

## Electrocardiographic Indices, Circulating Electrolytes and Cardiac Enzymes of Apparently Healthy Aged Layer Hens

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### Abstract

**BACKGROUND:** Information regarding the effects of physiological situation (age and egg laying) on heart electrical activities and blood profile of layer hens can aid in better understanding the cardiovascular system healthiness and performance in these birds.

**OBJECTIVES:** The present study was carried out to evaluate the electrocardiographic parameters and circulating levels of electrolytes and cardiac enzymes in apparently healthy 112 week-old Lohmann LSL-classic layer hens.

**METHODS:** Fifty apparently healthy 112 week-old Lohmann LSL-classic layer hens were selected. The electrocardiograms were recorded and blood samplings were performed for all birds. Serum concentrations of sodium, chloride, phosphorus, calcium, potassium, magnesium, aspartate aminotransferase, alanine transaminase and lactate dehydrogenase were assayed.

**RESULTS:** The mean±SD of heart rate was 333.5±28.6 beats/min. The P wave was seen in only 20% of birds and its duration and amplitude were 0.03±0.01 sec and 0.05±0.05 mV, respectively. PR, RR and QT intervals were 0.04±0.02, 0.18±0.02 and 0.13±0.01 sec., respectively. The circulating levels of calcium, phosphorus, and magnesium were 19.94±3.63, 5.02±1.15, 2.46±0.23 mg/dL and blood levels of sodium, chloride, and potassium were 146±2.5, 6.10±0.84 and 119.20±2.38 mEq/dL, respectively. Serum values of aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase were 240.20±51.26, 6.20±2.28 and 1262.40±344.18 U/L, respectively.

**CONCLUSIONS:** Based on the clinical healthiness of the studied birds, all of the electrocardiographic indices, circulating electrolytes and cardiac enzymes in aged layer hens were close to values in younger hens. So, increase of the laying period over 100 weeks had no harmful and adverse effect on the bird's physiology and welfare.

**KEYWORDS:** Cardiac evaluation, electrocardiography, circulating electrolytes, cardiac enzymes, aged layer hens

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## **Introduction**

Electrocardiography as a non-invasive and cost-effective procedure can be used to record heart electrical activities over a period of time. The electrocardiogram (ECG) is a record of sequential electrical depolarization-repolarization patterns of the heart that is used to monitor heart rate and detect arrhythmias, cardiac chamber enlargement and electrical conductance abnormalities in birds (Lumeij, 2001; Kostelanetz et al., 2009). The ECG can also be used to evaluate severe trauma, toxicosis, metabolic and electrolyte abnormalities (Çinar et al., 2006; Cushing et al., 2013). ECGs of poultry are recorded and evaluated by using bipolar (I, II, III) and augmented unipolar leads (aVF, aVR, aVL) (Strunk and Wilson, 2003); However, in birds, lead II is considered as the standard lead for analysis of ECG parameters (Lumeij, 2001). Avian ECG has a little difference from humans and some other mammals; because, in birds, the depolarization wave of ventricle moves from epicardium to endocardium and most of the birds show the inverted QRS wave in lead II which is indicative of negative mean electrical axis (Smith et al., 2000). The heart rate, the values of various waves and intervals in ECG are wide, depending on the bird's breed, type, size, age and sex (Mutibvu et al., 2017). Reference ECG values of various birds are available (Hassanpour et al., 2013, 2014 and 2016; Yogeshpriya et al., 2018) and environmental, physiological, nutritional and infectious agents can change the normal cardiovascular functioning and electrocardiographic patterns (Smith et al., 2000; Kostelanetz et al., 2009).

Blood profiling is a helpful diagnostic tool to evaluate the health status, nutritional and metabolic diseases of various birds (Al-

Obaidi 2015; Gattani et al., 2016). In avian medicine, determining the circulating profile is one of the common methods to evaluate the effect of different status (such as infectious and non-infectious conditions) on the health and production of farm birds and it provides valuable information about the relationship among the nutrition, age, growth and performance of birds (Aina et al., 2014; Dolka et al., 2014). In layer hens, the blood biochemistry and hematology are influenced by physiological conditions such as age and reproduction level (Suchy et al., 2004; Pavlik et al., 2007). The blood biochemical and hematological parameters of layer hens during egg production have been measured in various studies, but, information about ECG and circulating electrolyte and cardiac biomarkers in long life layers is very little. Cardiovascular system has an important role in health and production of layer hens and evaluating this system by practical, inexpensive and non-invasive methods may help veterinarians to monitor and manage layer herds. Hence, the present study was carried out to evaluate the electrocardiographic parameters and circulating levels of electrolytes and cardiac enzymes in clinically healthy 112 week-old Lohmann LSL-classic layer hens. The results of the present study may provide data to better understand the physiologic conditions of cardiac system of aged layer hens.

## **Materials and methods**

### **Birds and samples**

The current research was conducted in summer 2018 on 50 clinically healthy 112 week-old Lohmann LSL-classic layer hens (white-egg layers), weighing between 1300 and 1500 g, and reared in School of Veterinary Medicine, Shiraz University, Shiraz,

Iran. The birds were selected from a big flock and temporarily kept in 5 stainless steel cages (75×75×200 cm) (10 birds in each cage) with a 16-hlight:8-h dark photoperiod, 24-26% relative humidity and 22-25 °C tem-

perature. The birds were fed a balanced diet containing corn, soybean meal, barley, salt, dicalcium phosphate and a vitamin-mineral premix (Table1). Birds received water and food ad libitum.

**Table 1.** Ingredients and composition of feed mixture (g.kg-1) received by studied hens

Component	value
<b>Ingredients, %</b>	
Yellow Corn	53.0
Soybean meal (44%)	26.0
Dicalcium phosphate	1.3
CaCO <sub>3</sub> (38%)	9.8
Sunflower oil	2.5
Sodium chloride	0.3
Methionine	0.27
Lysine	0.2
Premix*	0.6
Total	100
<b>Calculated analyses</b>	
ME, kcal/kg	2820
Crude protein, %	16
Calcium, %	4.1
Available Phosphorus, %	0.5
Sodium, %	0.19
Lysine, %	1.29
Methionine, %	0.48
Threonine, %	0.66
Tryptophan, %	0.23

\* Vitamin and mineral content per kilogram of premix: vitamin A: 3,600,000 IU; vitamin D3: 800,000 IU; vitamin E: 7,200 IU; vitamin K3: 0.8 g; vitamin B1: 0.71 g; vitamin B2: 2.64 g; vitamin B3: 3.92 g, vitamin B5: 11.88 g; vitamin B6: 1.176 g; vitamin B12: 6 mg; folic acid: 0.4 g; biotin: 40 mg; choline chloride: 100 g; selenium: 80 mg; cobalt: 100 mg; iodine: 396 mg; copper:4 g; zinc: 33.88 g; iron: 20 g; manganese: 39.68 g.

### Electrocardiographic studies

The birds were evaluated clinically and ECG was recorded from clinically healthy ones. They were kept in a standing position without any sedation. When birds became calm, the alligator-type electrodes were attached to the skin after using electrocardio-

graphic jelly and then ECGs were recorded. Alligator clip electrodes were positioned at the base of the left and right wings and gastrocnemius muscle of the left and right limbs (Smith et al., 2000). All ECGs were recorded by a single channel electrocardiographic machine (Kenz-line EKG 110, Su-

zuken Co., Ltd., Japan) with paper speed of 50 mm/sec and calibration of 10 mm equal to 1 mV. The ECGs were recorded on I, II, III, and aVR, aVL and aVF leads for 30-40 s, but all measurements were analyzed in lead II as the standard lead. The values of P, R and T waves, duration of QRS complex, QT, R-R, P-R, and ST-intervals were determined (Reddy et al., 2016).

#### Blood samplings and biochemical assays

Blood samplings were performed after ECG recordings. All specimens were obtained from the wing vein and sera were separated after one hour by centrifugation at  $3000\times g$  for 10 min and stored in sterilized disposable plastic tubes at  $-20\text{ }^{\circ}\text{C}$  until assayed. The serum concentrations of sodium and potassium were measured by the flame photometry (Flame Photometer, FLM, Ontario, Canada). The serum levels of phosphorus and chloride were determined by using routine biochemical procedures (Burtis and Ashwood, 1994). The calcium and magnesium concentrations in serum were analyzed

by atomic absorption spectroscopy (Shimadzu AA-670, Japan). The activity levels of alanine transaminase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were determined by using Integra 800 auto-analyzer (Roche-Cobes, Switzerland) by commercial test kits.

#### Statistical analysis

Statistical analyses were performed by using descriptive analysis and data were expressed as mean  $\pm$  standard deviation (SD).

#### Results

Figure 1 shows ECGs recorded by different standard limb leads (6 leads) from an aged clinically healthy leghorn laying hen. In lead I, traces had very low amplitude. In lead II, the P and S waves were found in 20% and 54% of birds, respectively. The R wave in leads I, II, III, and aVF was observed positive and in lead aVR and aVL was negative (Fig. 1). The values of P-QRS-T waves are offered in Table 2 and the results of the serum biochemical analysis are presented in Table 3.



**Figure 1.** Electrocardiograms tracing from a 112-weeks old Lohmann LSL-classic Layer hen by 6 leads (with paper speed of 50 mm/sec and calibration of 10 mm equal to 1 mV).

**Table 2.** Electrocardiographic parameters (Mean±SD) recorded by lead II from 50 clinically healthy 112 weeks old Lohmann LSL-classic Layer hens

ECG parameters	Value	Range
P-duration (sec)	0.03±0.01	0.02–0.04
P-amplitude (mV)	0.05±0.0	0.05-0.05
R-amplitude(mV)	0.15±0.33	0.10-0.20
R-duration(sec)	0.04±0.10	0.02-0.06
T-duration(sec)	0.08±0.01	0.06–0.12
T-amplitude(mV)	0.10±0.02	0.05–0.15
QT-interval(sec)	0.13±0.01	0.12–0.16
ST-interval(sec)	0.02±0.01	0.02-0.04
QRS-complex(sec)	0.05±0.03	0.04–0.06
PR-interval(sec)	0.04±0.00	0.04-0.04
RR-interval(sec)	0.18±0.02	0.14- 0.26
HR (beat/min)	333±28.6	230- 428

**Table 3.** Circulating electrolytes and cardiac enzymes (Mean±SD) of clinically healthy 112 weeks old Lohmann LSL-classic Layer hens (n=50)

Biochemical parameters	Value	Range
Calcium (mg/dL)	19.94±3.63	14.2-23
Phosphorus (mg/dL)	5.02±1.15	3.8-6.3
Magnesium(mg/dL)	2.46±0.23	2.2-2.7
Sodium(mEq/dL)	146.01±2.5	143-150
Potassium(mEq/dL)	6.10±0.84	5.2-7
Chloride(mEq/dL)	119.20±2.38	116-122
AST(U/L)	240.20±51.26	182-305
ALT(U/L)	6.20±2.28	4-9
LDH(U/L)	1262.40±344.18	858-1660

## Discussion

According to Lohmann Tierzucht Company's recommendation (2014), the best age for laying in Lohmann LSL- layer hens is 85 weeks, but increasing persistency in lay to get more eggs is economic and valuable. Thus, information regarding performance and health of different organs and systems in these aged birds may aid in better managing the flocks. Cardiovascular system plays an important role in better laying performance in aged birds and the current research was conducted to evaluate and present the cardiovascular system activities in clinically healthy aged layer hens. Different methods have been suggested to assess the cardiovascular system in birds; however, electrocardiography and circulating cardiac biomarkers are the common and reliable techniques for this purpose. Hence, in this research the authors evaluated the electrocardiographic indices, circulating electrolytes and cardiac enzymes of clinically healthy aged layer hens to investigate the cardiovascular functions of clinically healthy 112 week-old Lohmann LSL-classic layer hens. The results of the current research may be used as a guideline to monitor and manage aged layer herds. This study attempted to address the issue concerning the lack of data on electrocardiographic and some blood biochemical parameters in old layer hens at over the suggested age by the company and incorporated a report of ECG, circulating electrolytes and some cardiac enzymes values linked to cardiac activity in old Lohmann LSL layer hens.

In this research, the mean $\pm$ SD of heart rate was 333.5 $\pm$ 28.6 beats/min. This result is consistent with report by Khalil et al. (2010) who showed that the heart rate ranged between 260 and 340 beats/min during a day in white Leghorn hens at 90 weeks old. Another

research assessed the mean of heart rate in tree line layer hens in a period from 78 to 95 weeks of age and this parameter was between 268.4 and 296.7 beats/min (Kjaer and Jørgensen, 2011). In the present study, RR-interval was 180 $\pm$ 20 milliseconds. The RR-interval in tree line laying hens was reported 204.0 to 228.1 milliseconds by Kjaer and Jørgensen (2011). The younger chickens had a higher heart rate (200 to 250 beats/min) than older ones (Mutibvu et al., 2017).

The results of the current study indicated that the mean serum level of calcium in 112-week layer hens was 19.94 $\pm$ 3.63 mg/dL. This result is consistent with the previous study that reported the concentrations of calcium in white leghorn layers at 74th, 75th, 76th and 77th weeks were 17.84 $\pm$ 1.04, 19.36 $\pm$ 0.94, 20.87 $\pm$ 0.72 and 21.07 $\pm$ 1.53 mg/dL, respectively (Kashap et al., 2017). In studied layer hens, the concentration of blood calcium was close to calcium level in the study of Kashap et al. (2017) at 75th week. In layer birds, calcium is mainly needed for the ossification of bones, regulation of muscular activity and regulation of enzymes and endocrine systems. Rath et al. (2017) argued that the calcium value in white Leghorn layers was 17.29 $\pm$ 0.72 mg/dL at 50 weeks of age. Gyenis et al. (2006) reported that the concentration of calcium was between 2 and 3 mmol/L from 3 to 72 weeks in leghorn hen.

The serum concentration of phosphorus in this research was 5.02 $\pm$ 1.15 mg/dL. Phosphorus is a structural constituent of essential biomolecules involved in both energy metabolism, such as ATP, and in the formation of key macromolecules such as nucleic acids and phospholipids. Also, calcium and phosphorus are the main components for egg production (Kebreab et al., 2009). Kashap et al. (2017) stated that the blood phosphorus lev-

els in white leghorn layers were  $5.87 \pm 0.26$ ,  $6.21 \pm 0.43$ ,  $6.93 \pm 0.27$  and  $4.87 \pm 0.46$  mg/dl at 74th, 75th, 76th and 77th weeks, respectively. In our study, the levels of phosphorus are nearly similar to the result of Kashap et al. (2017) at 77th week. Piotrowska et al. (2011) reported that phosphorus is not age dependent and had no significant change up to 76th week of age in layer hens. Therefore, in the present study, the blood phosphorus level at 112 weeks was closer to 77th in a report by Kashap et al. (2017). In layer hens, circulating calcium and phosphorus levels are also interrelated (Piotrowska et al., 2011) and have an effect on the body effort to regulate the pH of the blood with a consequence on the acid-base balance (Pelicia et al., 2009). Any changes in serum calcium and phosphorus profiles in hens cause a difference in eggshell quality (Nanbol et al., 2016). In the present research, the serum level of calcium and phosphorus were close to blood calcium and phosphorus range in normal reproduction age.

The circulating chloride and sodium concentration in a study by Nanbol et al. (2016) in layer hens at 52 weeks were 56-70 and 60-98 mmol/L, respectively. In our study, the high chloride ( $119.20 \pm 2.38$  mEq/dL) and sodium levels ( $146.01 \pm 2.5$  mEq/dL) in layer birds may be due to receiving water and food containing high chloride. Also, fatty liver disease in layer hens can cause hypercholemia (Funk et al., 2007). Gyenis et al. (2006) showed that the sodium concentration had a clearly increasing tendency with aging in leghorn hens and reported the serum concentration of sodium in 72-weeks of age was 165 mmol/L. The evaluation of the sodium concentration in serum was basically important to control and diagnosis of possible dehydration. There is a high positive

correlation between the serum sodium level with age and total body dry matter content (Szabó et al., 2005).

In the present investigation, the circulating level of potassium was  $6.10 \pm 0.84$  mEq/dL. This result is similar to the results of Gyenis et al. (2006) who stated that the serum potassium level varied between 4 and 6 mmol/L from 3rd to 72nd weeks in leghorn hens. Sodium, potassium, and chloride have an important role in physiological processes, such as osmotic and acid-base balance (Nobakht et al., 2006; Baloš et al., 2016). In layer hens, these minerals are vital for optimal growth performance, egg production and shell quality (Tunç and Cufadar 2015).

AST and ALT are enzymes found mainly in the liver, but are also found in skeletal muscle, heart, brain, kidney and red blood cells. AST in birds is relatively non-specific for hepatic problems while it is considered as a very good biomarker for cardiac injuries (Bodor, 2016). In the present study, the levels AST and ALT activities were  $240.20 \pm 51.26$  and  $6.20 \pm 2.28$  U/L, respectively. The levels of AST activity in Lohmann LSL-Classic in weeks 6 and 12 was 177 and 184 U/L (Neijat et al., 2014). In another study, the AST and ALT activities in layer hen at 6th, 32nd and 52nd weeks were similar and 1-3 and 1-2 U/L, respectively (Nanbol et al., 2016). High levels of AST and ALT in this study were probably due to liver damage or fatty liver disease.

The LDH is present in nearly all types of metabolizing cells, especially concentrated in the heart, liver, red blood cells, kidney, muscle, brain and lung. The high level of serum LDH is found following heart injury and myocardial infarction (Nigam, 2007; Bodor, 2016). In the present study, the LDH activity was  $1262.40 \pm 344.18$  U/L. This re-

sult was consistent with a study by Gyenis et al. (2006) that reported the LDH values range between 1000 and 2500 IU/L in medium heavy body layer hens during egg period from 2nd to 72nd week.

In conclusion, the results of this study indicated that the electrocardiographic parameters, circulating electrolytes and cardiac enzymes in 112-week old laying hens were within the normal reference range in younger hens. Furthermore, based on the results of this research and previous studies, there were no significant differences between aged and younger laying hens. So, according to the findings, it may be stated that increasing persistency in laying period did not have side effects on cardiovascular functioning in old Lohmann LSL-classic layer hens and it is economic.

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### Conflicts of Interest

The authors declare that there are no conflicts of interest.

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## شاخص های الکتروکاردیوگرافیک، الکتروولیت ها و آنزیم های قلبی در گردش خون مرغ های تخمگذار مسن به ظاهر سالم

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### چکیده

**زمینه مطالعه:** اطلاعات در زمینه اثرات وضعیت فیزیولوژیک (سن و تخم گذاری) بر روی فعالیت الکتریکی قلب و مشخصات خونی مرغ های تخمگذار می تواند به شناخت بهتر سلامت سیستم قلبی-عروقی و عملکرد این پرندگان کمک کند.

**هدف:** مطالعه حاضر به منظور ارزیابی پارامترهای الکتروکاردیوگرافیک و سطوح در گردش الکتروولیت ها و آنزیم های قلبی در مرغ های تخمگذار نژاد لوهمن LSL کلاسیک ۱۱۲ هفته به ظاهر سالم انجام شد.

**روش کار:** ۵۰ مرغ تخمگذار نژاد لوهمن LSL کلاسیک ۱۱۲ هفته به ظاهر سالم انتخاب شد. الکتروکاردیوگرام ثبت و خونگیری از تمام پرندگان انجام شد. غلظت سرمی سدیم، کلر، فسفر، کلسیم، پتاسیم، منگنز، آسپارات آمینوترانسفراز، آلانین آمینوترانسفراز و لاکتات دهیدروژناز مورد سنجش قرار گرفت.

**نتایج:** میانگین  $\pm$  انحراف معیار ضربان قلب  $333/5 \pm 28/6$  ضربان در هر دقیقه بود. موج P فقط در بیست درصد پرندگان دیده شد و مدت زمان و دامنه آن، به ترتیب  $0/03 \pm 0/01$  ثانیه و  $0/05 \pm 0/05$  میلی ولت بودند. فاصله های PR، RR و QT به ترتیب  $0/04 \pm 0/02$ ،  $0/18 \pm 0/02$  و  $0/13 \pm 0/01$  ثانیه بودند. سطح در گردش کلسیم، فسفر و منیزیم به ترتیب  $3/63 \pm 1/9/94$ ،  $5/02 \pm 1/15$ ،  $2/46 \pm 0/23$  میلی گرم در دسی لیتر و سطح خونی سدیم، کلر و پتاسیم به ترتیب  $146 \pm 2/5$ ،  $6/10 \pm 0/84$  و  $119/20 \pm 2/38$  میلی اکی والان در دسی لیتر بود. ارزش های سرمی آسپارات آمینوترانسفراز، آلانین آمینوترانسفراز و لاکتات دهیدروژناز به ترتیب  $51/26 \pm 24/0/2$ ،  $6/20 \pm 2/28$  و  $1262/40 \pm 344/18$  بود.

**نتیجه گیری نهایی:** بر اساس سلامت بالینی پرندگان مورد مطالعه، همه شاخص های الکتروکاردیوگرافیک، الکتروولیت ها و آنزیم های در گردش در مرغ های تخمگذار مسن به مقادیر موجود در مرغ های تخمگذار جوانتر نزدیک است. بنابراین افزایش طول دوره تخمگذاری بیش از ۱۰ هفته، اثرات مضر و سوء بر فیزیولوژی و رفاه پرندگان نداشته است.

واژه های کلیدی:

ارزیابی قلبی، الکتروکاردیوگرافی، الکتروولیت های در گردش، آنزیم های قلبی، مرغ تخمگذار مسن