Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2019.071

RESEARCH ARTICLE

Pre-Hatch Growth and Development of Selected Internal Organs of Japanese Quail (*Coturnix japonica*)

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ARTICLE HISTORY (18-329)

Received:	September 02, 2018
Revised:	March 23, 2019
Accepted:	April 07, 2019
Published online:	May 16, 2019

Key words:
Embryo
Incubation
Janoscheck growth curve
Japanese quail

A B S T R A C T

The objective of this study was to investigate the pre-hatch growth and development of the selected internal organs (brain, eye, heart, kidneys, tongue, oesophagus, proventriculus, gizzard, intestines, liver, trachea and lungs) of quail. Sixty eggs divided into ten groups (n=6) were weighed, labelled and incubated. One group of eggs was removed from the incubator on a daily basis for collection of samples. The weight of embryos and absolute weight of the organs along with the length of each selected organ of all embryos were recorded from day eight till hatch (day 17). The Janoscheck growth curve function was fit to evaluate group means and characteristic parameters of the growth curve. The growth curves based on measured and predicted means for each organ revealed sigmoid and exponential growth curves for most of the organs. During the course of incubation, the growth patterns grouped organs into two groups depending on the rate of growth. Tongue, esophagus, trachea, heart and eye showed early rapid growth while lungs, kidneys, intestine and other digestive organs exhibited late rapid growth. The results also revealed that the slop of the growth curve of each organ kept on changing, positively and negatively, with the embryo weight and duration of incubation steadily.

To Cite This Article: Sarfraz A, Qureshi AS, Kausar R, Usman M, Hussain M and Umar Z, 2019. Pre-hatch growth and development of selected internal organs of Japanese quail (*Coturnix japonica*). Pak Vet J, 39(3): 335-340. http://dx.doi.org/10.29261/pakvetj/2019.071

INTRODUCTION

There are 33 species of quails out of which seven are officially recognised (Tulobaev *et al*., 2012). Among them Japanese quail (JQ) gained attention because of its better growth rate, egg and meat production (Üçkardeş *et al*., 2015; Tahir *et al*., 2017), shorter incubation period (Ainsworth *et al.*, 2010; Ben-Ezra, 2017; Butt *et al*., 2018), earlier age of maturity, hence shorter generation gap (Tarhyela *et al*., 2012) and easier maintenance. JQ is a small sized dual purpose bird that belongs to Aves class of Galiformes order from Phasianidae family. These migratory terrestrial birds live for 2-3 years in the wild and 1.5-2.5 years in laboratory conditions. The average body weight of the hatchling is 5-6 grams that increases rapidly to 160-250 grams in 5-6 weeks and even more (~ 600 grams) in the birds that are bred for meat purpose. The JQ is reared on commercial scale because of its high dressing percentage of 72.36% (Daikwo *et al*., 2013; Khan *et al.*, 2016) and a low feed conversion ratio is 2.52 (Khan *et al.*, 2016). Therefore, JQ is frequently used in research areas like histology, embryology, physiology, pathology, toxicology, nutrition and genetics (Banerjee *et al*., 2016; Kausar *et al*., 2016; Coskun *et al*., 2017).

An avian embryo model has successfully been used for centuries to understand developmental biology. They represent similar developmental changes as mammalian embryo does, except the initial stages. Avian embryo can develop *in vitro,* and can be manipulated using macro, micro, and molecular techniques (Le-Douarin, 2018).

Growth curve analysis gives perception of the growth pattern and unbiased assessment of the growth progression as well as removes random probability distribution errors. Characteristics and derivatives of a growth curve yield measurable values that may be utilized towards the comparing the growth process (Gille and Salomon, 1998). A number of growth curve models and their alternative are used to describe the growth pattern (Gürcan *et al*., 2017). Since long, the Gompertz model was practised in time-growth related data studies

(Üçkardeş and Narinç, 2014) but now the Janoscheck growth curve model is commonly used because it is considered the best fit to most types of growth related data of diverse origin and its flexibility, therefore, is preferred over Gompertz, logistic and Bertalanffy growth curves. These properties are also ascertained by the empiric function and objective interpretation of experimental results that enables the acquiescence of the experimental data (Narinç *et al*., 2014a). Although, the growth data on post-hatch and sexual maturity of JQ is available (Narinç *et al*., 2014b). There is a dearth of literature on allometric growth information during pre-hatch growth period, hence, the present study was designed to study the allometric growth of selected internal organs of JQ during the pre-hatch spell using Janoscheck growth curve model.

MATERIALS AND METHODS

A total of 60 freshly laid eggs of JQ, having uniform weight and size, were obtained from the Avian Research and Training Station (ARTS), University of Veterinary Animal Sciences. The managemental practices, genotype and age of the birds in the randomly breeding colony of JQ were uniform at ARTS. Water and commercially available feed were provided *ad libitum*.

The eggs were cleaned, labelled, weighed to nearest milligram and then divided into ten equal groups (n=6). Then they were incubated at 99-100°F and 65-70% relative humidity in the incubator tray at random by their sharp end pointing downwards. The incubator was set on automatic turning at 45° after every half an hour for the whole course of incubation.

Starting from the eighth day of incubation (the first day of sampling), one group of eggs (n=6) was removed from the incubator on daily basis. They were opened following the methods described by Peebles *et al*. (1998). Briefly, the eggs were opened into a petri dish containing normal saline solution. Only those embryos were selected which appeared physically normal (Fig. 1). The embryos were cleared from the yolk at the point of yolk stalk attachment, washed with fresh saline and weighed individually. The embryos were opened; internal organs were removed and weighed. The hatchlings (on day 17) were slaughtered, cleared of yolk and the membranes. They were weighed in similar fashion as that of pre-hatch embryos. The Gender discrimination of the embryos and hatchlings was overlooked as it is not easily possible to differentiate between sexes during the embryonic period.

The Organs including brain, eye, heart, kidneys, tongue, oesophagus, proventiculus, gizzard, small and large intestines, liver, trachea and lungs were studied for morphometric measurements.

The length of the tongue was measured from base to apex. The length of the oesophagus was measured till the point of entry into the gizzard. The trachea was measured for its length from the point just below the larynx to the point of its bifurcation into bronchi. The heart was removed by removing its major blood vessels from the base and was rinsed properly. The liver, after removing from the body, was left for some time for the removal of blood. The kidneys were removed from their sockets and lungs were taken out of the rib cage. The eye ball diameters were measured at two locations, perpendicular to each other. The organs were weighted carefully to the nearest milligram using an electronic weighing balance (CHYO-MK-300, Japan having readability up to 0.001g). The length of the organs was measured to the nearest millimetre using Vernier caliper (Tailan, Japan, having least count 0.001mm). Stereo microscope (Beck Kassel CBS-45357, Germany) was used to dissect the small organs like tongue, eye ball and brain under it.

Means and standard error of means were calculated for each age group of embryos. The Janoscheck growth curve function (Janoschek, 1975) following Gille and Salomon (1999) was fitted to age group means by the nonlinear regression procedure of Paul (1975).

$$W = A - (A - W_o) \cdot Exp(-k \cdot t^{p})$$

Here, "W" is the growth size (in grams/ millimetres) at certain time point "t" (in days) and "A" the asymptotic value (in grams/ millimetres), which is called theoretically calculated end value and "W_o" is the weight/ length at t=0. The parameter "k" and "P" are without direct biological organization. The parameter "P" determines the form of the growth curve. The growth curve parameters u_o and u_i were also calculated. u_o shows degree of maturity at eighth day of incubation and u_i shows degree of maturity at the Point of Inflection (POI) of an embryo or organ. They are percentage values of W_o and W_i, respectively, to "A". These parameters are calculated as follows:

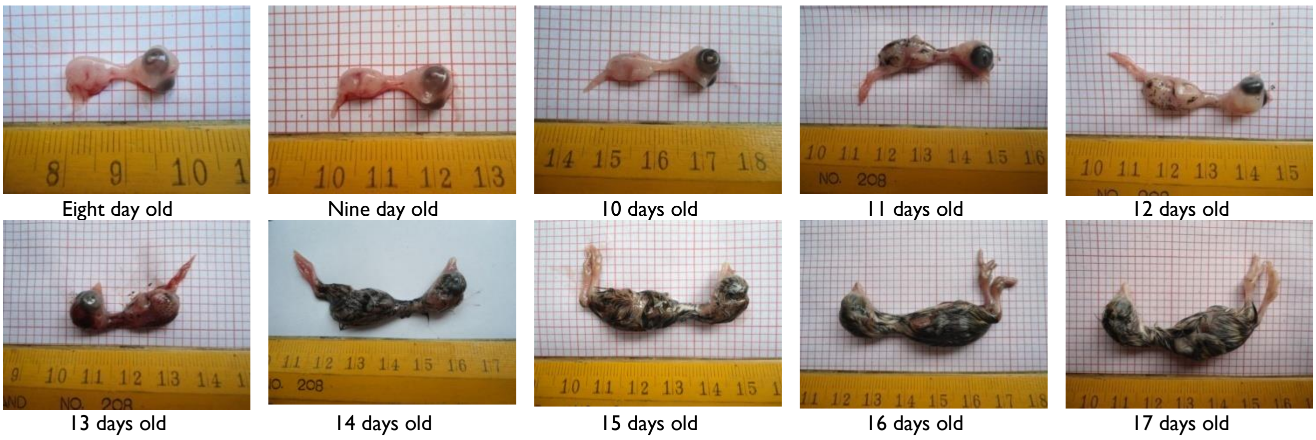

Fig. 1: Micrographs of the embryos of Japanese quail (*Coturnix japonica*) at different days during incubation period.

$u_o = (W_o / A) \times 100$
$u_i = (W_i / A) \times 100$

The data were analysed by the allometric regression model as described by Snecdecor and Cochran (1991) while the level of significance was kept ≤0.05. The Goodness-of-Fit (R^2) was also calculated to determine accuracy of the model applied.

RESULTS

The present study was focused on the measurement of the weights and lengths of selected internal organs of JQ during incubation. The Janoscheck growth curve model was applied to the measured mean values of the organs including brain, eye, heart, kidneys, tongue, oesophagus, proventiculus, gizzard, small and large intestines, liver, trachea and lungs. The resulted growth curves and its characteristics parameters are shown in Fig. 2, 3, 4 & 5 and Tables 1 & 2. The resulted curves revealed a variety of growth patterns of different organs. On detailed examination of the data showed under mentioned types of the growth curves:

a. Most of the organs including; eye, heart, brain, trachea, esophagus and tongue length exhibited a constant growth with the increase in the embryo weight and plotted a sigmoid course of growth curve.
b. On the other hand, exponential growth curve was presented by intestines, proventriculus, gizzard, liver, lungs and tongue width.
c. The isometric growth curve with the body weight of the embryo was depicted by rest of the organs.

Some of the internal organs were very mature during the early periods of incubation and showed a very early POI, between 8-11 days. These organs included eyes, kidneys, gizzard and proventriculus. The organs with late POI (15-17 days) included lungs, intestines, tongue width and liver. Other organs included brain, esophagus, trachea and tongue length with POI (12-14 days) that fall in between these two extremes.

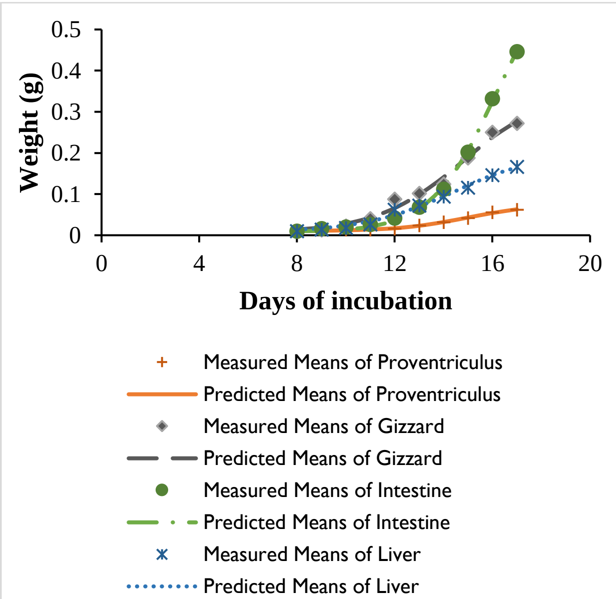

Fig. 2: Measured and predicted mean values and resulting growth curves of various digestive organ weights of Japanese quail (*Coturnix japonica*).

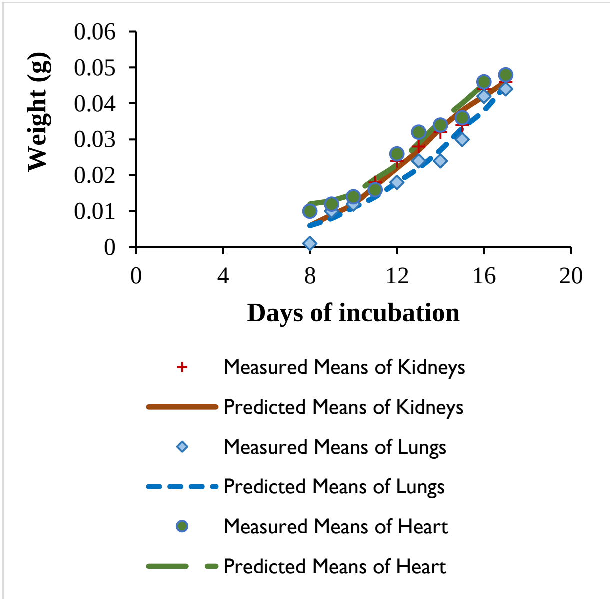

Fig. 3: Measured and predicted mean values and their resulting growth curves of weights of heart, kidney and lungs of Japanese quail (*Coturnix japonica*)

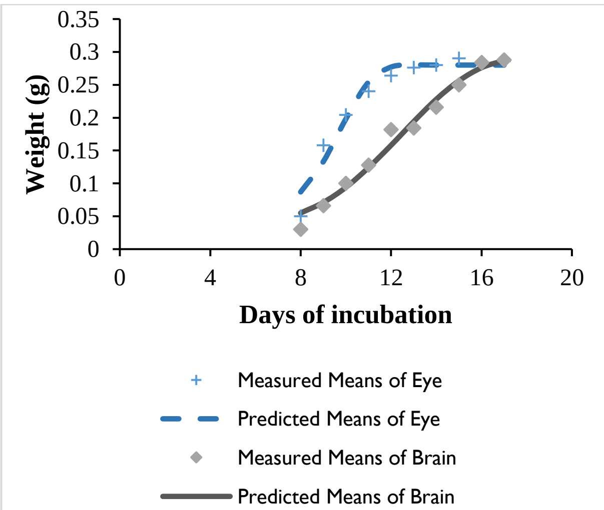

Fig. 4: Measured and predicted mean values and their resulting growth curves of weights of eye and brain of Japanese quail (*Coturnix japonica*).

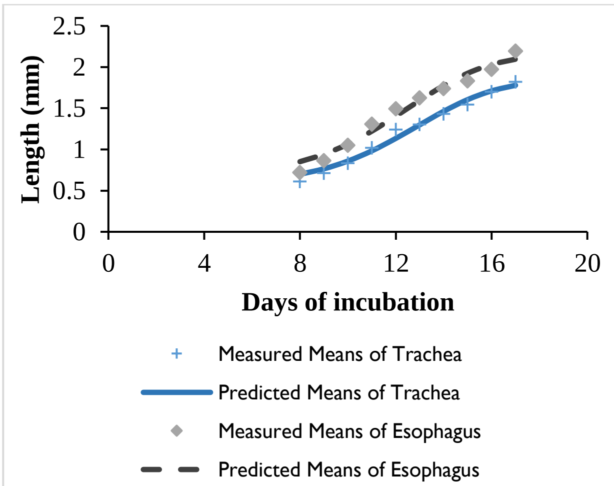

Fig. 5: Measured and predicted mean values and their resulting growth curves of lengths of trachea and esophagus of Japanese quail (*Coturnix japonica*).

Table 1: Characteristics parameters of growth curve of organs length/width

Organs	A (mm)	W_o (mm)	$W_{i(mm)}$	$u_{o(\%)}$	$u_{i(\%)}$	$t_{i(days)}$	R^2
Tongue Length	0.526	0.150	0.359	28.517	68.199	13.152	0.994
Tongue Width	0.526	0.030	0.217	8.130	58.761	15.958	0.996
Esophagus Length	2.145	0.720	1.497	33.566	69.770	12.484	0.970
Trachea Length	1.833	0.610	1.282	33.279	69.929	12.898	0.980

A = Asymptomatic value in millimeters; W_o = Length at 8th day of incubation; W_i = Length at the point of inflection; t_i = Age in days at the point of inflection; u_o = Degree of maturity at day eight; u_i = Degree of maturity at the point of inflection.

Table 2: Characteristics parameters of growth curve of organs weights

Organs	A	$W_{o\,(g)}$	$W_{i(g)}$	$u_{o(\%)}$	$u_{i(\%)}$	$t_{i\,(days)}$	R^2
Embryo	6.271	0.350	3.603	5.581	57.461	13.649	0.989
Brain	0.297	0.030	0.176	10.101	59.118	12.483	0.978
Eye	0.280	0.050	0.184	17.857	65.713	9.795	0.952
Heart	0.050	0.010	0.032	20.000	64.923	13.610	0.971
Kidney	0.051	0.001	0.027	1.961	53.872	10.413	0.974
Proventriculus	0.680	0.010	0.401	1.471	59.043	11.370	0.995
Gizzard	0.321	0.010	0.188	3.115	58.477	10.521	0.987
Intestine	0.578	0.010	0.347	1.730	60.028	16.238	0.999
Liver	0.183	0.010	0.108	5.464	59.143	14.529	0.989
Lungs	0.132	0.000	0.065	0.076	49.319	20.023	0.961

A = Asymptomatic value in grams; W_o = Weight at 8th day of incubation; W_i = Weight at the point of inflection; t_i = Age in days at the point of inflection; u_o = Degree of maturity at day eight; u_i = Degree of maturity at the point of inflection.

DISCUSSION

During the development, a variety of growth curve data was recorded. In order to explain this, the Janoschek growth curve model was used. It is an optimum model to elucidate such type of data (Narinç *et al*., 2014a). Similar to the Richards growth curve model, the Janoschek model enables its application to exponential and sigmoid developmental patterns. Both of these models provide inflection ordinate ratio and flexible asymptote which are not rendered by Bertalanffy and Gompertz logistic models. There is also easy availability of initial parameters with least procedural complications; therefore, we choose to use the Janoschek model.

Up to day 10, eyes were the most prominent and heaviest organs of the embryo and showed the earliest Point of Inflection (POI) (9.795) with higher degree of maturity (65.713) than most of other organs. After day 10, they gained weight at a slower rate compared to earlier days. The advanced maturity of eye may be linked to its development start earlier during incubation. Moreover, retina starts to form neuronal linkage with the brain. These findings are in agreement to Gille *et al*. (2000) in geese and Qureshi *et al*. (2016) in domestic duck.

After eye, the brain was the second organ that showed a rapid growth. The curve indicated almost a sigmoid trend. The Brain size increased rapidly as compared with body size in the initial stages of development, however, slowed down later on. It slowed down gaining weight after day 13. Striedter and Charvet (2008) also found similar observations in two species, parakeet (*Melopsittacus undulates*) and quail (*Colinus virgianus*). Finlay and Darlington (1995) have reported the similar findings in various species of the birds that the brain development is related to the duration of development. The rapid growth of brain in JQ is directly related to the higher degree of cerebralization which is a typical characteristic of Anseriform birds. These birds are precocial, i.e. they are largely independent of maternal care soon after hatching. This is due to higher degree of neurogenesis during the incubation period. Such development and growth is not observed in altricial birds (Gille *et al*., 2000; Iwaniuk and Hurd, 2005).

After eye, the most prominent tissue in the embryo at initial stages of incubation was the heart. There was the formation of blood vessels that covered the whole embryo around day 4. The Blood to organs was supplied by the pumping action of heart. Heart presented quite high rate of development as early as day 8. It kept on gaining its mass with an increase in the mass of the embryo and time of incubation linearly till day 14 of incubation. Similar findings have been previously published (Arora, 2011). The Chick embryos also present high initial development rate (Yang, 1998). The higher initial weight gain may be due to its role as a supply organ and also due to its function of storing glycogen which may contribute towards its weight. There was a linear trend of heart weight with the weight of the embryo as well as duration of incubation. However, heart weight did not increase just before the hatch as recorded by Yang (1998) in White Leghorn.

Following the eye ball, brain and heart, the digestive organs were among the fastest growing organs in the embryo especially gizzard, liver and proventriculus exhibited accelerated initial growth rate. The growth curves of all these three organs were sigmoid. Proventriculus, though it was a rapidly growing organ, its proportion to the body mass decreased after day 16. A similar trend was expressed by gizzard and liver. Their POIs were as late as 11 and 15 days.

The initial higher growth rate of liver may be attributed to its role as a supply organ (Christensen *et al*., 2002) that contributed towards its weight gain. Like heart, liver also stores glycogen which helped in increase in weight. Liver gained weight in a very sustainable way and inflected at a very late stage (14 days). The late POI may be due to high energy production, consequently, higher metabolic activity. However, weight gain was not proportionate to the embryo weight, similar to White Leghorn lines of chickens (Yang, 1998).

The intestines presented quite high rate of development near the hatch. There was an enormous weight gain after day 13 and this pace was kept until hatching. However, the intestine was not fully mature at the time of hatching. Our findings are in agreement with Sell *et al*. (1991) in poultry embryos where they recorded a high growth rate towards the end of the incubation and after hatching. Immaturity of the intestines at the time of hatch is also previously reported in ostrich (Iji, 2008) and in turkeys (Sell *et al*., 1991). The intestines were approximately 3% of the total embryo weight that raised to more than 9% at the point of inflection POI. Similar reports have been published mentioning an increase in the percentage of intestine from the beginning towards the end of the incubation period in chicken (Uni *et al*., 2003; Tong *et al*., 2013). This may be associated with preparation of the organs for post-hatch digestion of the ingesta and nutrient absorption. This enlargement is necessary in order to utilize the nutrients ingested. The Mucosa is developed and the wall of the intestine is equipped with the mucous and enzyme producing cells as well as the absorptive capacity is increased. Birds also consume their amniotic fluid orally before the hatch that triggers the activation of the intestinal mucosa (Moran, 2007). Many other studies indicated that intestines get fully mature after hatch (Sell *et al*., 1991).

Among the respiratory organs, the lungs were a paired structure. No rapid development was observed in them in early incubation period. Based on the statistical data, their maturity was recorded towards the end of the incubation period. They developed faster than the body mass of the embryo as were recorded in Australian pelican (Runciman *et al*., 2005) during later stages of incubation. The growth curve of the lungs touched to its maximum value towards day 16, just one day before the hatch. Similar delayed maturity of the lungs in domestic ducks was also recorded by Qureshi *et al*. (2016). On the other hand, the trachea settled on an accelerated rate from the very beginning of the incubation period and a sigmoid growth curve pattern was displayed by the trachea it. Kidneys were also paired. Although they did not attain an early point of inflection POI, but they were matured organs at the time of hatch. Their growth rate declined after the 10^{th} day of incubation.

At the eighth day of incubation, oesophagus (33.566), trachea (33.279) and heart (20.000) were quite mature and the lungs (0.076), proventriculus (1.471), intestine (1.730), kidneys (1.961) and gizzard (3.115) were the least matured (<5%) as calculated by u_o and u_i as previously mentioned in materials and methods section. The heart, eye, brain and other digestive organs, stood between the two extremes. At POI, there was a slight change in the degree of maturity. Trachea (69.929) superseded oesophagus (69.770) which was followed by tongue (68.199), eye (65.713), heart (64.923) and intestine (60.028). The least matured organ was lungs (49.319) and kidneys (53.872).

Conclusions: This study showed that every organ participates in the embryo weight at a different percentage from day 8^{th} of incubation till hatch. The percentage of the organ weights to the embryo weight kept on altering with the days of incubation, as they have dissimilar growth rates. This depends on the energy requirements and the growth plan of the organs. With the understanding of growth dynamics, this work presents as reference material for scientists dealing with embryo growth studies. This method of growth studies enables researchers to compare the avian growth statistics which may play a significant role in the manipulation of avian embryos for the betterment of poultry sector.

Authors contribution: ASQ and RK made the concept and design of the project. AS and MH did the experimental trials. AS also collected the data and prepared the rough draft of the manuscript. MU and ZU did the analysis of the data as well as interpretation of the results. ASQ also critically supervised the entire process and reviewed the manuscript.

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