

## The Effect of Simulated Transportation on Biochemical Plasma Parameters of Japanese Quails

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**Abstract:** The effect of the duration of simulated transportation on blood SOD (Superoxide Dismutase), GSH-Px (Glutathione Peroxidase), AST (Aspartate Transaminase), ALT (Alanine Transaminase), GGT (Gamma-Glutamyl Transpeptidase), LDH (Lactic Dehydrogenase) enzyme levels and NO (Nitric Oxide) and MDA (Malondialdehyde) product levels was investigated in Japanese quails. Simulated transportation was carried out with a road simulator which was calibrated for Turkish roads conditions. Animals were loaded into well ventilated carrier boxes and carried at 2 and 5 h on the simulator. Blood samples were collected from 10-weeks-old Japanese Quails just after transportation. A significant ( $p < 0.05$ ) increase in all measured plasma parameters (except GGT) at 5 h transportation was observed. However, all of the plasma parameters have not shown a significant increase at 2 h transportation. This transportation duration might be the evidence of the time duration in still safe zone for Japanese Quails for that condition.

**Key words:** Japanese quail, stress, plasma parameters, road simulator, simulated transportation, Turkey

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### INTRODUCTION

The welfare of domestic animals during transportation has recently been emphasized (Adams, 1994; Fazio and Ferlazzo, 2003). It is well recognized that commercial transport of poultry imposes many physical and biological stressors, which adversely affect bird welfare. Animal transportation is one of the most important activities of the farming industry. However, it is also contemporary controversial subject of animal welfare (Ali *et al.*, 2006). Many researchers have confirmed that transportation duration may impose stress on animals (Ali and Al-Qarawi, 2002). The occurrence of vibrational and thermal stressors during transport and their effect on fowl have been well documented (Randall *et al.*, 1997).

Transportation stress may be more or less severe depending on a number of factors, such as crowding temperature feed and water deprivation and length of travel (Odore *et al.*, 2004). According to Mitchell *et al.* (1988) stress may be divided into two phases: a hypothalamic-adrenocortical phase, which is associated with perceived environmental stress such as noise and a sympathetic-adrenal-medulla phase associated with neurogenic stress such as transport. Many studies often deal with short term transportation or simulated transport.

In long term transportation, animals are exposed to repeated stress events during a long period of time and the activation of different hormonal pathways may cause biochemical changes in target tissues (Odore *et al.*, 2004).

Due to excessive tiredness and exposure to stress, can cause Free Radicals (FRs) or Reactive Oxygen Species (ROS) continuously arise in the body as a result of ongoing stress conditions. The cellular anti-oxidant defense system consists of Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GSH-Px). The shift of the delicate balance between FRs and cellular antioxidant defense system in favor of FRs might lead to development of oxidative stress (Elsayed and Bendich, 2001). ROS cause lipid peroxidation of cellular membranes, oxidation of protein and proteolysis and deoxyribonucleotide fragmentation and hence, disruption of the structural integrity and cell functions such as capacity for cell transport and energy production and ion balance (Ozyurt *et al.*, 2006).

Superoxide Dismutase (SOD) is a protective enzyme that can selectively scavenge ( $O_2$ ) by catalyzing its dismutation to  $H_2O_2$  and molecular oxygen ( $O_2$ ). The other antioxidative enzyme, Glutathione Peroxidase (GSH-Px) catalyzes the conversion of  $H_2O_2$  to water by using reduced Glutathione (GSH) as cofactor (Ozyurt *et al.*,

2006). Malondialdehyde (MDA) plasmatic concentration, the marker of oxidative stress and appears in the plasma after the ROS gave damage to cell membrane (Ozyurt *et al.*, 2007). Nitric Oxide (NO) can cause cellular injury, when they are generated excessively (Ozyurt *et al.*, 2007). Presence of enzymes such as AST, ALT, GGT and LDH at atypical concentrations in serum is generally indication of pathologic modifications. AST, ALT, GGT and LDH usually appear in serum when there is damage on the liver and muscle tissues caused by excessive stress (Mengi, 1997). These plasma parameters have been well documented on animals such as chicken, cattle, pig, goat and horse (Boyd, 1983; Randall *et al.*, 1997). However, no studies have been done on biochemical stress parameters in plasma of Japanese Quails. In this study, the effect of the duration of simulated transportation on blood parameters was investigated in Japanese Quails.

## MATERIALS AND METHODS

Transportation simulator have been developed and calibrated to simulate Turkish highway road conditions (Fig 1). Primarily, vibration signals have been captured during transportation by using accelerometers (Endevco 752A12 model) on a track which is normally being used for animal transport. The several Power Spectral Density (PSD) profiles were weighted in the time considering the duration of transit of the mean on different kinds of road. The overall PSD profiles obtained from this data processing was used to drive the simulator. Secondly, measured vibration signal was analyzed and the average Root Mean Square (RMS) values of the (PSD) of signal was calculated. Then, the simulator was calibrated with the average RMS value (0.35 g) to simulate transporting road conditions.

In this study, 6 weeks old male Japanese quails were transported on simulator. Animals were divided into 3 groups seven by seven. The control group was not transported but they were kept in the same room with the other groups to be affected with the same conditions (temperature, noise etc. exclude transportation). The 2 h group was transported 2 h. Transportation process carried out up to 5 h for only 5 h group. Containers (0.65×0.45×0.35 m), which have good air ventilation were tightly placed on the simulator and then Japanese quails were placed in to containers. Containers have sufficient height to allow Japanese quails, to stand and move about during transport. There was more sufficient space than recommended minimum floor space. During simulated transportation, room temperature was about 18°C and noise level was >72 decibel. These values were optimum for comfortable living area (Tao *et al.*, 2005).



Fig 1: Road condition simulator

Japanese Quails were euthanatized by decapitation at the end of experimental period under anesthetics and blood samples were collected (ethical report by University of Gaziosmanpasa, ethic committee for experimental animals). Blood samples were collected from each group at the end of the each transportation period. Control group samples were collected together with the third group.

Samples were collected in 5 mL tubes. Samples were immediately stored at -80°C until assayed. Then, plasma was separated (2500 g for 5 min at 4°C). In this study, measured plasma parameters were studied for indication of stress on animals (Ozyurt *et al.*, 2006). The studied SOD, GSH-Px, MDA and NO parameters are measured by spectrophotometry technique and ALT, AST, GGT and LDH were measured using the commercially available kits.

Data were analysed by using a commercially available statistics software package (SPSS® for Windows). One-way descriptive ANOVA test was performed and post hoc multiple comparisons were done with LSD. Results were presented as mean values ± SEM.  $p < 0.05$  were regarded as statistically significant.

## RESULTS

Stress associated with transportation was determined by measuring the level of SOD, GSH-Px, MDA, NO, AST, ALT, GGT and LDH in serum of Japanese quails. All results are presented in Table 1 and 2. All measured plasma parameters showed that there were no relationship between control group and 2 h transported group of Japanese quails ( $p > 0.05$ ). However, for all measured plasma parameters (excluding GGT), there were

**Table 1: Enzyme activities of SOD, GSH-Px, MDA and NO in all experimental groups measured by spectrophotometer technique**

Groups	SOD (U mL <sup>-1</sup> )	GSH-Px (U L <sup>-1</sup> )	MDA (µmol L <sup>-1</sup> )	NO (µmol L <sup>-1</sup> )
Control	2.17±0.742a	427.00±103.42a	1.90±0.191a	19.78±1.442a
2 h	2.48±0.280ab	407.44±70.89a	1.90±0.439a	20.98±2.610ab
5 h	2.99±0.592b	554.77±125.12b	3.09±0.847b	26.36±7.799b

Values in the table are means±SEM (n = 7). Means with different letters in each sub-group are significantly different from each other (2 h group: 2 h transported, 5 h group: 5 h transported, SOD: Superoxide Dismutase, GSH-Px: Glutathione Peroxidase, MDA: Malon-Dialdehyde and NO: Nitric Oxide)

**Table 2: Enzyme activities of AST, ALT, GGT and LDH in all experimental groups**

Groups	AST (U L <sup>-1</sup> )	ALT (U L <sup>-1</sup> )	GGT (U L <sup>-1</sup> )	LDH (U L <sup>-1</sup> )
Control	180.0±23.80a	8.71±2.138a	16.86±7.690a	476.6±212.32a
2 h	191.7±39.24ab	9.71±3.147a	16.57±5.884a	383.9±130.27a
5 h	226.9±30.58b	15.29±7.365b	21.57±7.850a	723.1±210.61b

Values in the table are means±SEM (n = 7); Means with different letters in each sub-group are significantly different from each other; (2 h group: 2 h transported group, 5 h group: 5 h transported group, AST: Aspartate Transaminase, ALT: Alanine Transaminase, GGT: Gamma-Glutamyl Transpeptidase, LDH: Lactic Dehydrogenase)

statistically significant increase between control group and 5 h transported group (p<0.05). Furthermore, there were statistically significant increase in GSH-Px and MDA levels between 2 h transported group and 5 h transported group (p<0.05).

There was no significant difference on AST, ALT and LDH values between control group and 2 h transported group however, there is significant difference between 5 h transported group and non-transported control group on the same plasma parameters (p<0.05). On GGT enzyme there were statistically insignificant relationships among the treatment groups. However, distinguishable increase can be seen on GGT enzyme between 5 h transported group and the others (p>0.05).

## DISCUSSION

In the study, simulated transportation effect on some of plasma parameters could be significantly observed especially, when the transportation duration for Japanese quails extended to 5 h. In long term transportation in previous studies (Re *et al.*, 1997) showed that animals are exposed to repeated stress events during a long period of time and activation of different hormonal levels may cause biochemical changes in target tissue.

The results of this study have shown consistency with previous experiments. As indicated by Elsayed and Bendich (2001) the cellular anti-oxidant defense system consists of Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GSH-Px). The shift of the delicate balance between FRs and cellular antioxidant defense system in favor of FRs might lead to development of oxidative stress.

As a result of excessive prostration and to be exposed to abnormal stress, the amount of SOR in the body increases. When the amount of SOR increases, defense system starts to produce enzymes of SOD and GSH-Px to protect the body.

Based on the results, it is possible to conclude that measurement of 8 plasma parameters studied in this research may be a useful indicator of transportation stress for Japanese quails and farm animals.

Enzymes of AST, ALT, GGT and LDH generally appear in muscular tissue under the excessive stress, especially when the muscular tissue was damaged (Ozyurt *et al.*, 2006). Values of AST, ALT and LDH enzymes were presented in Table 2. Investigated plasma parameters given in Table 1 and 2 that are indication of stress, there were no significant affects of 2 h transportation on none of the plasma parameters of Japanese quail. However, when the transportation duration extended to 5 h, all the measured plasma parameters have significantly increased except GGT.

## CONCLUSION

The measured plasma parameters may be a useful indicator of transportation stress in Japanese quails. Studies of the effects of transportation in Japanese quails have shown that measured plasma parameters except GGT have significantly increased at extended transport. It could be said that Japanese quails may be transported for short period of time without stress. But, extension of transportation duration may cause to stress. Level of starting point of stress has not been known yet. Therefore, investigations between 2-5 h should be carried out for Japanese quail to find out stress starting point. The developed road simulator gives also opportunity to investigate and carry out comparative studies on animal transportations under the controlled conditions. The investigations on Japanese quails and transported animals on stress related plasma parameters for post transportation should be carried out. The investigations should also be carried out on density of animals and temperature and damp factors to find out and to improve the most suitable transportation environment and conditions.

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