic effect on bone calcium (BC; *p*=0.002), bone phosphorus (BPH; *p*=0.009), bone ash (BA; p<0.001), femoral bone resistance (FBR; *p*=0.04), tibial bone resistance (TBR; *p*=0.05) and femoral Seedor index (FSI; *p*=0.009). Meanwhile, vitamin D levels had an increasing linear effect on bone densitometry (BD; *p*=0.01).

The best values for the bone variables were BC (11.84%), BPH (7.48%), BA (40.98%), FBR (5.25 kgf), TBR (5.16 kgf), and FSI (5.78 mg/mm), which corresponded to 0.78, 0.71, 0.78, 0.77, 0.83, and 0.71% Ca, respectively.

Dietary Ca levels demonstrated a quadratic effect (*p*=0.004) on blood Ca (BLC) levels, with the greatest value associated with 0.64% of Ca in the diet (Table 3). However, there was no effect of vitamin D on blood Ca levels during this production phase.

DISCUSSION

Growth performance variables indicate that animals are dependent on Ca and vitamin D supplementation due to the accelerated development of muscle and skeletal tissue during the early phase of life. According to Edwards (2000), Ca deposition in the skeleton is greater during the initial growth phase. Thus, Ca content in the body increases quickly during the initial phase, so that at the end of the first month of age, the chicks have 80% of the total Ca of an adult bird; this demonstrates the importance of these nutrients to the structural development of quails in the first days of life, since skeletal support is essential for protein deposition.

Regarding feed conversion, there was no significant effect due to supplementation, which is probably due to the offsetting effect of this index; a reduction or increase in feed intake was accompanied by a decrease or increase in the weight gain of the quails.

According to Silva & Costa (2009), the Ca supplementation requirement for Japanese quail from 1 to 21 d of age is 0.60%, whereas for European quail, the requirement is 0.85%. However, Rostagno *et al.* (2011), recommended the supplementation of 0.90% Ca for Japanese quail during the initial and growing phases. Moreover, the National Research Council (NRC, 1994) reported a requirement of 0.80% Ca in diets containing 2,900 kcal of ME/kg.

Silva *et al.* (2009), evaluated meat-type quail from 1 to 14 d of age, but did not observe the effects of interactions between Ca and phosphorus levels on growth performance variables. They concluded that 0.65% Ca, the lowest level provided, was enough to meet the nutritional requirements of these quails.

However El-Katcha *et al.* (2014) recommend a diet with 21% crude protein and 1.0% calcium (or 25% more calcium than NRC recommendation) in growing Japanese ration to improve growth performance and feed efficiency parameters.

The literature shows several works that relate the interaction of calcium with other nutrients in determining the requirement, such as phosphorus, crude protein, vitamin D, among others. It is important to completely understand dietary levels because the balance between the various nutrients is crucial for maximum performance.

The best Ca: P ratio for WG was 1.78:1, in which the diet contained 0.73% Ca and 0.41% available phosphorus. The phosphorus concentration was maintained in the different treatments, such that the Ca: P ratio varied from 1:1 to 2.20:1. In the literature, different requirements can also be affected by the Ca: P ratio of the diet, because they are controlled by the actions of the same hormones: calcitonin and PTH; together with vitamin D, they act on metabolism. Thus, diets with lower phosphorus contents also require a lower Ca content to maintain the ideal ratio.

There are similarities as well as discrepancies between the nutritional requirements of meat-type (European quail) and egg-laying quail (Japanese quail) during the growing and oviposition phases. Silva *et al.* (2011), suggests that despite the fact that crude protein and energy requirements are equal, Ca, available phosphorus levels and the amino acids profile are greater in heavy-strain quail. This may be justified due to the greater weight gain and muscle growth rates of these quails, especially during the first four weeks of age.

Vitamin D3 requirements in growing broilers are 200 IU (NRC, 1994), 2,375 IU (from 1 to 7 d of age) and 2,090 IU (from 8 to 21 d of age) (Rostagno *et al.*, 2011). Only high vitamin D3 levels decrease the incidence of bone abnormalities in chicks (Edwards *et al.*, 1992; Silva *et al.*, 2001; Whitehead *et al.*, 2004).

In terms of the BD variable, there was an increasing linear relationship with vitamin D levels. Bone mineral density stands out as an important factor in assessing bone quality and has been used in poultry production (Almeida Paz & Bruno, 2006). An inadequate amount of Ca and vitamin D supplementation during the initial phase may cause an imbalance in mineral homeostasis, inappropriate bone development and abnormal calcification.

Bonagurio *et al.* (2020), evaluated that die-tary supplementation with canthaxanthin and 25-hydroxy-cholecalciferol has beneficial effects on bone (femoral and tibiotarsal dry weight and calcium content) in European quail breeders, but production performance was not influenced by diet.

Sheikhlar & Navid (2009), evaluated four different levels of 1,25-Dihydroxycolecalciferol supplementation (from 2.5 to 8.5 ug/kg 1,25 $(OH)_2D3$) in diets containing 0.71% Ca and 0.37% total phosphorus in quails between 0 to 3 weeks of age; they did not observe an effect of vitamin D supplementation on WG or FC. However, they verified that the Ca content in the bones and the plasma significantly increased with an increase in vitamin D levels.

Bone tissue rigidity results from the deposition of Ca and phosphorus as hydroxyapatite in the bone mineralization process. Both minerals make up about 70% of the bone and 30% consist of organic matter (Kälebo & Strid, 1988).

Zhang & Coon (1997) found in white Leghorn chickens, that bone strength, is the result of bone volume (taken into account in the Seedor index) and not the percentage of bone ash.

In a study conducted by Silva *et al.* (2009) with meat-type quail at 14 d of age, the estimated Ca level needed for the maximum resistance was 0.89% in the diet. And the optical density was not influenced by the Ca levels during this phase.

The results of this work indicate that, in order to attain optimum growth performance, quail will have a lower requirement than needed for the bone variables. This coincides with the results of Schoulten *et al.* (2003), who estimated values of 0.46% Ca for WG and 0.59% Ca content in the tibia.

Ca and vitamin D requirements affect the accelerated muscle and bone development in meat-type quail between 1 to 14 d of age. By studying the growth curves of meat-type quail, Grieser *et al.* (2015), estimated that the ash deposition rate was 0.053 g/quail/d at birth and 0.245 g/quail/d at 14 d of age; this demonstrates the importance of estimating ideal supplementation levels based on the physiologic requirements for quail to maintain metabolism, increase bone development and attain maximum growth performance.

CONCLUSIONS

Requirements of Ca and vitamin D for the maximum weight gain of meat-type quails in the growing period from one to 14 days of age were 0.73% and 2,883 IU, respectively.

ACKNOWLEDGMENT

This study was financed in part coordination for the improvement of higher education personal - Brazil (CAPES) - Finance Code 001".

AUTHORS' CONTRIBUTIONS

TPP, SMM and ACF designed the experiment. TPP, APST, EB, VTZ, CES and DOG conducted the experiment. TPP, SMM and ACF analysed the data and interpreted the results.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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