Effect of injection of diazepam, melatonin, methocarbamol and glucose on physiological responses of neonate chicks subjected to road transportation stress

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Received; 2019/02/17 revised; 2019/03/8 accepted; 2019/05/30

Abstract

Introduction: Transportation causes stress in chicks that may make numerous physiological changes with a negative impact on their production and health. This study was conducted to examine the effects of injection of diazepam, melatonin, methocarbamol and glucose on Serum biochemical parameters include: glucose, total protein, albumin, uric acid, cholesterol, triglycerides, Ca, P, Zn, Fe, Na and K in neonate chicks subjected to road transportation stress.
Materials and methods: The research was carried out in the form of factorial experiment (9×5) based on a completely randomized design with 4 replications. A total of 940 one-day old Ross 308 Male-sex broiler chicks were bought from a commercial hatchery. Immediately after the hatching, broiler chicks were traveled to about 1200 km far from the hatchery. At hatchery (40 samples) and after 300, 600, 900, and 1200 km journey, a random sample (From each treatment 8 birds) were sampled for blood collection.
Results: The mean concentration of glucose, protein, albumin, cholesterol, triglyceride, calcium, phosphorus, iron, sodium, and potassium was not affected by the treatments (P>0.05), but concentration of uric acid and plasma Zn was influenced (P<0.05). The effect of road distance on all serum parameters except Zn was significant. Furthermore, the mean concentrations of uric acid, Zn, Sodium and Potassium in serum showed an interaction between treatments and transport distance (P<0.05).
Conclusion: These results clearly show the physiological responses of chickens under long-distance transport and the effect of some treatments on stress indicators.

Keywords: Neonate chicks, Transportation stress, Physiological parameters

Introduction

In animals, factors such as transportation and high environmental temperatures and population density cause stress. To assess the stress or welfare of animals, immune parameters, plasma hormones and sympathetic adrenal agents such as heart rate are commonly used. Transport stress changes the physiological state and many metabolic factors of animals like: Lowering body weight, increasing serum creatine kinase activity, the amount of nitric oxide in the heart, and the transcription of nitric oxide isoforms (1). Movement increases serum albumin, keratin kinase, HSB70 (severe stress index), mRNA expression of cortisol, mRNA expression of inflammatory cytokines and serum inflammatory proteins such as lysozyme and entephalin (2). The performance and resistance of chickens for long time exposing to stressors were decreased with significant rate in their mortality (3).

Transportation caused chicken weight loss (4) The transfer of birds increased corticosterone and carnosine concentration in the breast and thigh muscle (5). Several stress-related behavioral parameters change during transportation (7,12). Transport stress have been confirmed to change in metabolic, hematologic and immunological parameters (such as change in plasma cortisol, aspartate aminotransferase, no esterified fatty acids, glucose, lactate, albumin, creatine kinase, triglycerides, and uric acid, IFN-gamma, neutrophil: lymphocyte ratio, eosinophils, packed cell volume, red blood cell numbers , hemoglobin, haptoglobin, saliva , serum and animal mortality, meat quality, body weight, heart rate) of various animals under road transport (13,23). Diazepam was effective in antagonizing the hematological and biochemical changes induced by immobilization in pigs (24) that reduced the cortisol concentration and heart rate (25). The sedative effects of diazepam and xylazin were observed in two groups of stressed and non-stressed chicks (36). A study found that Xylasein can well reduce stress (27). Melatonin supplementation increased the levels of high density lipoprotein and decreased the thiobarbituric acid (28). Significant increase in goblct cells and mucins gene expression, increased the digestive enzymes (maltase, sucrose, and lactase), the expression of the nutrient transporter genes in the duodenum or jejunum. On the other hand, they reduced the Notch receptors (Notch1 and Notch2) and their ligands after melatonin administration (29). Melatonin decreased the inflammation of the small intestine of chicks by reducing the expression of inflammatory cytokines, increasing the proliferation of the epithelial cells, and inhibiting epithelial apoptosis (with TLR4 mediation) Melatonin (the pineal gland hormone) is probably effective on daily secretion of catecholamines (epinephrine and norepinephrine) in chickens (30). Melatonin is a hormone that plays a role in cooling body temperature and regulating heat dissipation (31,32). Melatonin exhibits potent anti-inflammatory properties, it plays a fundamental role in neuroimmunomodulation (33), strong analgesic (34), modulator of inflammation and apoptosis (35), an antioxidant (36,37) and an effective medication for sedation (38). Typically, a muscle relaxant drug is a medicine that affects muscle function and reduces muscle tone. Methocarbamol is a skeletal muscle relaxant that is usually formulated as a single entity or in combination with other analgesics such as aspirin, acetaminophen (paracetamol) and codeine (39). Despite the extensive knowledge on the impact of transport on stress and animal welfare, no significant studies have been undertaken to moderate the effects of stressors on newly hatched chicks. Therefore, this study aimed to evaluate the effect of injection of diazepam, melatonin, methocarbamol and glucose on physiological responses of broiler chicks to road transport stress.

Materials and methods

Birds and transportation: About 940 one-day old Ross Male-sex broiler chicks with a mean weight of 43.06 ± 2.48 g were provided from a commercial hatchery (Arak, Iran-34°.5 N and 44°.41 E). After collection of chicks from hatcher baskets, they immediately were transported to Khorramabad (33° 48 N and 48° 35 E) over 1200 km. All the chicks were wing banded and individually weighed within an hour after hatch and kept in boxes of 100 chicks. The birds inside each box were kept in four groups of 25 chicks each. Just after release from hatcher, 25 randomly selected chicks were killed by severing the jugular vein and carotid artery for blood collection within the same time at hatchery. The chicks were transported by a regular commercial chick delivery vehicle. During transport, air temperature was kept constant at 27°C to 30°C with a relative humidity of 55 to 65 percent inside the vehicle. The vehicle moved with a speed of 50 to 60 km/h throughout the 1200 km journey. Therefore, the travel distance (300 km) and duration (300±5 min) were constant in each segment of the journey. All procedures used in this experiment were based on the Animal Care Guidelines, Animal Ethics Committee at the Shiraz University, Iran.

Blood samples: During the 1200 km journey, individual\ weighing and blood collection were repeated for a random sample of 72 chicks (8 samples from each treatment, two chicks from each box replicate) at 300, 600, 900, and 1200 km distance from the hatchery. Blood samples were kept in 35°C temperature for an hour for coagulation and then along with all the killed hatchlings were kept at 4ºC on slush-ice. By arriving at lab, all killed hatchlings were dissected and examined for sex and yolk sac weight and individual samples of coagulated whole blood were centrifuged at 1800× g for 15 min. The serum was collected and stored at -20°C for further chemical analyses. Concentrations of serum biochemical constituents including Glucose, Uric acid, total protein, Albumin, total cholesterol, total Triglycerides and major electrolytes Contains Calcium, Phosphorus, Zinc, Iron, Sodium, and Potassium were determined using an autoanalyser (Selects E Autoanalyzer, Sr. No. 8-7140, Vital Pharma BV, Maarheeze, The Netherlands).

Experimental design: A completely randomized design with a 9×5 factorial arrangement of treatments was used to determine the response of broiler chickens to 9 treatments. Nine hundred birds distributed into nine chick carton boxes with 25 birds in each quarter. Four quarters in different boxes assigned to each treatment. Four quarters holding total number of 100 birds considered as control birds and the same number of birds and quarters were subjected to parenteral administration of either 0.5 ml saline (NaCl 0.9 g/L; control group) or the same volume of diazepam at two concentrations of 100 and 200 µg/bird, methocarbamol (10 mg/bird), melatonin (500 µg/bird), glucose at three concentrations of 200, 300 and 400 mg/bird. All solutions were administrated through subcutaneous injection in posterior area of neck within 60 min post hatch. The data were analyzed using PROC GLM of SAS software 9.1 (SAS, 2002) and the means comparison was done at a significant level of 0.05 using the Student-Newman-Keuls test. Linear and quadratic contrasts were used for the effects of distance on the measured variables.

Results

In the present study, the effects of transport stress and the possibility of modulating it by melatonin, methocarbamol, diazepam and different levels of glucose were studied and the results were presented as follows (Tables 1 and 2). The mean concentration of glucose, protein, albumin, cholesterol, triglyceride, calcium, phosphorus, iron, sodium, and potassium was not affected by the treatments ($P>0.05$), but concentration of uric acid and plasma Zn was influenced ($P<0.001$). The effect of road distance on all serum parameters except Zn was significant ($P<0.001$). Furthermore, the mean concentrations of uric acid, Zn, Sodium and Potassium in serum showed an interaction between treatments and transport distance ($P<0.05$).

Table 1. The main effects of subcutaneous injection of diazepam, melatonin, methocarbamol and glucose on serum concentration (mg/dL) of glucose, total protein, albumin, uric acid, Cholesterol, Triglycerides in neonate chicks subjected to road transportation stress.

Treatment	GLU (mg/dl)	UA (mg/dl)	TP (mg/dl)	ALB (mg/dl)	CHOL (mg/dl)	TG (mg/dl)
Injected chemicals (IC)						
Control	254.33^{A}	8.34^{AB}	3.86^{A}	1.06^{A}	608.00^{A}	149.75^{A}
Saline	256.17^{A}	7.59^{AB}	3.86^{A}	1.05^{A}	626.92^{A}	148.33^{A}
Diazepam 0.5	254.67^{A}	6.83^{BC}	3.78^{A}	0.98^{A}	619.75^{A}	152.58^{A}
Diazepam 1	259.58^{A}	6.63^{BC}	3.78^{A}	0.96^{A}	623.50^{A}	143.50^{A}
Melatonin	254.66^{A}	8.87^{A}	3.91^{A}	1.05^{A}	628.14^{A}	157.23^{A}
Methocarbamol	241.89^{A}	7.14^{BC}	4.00^{A}	1.10^{A}	615.49^{A}	151.54^{A}
Glucose 1	262.25^{A}	6.70^{BC}	3.70^{A}	0.98^{A}	604.00^{A}	156.42^{A}
Glucose 2	269.33^{A}	7.40^{AB}	3.91^{A}	1.08^{A}	633.08^{A}	159.83^{A}
Glucose 3	258.67^{A}	5.30^{C}	3.50^{A}	0.89^{A}	603.33^{A}	149.17^{A}
SEM	6.45	0.51	0.12	0.60	20.46	6.77
Transportation distance (TD)						
0	223.49^{D}	5.41^{C}	2.70^{D}	0.68^{C}	448.77^{D}	130.77^{B}
300	230.08^{DC}	7.48^{B}	3.26^{C}	0.88^{B}	539.13^{C}	139.71^{B}
600	269.25^{B}	6.78^{B}	3.86^{B}	1.15^{A}	635.01^{B}	167.31^{A}
900	241.78^{C}	5.65^{C}	3.91^{B}	0.98^{B}	609.59^{B}	144.34^{B}
1200	305.27^{A}	9.89^{A}	4.83^{A}	1.25^{A}	784.37^{A}	168.60^{A}
SEM	4.76	0.37	0.09	0.04	14.09	5.00
ANOVA results						
IC	0.1731	0.004	0.5814	0.4527	0.9772	0.7407
TD	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
IC × TD	0.8647	0.0003	0.3361	0.4246	0.6433	0.9139
Regression analysis						
Liner	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Quadratic	0.0112	0.0107	0.7026	0.0843	0.3884	0.3243

Means within a column with no common superscript differ significantly (P<0.05). GLU (Glucose), UA (Uric acid), TP (total protein), ALB (Albumin), CHOL (cholesterol), TG (Triglycerides).

Discussion

Although none of the injectable treatments had an effect on the amount of glucose (P>0.05), the results indicated that transport had an effect on glucose concentration (P<0.001). Serum GLU level was increased from 223.49 mg/dL at hatchery to 305.27mg/dL at the end of journey. Our results were in agreement with (27, 40) that showed an increase of glucose after a road transportation and in contrast with (2). In birds, a common physiological response to stressors is the activation of the hypothalamic-pituitary-adrenal axis (40). By activating this axis due to stress, the initial response is to increase the concentration of stress-related hormones: glucocorticoids, catecholamines and beta-endorphins. Secondary response is an increase in blood glucose due to glycolysis and gluconeogenesis (3, 40). This increase in glucose is probably due to the role of glucose in energy metabolism and the overcoming on the challenge facing by birds (40, 42).

The concentrations of uric acid were significantly affected by treatments and transport distance (P<0.001). Compared to control treatment, a significant decrease was observed in Serum uric acid concentration in Treatment 9 (8.34 versus 5.30 mg / dL). Serum uric acid concentration was 5.41mg/dl in the birds killed at hatchery and increased to 9.89 mg/dL after 1200 km transportation distance (P<0.001). The results of the present study showed that birds with high serum glucose had high levels of uric acid in their blood. This high uric acid density pattern after road transport can be interpreted as a part of the physiological responses of birds to stress (reflecting increased protein/amino-acid catabolism). Following previous discussions, one of the important effects of corticosterone on chicks is increasing the cellular oxidation, proteolysis, gluconeogenesis and ultimately no protein nitrogen (NPN) and uric acid concentration (43).

Table 2. The main effects of subcutaneous injection of diazepam, melatonin, methocarbamol and glucose on serum concentration of Ca, P, Zn, Fe, Na and K ions in neonate chicks subjected to road transportation stress.

Treatment	Ca (mg/ dl)	P (mg/ dl)	Zn (mg/dl)	Fe (mg/dl)	Na (mEq/l)	K (mEq/l)
Injected chemicals (IC)						
Control	9.08 A	9.17 A	235.25 AB	189.83 AB	286.33 A	8.15 A
Saline	9.49 A	9.09 A	213.17 ABC	170.33 AB	276.50 A	7.49 A
Diazepam 0.5	9.18 A	9.13 A	208.42 BC	141.58 B	285.00 A	8.43 A
Diazepam 1	8.87 A	9.07 A	206.56 BC	166.75 AB	289.08 A	8.30 A
Melatonin	9.16 A	9.14 A	218.26 ABC	191.09 AB	289.71 A	8.21 A
Methocarbamol	9.49 A	10.02 A	248.43 A	201.89 A	274.51 A	7.73 A
Glucose 1	8.98 A	9.43 A	205.33 BC	194.67 AB	274.83 A	7.94 A
Glucose 2	9.43 A	9.51 A	210.58 ABC	167.17 AB	292.08 A	7.71 A
Glucose 3	9.06 A	9.28 A	190.75 C	162.83 AB	283.67 A	7.57 A
SEM	0.32	0.34	10.13	13.21	5.81	0.34
Transportation distance (TD)						
0	7.68 D	8.62 B	231.08 A	168.38 B	223.51C	7.43 C
300	8.80 C	10.20 A	217.03 A	150.00 B	282.08B	10.58 A
600	10.02 A	10.17 A	220.14 A	169.93 B	292.04B	6.54 D
900	9.62 AB	9.00 B	205.56 A	152.41 B	278.49B	8.28 B
1200	9.16 BC	8.25 B	209.57 A	238.41 A	314.57A	6.63 D
SEM	0.24	0.24	7.48	9.76	4.00	0.23
ANOVA results						
IC	0.8756	0.7181	0.0009	0.0732	0.3233	0.1788
TD	<.0001	<.0001	0.2179	<.0001	<.0001	<.0001
IC × TD	0.8657	0.8925	<.0001	0.9085	0.0158	0.0199
Regression analysis						
Liner	<.0001	0.0252	0.0366	<.0001	<.0001	<.0001
Quadratic	<.0001	<.0001	0.5698	<.0001	<.0001	0.0002

Means within a column with no common superscript differ significantly (P<0.05). Ca (Calcium), P (Phosphorus), Zn (Zinc), Fe (Iron), Na (Sodium), and K (Potassium)

In addition, road transport and lack of access to water that is necessary for uric acid removal, exacerbates bird stress, which results in increase in concentration of uric acid in the blood (40). However, it was observed that injection of 200 mg glucose significantly reduced the amount of uric acid in the plasma. It is possible that the treatment of 3 glucose by reducing the glucose required by the bird reduces the proteolytic and gluconeogenesis process with having an operative support role. The mean concentration of protein and Albumin were not significantly affected by the treatments (P>0.05), while the effect of distance was significant (P<0.001). Total proteins concentration was 2.70 mg/dl in the birds killed at hatchery (0 km) and increased to 4.83 mg/dL after 1200 km transportation distance. Serum albumin concentrations reached the highest at the end of the travel, despite a significant decrease at 900 km. Corticosteroids created by stress, influence protein metabolism with decreases in protein synthesis and increases in degradation in skeletal muscle (40, 45). In addition, the production of free radicals also is increased, which results in oxidation of proteins, DNA alteration and solos injury (6). It should be noted that birds are dehydrated (through breathing and dying) during road transport. Water scarcity appears to increase with increasing osmotic pressure and increasing plasma protein concentrations. Increased serum protein and Albumin concentrations due to stress or transport have also been reported in other researchers' reports (20, 21, 22, 44). However, some controversial results were reported on cattle and calves with their own specific conditions (23).

The mean concentration of Cholesterol and Triglycerides were not affected by the treatments (P>0.05), but the transport distance had a significant effect on both concentrations (P<0.001). Serum cholesterol concentration linearly increased from 448.77 mg/dl in the chicks killed at hatchery to 784.37 mg/dL in those experienced after 1200 km journey. Also, lowest concentration (130.77 mg/dl) of triglyceride was from the chicks killed at hatchery and the maximum (168.60 mg/dl) was those experienced after 1200 km journey (Table 1). The report of other researchers (41, 44) confirms the results of this study. Normally, when the chick is placed under stress, the levels of corticosteroids rises to provide energy to cope with such conditions. Supplying energy in these conditions is done by increasing the metabolism of carbohydrates (glycolysis), proteins (with decreases in protein synthesis and increases in degradation in skeletal muscle) and lipids (increase lipolysis). Therefore, the concentration of glucose, uric acid, cholesterol, and triglyceride increases (3, 40). In addition, a role of CORT is likely to be increasing the synthesis of NEFA and both expression and levels of triglyceride lipase in chicken adipose tissue (15, 47). It seems that these changes are necessary for bird to adapt with the stressful conditions and providing the needed energy.

The injected treatments did not have a significant effect on serum calcium concentration (P>0.05), but the transport distance was effective (P<0.001). The highest concentration (10.02 mg/dl) of calcium was recorded at a distance of 600 km and the lowest value (7.68 mg/dl) was at the origin of the movement. The consequence of stress and glucocorticoid secretion decreases calcium absorption (47). Increasing the concentration of corticosterone in the blood prevents the complete deposition of calcium on the bone. Discharging bone calcium under stress causes increased calcium in the blood (44). The results of this study confirm the findings of Khosrowinia. In addition, albumin acts as a calcium ligand, therefore it is expected that a higher concentration of calcium in the serum will be observed with increasing serum albumin concentration due to the positive correlation between calcium and albumin (48). Although there is not much report on the study of calcium in the blood of day-old chicks, a report showed that stress had no effect on calcium concentration (41).

Serum Phosphorus was not affected by any of the treatments (P>0.05), but it increased significantly due to the transport distance(P<0.001). The P concentration increased in the birds travelled up to 600 km, then decreased to 1200 km. According to these results and the report of Khosravinia (2015), changes in plasma phosphorus can be attributed to the road stress.

Unlike other serum variables, Zn concentration was affected by the treatments (P<0.001), while the distance had not significant effect (P>0.001). Administration of 200 mg glucose (Treatment 9) significantly reduced serum zinc, but the average zinc concentration for other treatments was a severe one (Table 2). Zn is an element that plays an important role in biological processes and the activity of more than 300 enzymes in the body (49). In addition, in the immune system, skeletal maintenance and antioxidant enzymes activity such as superoxide dismutase and glutathione peroxidase play a major role (50). The activity of these antioxidants depends on the provision of cofactors such as selenium for glutathione peroxidase, zn, Cu and Mn for superoxide dismutase (51). During environmental stress, the mobilization of minerals and vitamins is removed from the body. Stress reduces the maintenance of zinc, iron, sodium and calcium from the body (51). The results of this study showed that the distance did not change significantly the zinc concentration, but glucose injection (200 mg) significantly reduced serum zinc, and glucose probably provided a suitable protective role for stressed chicks.

Serum Iron was not affected by any of the treatments (P>0.05), but it increased significantly due to the transport distance (P<0.001). Iron concentration did not change to the original level until the end of 900 km, but then it increased significantly. The maximum concentration (238.41 mg/dl) of iron belonged to a distance of 1200 km (Table 2).

Serum Sodium was not affected by any of the treatments (P>0.05), but it increased significantly due to the transport distance (P<0.001). Fe concentration in an incremental process reached its highest value at a distance of 1200 km. Serum Potassium was not affected by any of the treatments (P>0.05), but it increased significantly due to the transport distance (P<0.001). The trend of potassium changes was irregular compared to the initial level, so that the maximum concentration of potassium was 300 km and the minimum value was 1200 km. Increasing the concentration of Fe, Na and K in the plasma can be a sign of the stress and tough conditions of the birds. Reduction in the storage and absorption of iron, sodium, potassium and phosphorus and calcium by stress has been confirmed by other researchers (47, 51).

Conclusion

While most of the stress indicators were affected by transport, glucose was able to partially play a protective role for the broiler chicks. It is recommended that other levels of sedative and relaxant compounds are evaluated to reduce transport stress.

Acknowledgments

The authors are grateful to the laboratory staff and the members of the Animal Science Department of Lorestan University.

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