Useful managemental practices for mitigating the effect of transportation stress on broiler under Egyptian conditions

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Abstract: Broiler birds are transported to slaughter house sites within one to 2 days before slaughtering. Possible effects of this transportation are poorly understood and could vary among birds from breeder flocks of different ages. The aim of the present study was to investigate the role of some managemental practices in alleviation of the harm effects of transportation stress on broilers. A total of 300 of 28 day old broiler Cobb birds were allocated randomly into equal five treatment groups (n = 60). The birds in the first group (C) were transported without treatment and kept as a control group. The birds in the second group (V) were supplied with vitamin E & selenium with a dose of 1 ml per liter of the drinking water from 35th to 38th day (day of transportation)**.** The birds in the third group (FW) were treated by feed withdrawal 12 hours before transporting. The birds in the fourth group (BL) were reared under blue light color. While, the birds in the fifth group (D) were reared in the darkness three hours before transporting. The obtained results revealed that live body weight, internal organs weight loss, H/L ratio, T3, T4, respiratory and heart rates were increased after transportation and decreased by different treatments except feed withdrawal. From the obtained results in the current study, it is strongly recommended to use an alternative management between darkness and blue light colors or adding vitamin E & selenium in the drinking water in broiler farms before transportation to reduce the harm effects of transportation stress and improve the bird welfare. But, feed withdrawal is contraindicated.
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1. Introduction

Poultry production and consumption exhibit a global trend towards overall increase despite the temporary effects of the current recession. It is reasonable to predict that in the next decade the demand for poultry meat production will expand further **(Mottet and Tempio, 2016).** This commercial expansion of the broiler industry has been associated with the development of concentrated large-scale hatcheries and incubators. This expansion modified the distance between the hatcheries and the growout farms, so, the transportation distances and durations were increased (**Fairchild *et al.*, 2006).** Current European Union legislation (Council of EU, 2005) specifies the transportation duration for broiler for a maximum of 24 hours.

All poultry species and major breeds employed in the main intensive production systems are transported at least twice during their lifetimes over distances (e.g. From hatchery to production site or from farm to processing plant). Despite efforts to improve the design of transportation means (truck, plane, or train), birds still may be transported for extended periods under suboptimal environmental conditions **(Mitchell, 1998)**. Fast- and slow-growing strains chickens seemed to be different in the sensitivity to transportation stress due to the high kinetic behavior of slow-growing strains **(Rimoldi *et al.*, 2015; Castellini *et al.*, 2016**).

All the procedures and practices involved in transportation and the micro-environments prevailing in containers and vehicles may cause stress and adversely affect the bird welfare status, health and productivity **(Jayapashrak *et al.*, 2016).** For instance, dehydration has been reported to be a problem in birds transported for long periods **(Fairchild *et al.*, 2006).** Bird mortality occurs mostly due to the combined stress of handling, transportation, and poor adaptation to the farm conditions **(Heier *et al.*, 2002).**

When the transportation distance was increased from 50 to 300 km, mortality also increased from 1.2 to 1.4% **(Chou *et al.*, 2004).** However, when water and feed were removed before and during the transportation period, the bird mortality was reduced and body weight gain improved **(Xin and Rieger, 1995).** This practice is widely used due to the birds are transported in total darkness and the birds are less active in the darkness **(Archer *et al.,* 2009).** To cope with transportation stress, bird develops physiological responses, which may subsequently affect meat quality and mortality **(Qi *et al.,* 2017).**

Accordingly, the aim of the present study was planned to compare between the using of blue light, feed withdrawal, darkness and adding vitamin E & Selenium in the drinking water prior to transportation in alleviation of transportation stress on broilers under Egyptian conditions**.**

2. Materials and Methods

General layout of the experiment:

The present study was carried out in the laboratory of the Department of Hygiene and Preventive Medicine, Faculty of Veterinary Medicine, Kafrelshiekh University, Egypt, (from 4^{th} April till 20^{th} May, 2017) and in accordance with our institutional recommendations of the guidelines of Animal Care and Use of laboratory animals in the research.

Birds, housing and management:

A total of 300 of 28day old broiler Cobb birds was selected from a broiler farm at Gharbyia city, Egypt. The birds were inspected for any deformities and health problems such as lameness, crooked legs and beaks. All birds were reared with each other as one group for one week for adapting to the new place. Identical care and management was provided to all birds in the different treatments throughout the duration of the study according to **Xie *et al.* (2011)** and **Kim *et al.* (2014)**. Broilers had free access to a finisher ration pellet (EL-Wadi Company, Cairo, Egypt) 19% crude protein (CP) and 2,900 kcal metabolizable energy (ME)/kg, and water was supplied at all times.

Experimental design:

The birds were allocated randomly into equal five treatment groups (n = 60). Each group was kept in a separate free-run environmentally controlled room at a density of 10 bird/m^2 according to **Firouzi *et al.* (2014)**. The birds in the first group (C) were transported without any treatment and kept as a control group. The birds in the second group (V) were supplied with vitamin E & selenium (Myo Gaster E, VMD, Belgium) with a dose of 1 ml per liter in drinking water from 35^{th} to 38^{th} day (day of transportation)**.** The birds in the third group (FW) were treated by feed withdrawal 12 hours before transportation according to **Nijdam *et al.* (2005)**. The birds in the fourth group (BL) were reared under blue light color using colored incandescent bulbs lighting (480 nm). While, the birds in the fifth group (D) were reared in the darkness three hours before transporting.

Transportation conditions were done according to **Mousa-Balabel and Mohamed (2011).** In this study, transportation was used as a stress and the broilers were transported on a country road with stocking density 10 birds from each group were held in one crate (1.3×0.7×0.25m). All 24crates containing birds undergoing the same treatment and marking and were randomly distributed in the truck. The transportation was occurred during the period from 07.30 to 10.30h a.m. withan average outside temperature of 21±4°C and inside temperature of 26±2°C. As a result of appropriate ventilation, the average of inside RH remained at about 75% during the transporting period with an average speed of 60 ± 5 km/h. The crates were covered with black plastic sheets during loading, transport, and unloading to protect birds from exposure to bright light and to reduce possible excitement of the birds during these procedures. When crated for experiments, individual birds were picked up in an upright position with both hands and placed in the crates as gently as possible in order to avoid physical damage or stress to the birds due to inverted handling. Only a few minutes from arriving feed was added and water was available for *ad libitum.*

Data collection:

All birds were individually weighed on the day 38immedaitely after transportation (using the Sartorius balance produced by Sartorius– universal, made in Germany). Individual live body weights were totaled and divided by the number of birds to obtain the average of live body weight (BW). The body weight (BW) was recorded directly after transportation for all birds for all groups.

Tonic immobility (TI) test:

25 randomly selected birds (5 birds from each group) were tested individually for the duration and induction of TI, before the directly after unloading process of transportation for the birds. The birds were carried to a separate room (no auditory and visual contact with other birds) and subjected to TI measurements. TI was induced by manual restraint. The bird was placed on its back in a U-shaped cradle covered with clothes. Then the bird was restrained with one hand on its sternum for 45 seconds while holding the head and neck with the other hand. Towards the end of the induction period, hand pressure was gradually lifted and if the birds till moved, another induction period was started immediately, until the movement stopped. After removal of the hands, as top watch was started. The experimenter then retreated, moving out of sight of the bird and observed the behavior of the bird. The number of induction trials and the duration of the tonic

immobility reaction, that was, the latency until self righting. If the bird righted itself in less than 20 seconds, it was considered that tonic immobility had not been induced and the restraint procedure was repeated. Conversely, if a bird did not show a righting response over the 10 min. test period, a maximum score of 600 seconds was given for the duration **(Ghareeb and Awad, 2008).** One day later, another 5 birds per group were subjected to tonic immobility test to measure the level of fearfulness of birds after the adaptation period of birds to stress had finished. The number of inductions required to perform TI were also recorded.

Respiratory rate (RR):

Respiratory and heart rates were recorded for 5 randomly selected birds/group, according to **Kassim and Norziha (1995)** directly after transportation.

Blood sampling:

Blood samples (3mL/bird) were aspirated from the wing vein immediately after unloading processes from crates for 5 birds from each group and transferred into vacuum tubes with and without ethylene diamine tetra acetic acid (EDTA).

Heterophils/ lymphocytes (H/L) ratio:

Heterophils, lymphocytes and H/L ratio in the whole blood were measured using an automatic blood cell counter (Exigo-Vet., Boule Medical AB Inc., Stockholm-Sweden). Blood with EDTA was centrifuged at 3000 rpm for 10 minutes, and plasma samples stored at –20°C in Eppendorf test tubes until the analyses were performed. Some selected plasma biochemical indices: glucose, total protein (TP), T3 and T4 were measured by a Cobas Plus Mira biochemical analyzer using commercial test kits **(Vosmerova *et al.,* 2010).**

Weight of internal organs:

Immediately after transportation and unloading processes, 5 birds from each group were carefully euthanized via exsanguination from a neck cut that severed the carotid artery and jugular vein. This method is considered humane when performed by a trained person **(Gracey, 1986)**. The birds were eviscerated to harvest the liver, spleen, heart, gizzard and proventriculus. The organs were gently soaked in 0.9% saline to remove the remaining blood. Harvested organs were immediately weighted by digital balance (PW Balance, ADAM equipments Co., USA).

Statistical Analysis:

Statistical analysis was carried out by using the SPSS software, version 16 (SPSS Inc., Chicago, IL, USA). Groups data were compared by one-way analysis of variance (ANOVA), followed by LSD test. The statistical significance was accepted at ($P<0.05$).

3. Results and Discussion

The increased rearing number of poultry and the demand for poultry to be centered and slaughtered in processing plants make transport a critical constituent in poultry industry all over the world. Recently there are various concerns about poultry transport due to these three major facts **(Spurio *et al.,* 2016).** Firstly, mortality may be raised by different transport. Secondly, stressful transport reduces poultry welfare. Thirdly, transport decreases meat quality. However, transport stressors include feed and water withdrawal, handling, (un) loading, crating density, transport time, ambient temperature, vehicle design, trailer microclimate and lairage time. Among them, transport duration, trailer microclimate and loading density are the three main causes of mortality of birds **(Schwartzkopf-Genswein *et al.,* 2012; Qi *et al.,* 2017).**

Geographical, climatic and seasonal aspects, many other factors affect broiler mortality and live weight losses during transportation in the broiler industry, including transportation conditions, transportation infrastructure, and transportation vehicles. According, the transportation operations should be carried out for short distances, taking into the economically optimal to ensure a minimum level of impact of environmental and climatic conditions on the birds. These factors should be taken into consideration for broiler transportation **(Arikan *et al.,* 2017).**

In some previous studies on broiler transportation, catching method **(Knierim & Gokce, 2003),** transportation distance **(Sowinska *et al.,* 2013),** time of transportation **(Luptakova *et al.,* 2012)** is the main factors affecting the mortality rate and live weight loss during transportation between the farms and the processing plants.

The present study was conducted to evaluate the role of some managemental practices in relieving the total loss (live weight loss and mortality) of broilers transported from the farms to the processing plant. There was a significant difference between the treated groups and the control in the body weight loss. As, the average live weight loss during transportation throughout the experiment was 107g in the control group in comparison with 80, 115, 80 and 75g for V, FW, BL and D, respectively. In addition, the weight loss in the internal organs was higher in the control group than those in the treated groups except for the FW group was recorded higher values than those in the control group. This may be attributed to the feed withdrawal is considered as a second stress beside transporting one. Moreover, the most organ affected by the transportation was the proventriculus in all groups as shown in table (1). These findings are in agreement with that recorded by **Oba *et al.* (2009)** who observed that the decrease in the body weight in broilers during the short transport period 30, 90 and 180 min were 2.11, 3.03 and 4.82 percent, respectively, and during the long transport period was higher weight loss if compared with a short transport period. Also, **Karaman (2009)** observed a significant reduction in body weight of broiler chicken during transportation. An experimental study conducted in Slovakia in 2008 reported that transportation of 6 to 7week old broilers from two different farms 30 km and 120 km distance from the processing plant resulted in a live weight loss of 100 and 306 g per bird, respectively **(Ondrasovicova *et al.,* 2008).** In another study conducted in Turkey in May 2012, six transportation durations were evaluated (Group 1: 0-120 minutes, Group 2: 121-240 minutes, Group 3: 241-360 minutes, Group 4: 361-480 minutes, Group 5: 481-600 minutes and Group 6: 600 minutes or longer above) the mortality rates were 0.29, 0.38, 0.40, 0.43, 0.42, and 0.46%, respectively, and average live weight losses of 4.33, 4.95, 5.55, 5.73, 6.02, and 6.63%, respectively **(Aral *et al.,* 2014).** Under this experiment, only one bird was died (from FW group) during transportation, thus no statistical analysis was done for one dead bird on arrival. This result is coincided with **Vecerek *et al.* (2006)** who recorded that short duration of transportation (up to 50km) were less mortality (0.15 %) compared than long duration of transportation (300 km or more) were high mortality (0.86 %).

Table 1. Effect of transportation on body and internal organs weight loss in broilers.

	C	Treatment			
		V	FW	BL	D
BW loss (g)	**107**± 0.400[b]	**90**± 0.606[c]	**115**± 0.205[a]	**80**± 3.601[c]	**75**± 0.520[d]
Proventriculus weight (g)	**0.93**± 0.201[b]	**0.67**± 0.321[c]	**0.97**± 0.311[a]	**0.56**± 0.155[c]	**0.36**± 0.145[d]
Gizzard Weight (g)	2.83± 0.210[b]	1.96± 0.226[c]	3.03± 0.206[a]	1.67± 0.416[c]	1.10± 0.240[d]
Heart Weight (g)	1.50± 0.321[b]	0.96± 0.410[c]	1.80± 0.450[a]	0.87± 0.422[c]	0.56± 0.371[d]
Liver Weight (g)	1.83± 0.132[b]	1.40± 0.210[c]	2.20± 0.231[a]	0.94± 0.321[c]	0.40± 0.146[d]
Spleen Weight (g)	0.34± 0.160[b]	0.32± 0.210[c]	0.40± 0.205[a]	0.20± 0.320[c]	0.10± 0.120[d]
Mortality rate	0	0	1	0	0

*Means, in the same row, which superscript with different small letters (a, b, c,…..) differ significantly at ($P<0.05$)

Stress is regarded to be adaptive or protective responses in the body, which protect animals from the adverse effects of the stressor. Transporting stress responses are mediated by the two major physiological systems, the Sympatho Adrenal Medullary System (SAMS) and the Hypothalamic-Pituitary-Adrenal Axis (HPA). This can alter the physiological states of animals. Transport stress impacts on some physiological parameters of bird, like corticosterone (CORT), glucose and creatine kinase (CK) before slaughter **(Nijdam *et al.,* 2005)**. According to these indicators, we can evaluate the stress level of poultry, for improving the management of transport conditions to meet the needs of poultry.

Data on biochemical indices concentration in blood sampled during exsanguination are given in table 2. On the farm, feed deprivation is done to reduce fecal contamination of the carcasses. During transport, the birds also don't have access to feed or water. Feed deprivation for 4 h or longer is needed for emptying the gastrointestinal tract. Combined with 2 to 3 h crating time, 1 h transport and 1 h lairage this makes a total of 9 to 10 h of feed deprivation. Much longer time have been reported **(Warris *et al.,* 1990),** which will lead to a reduction in carcass yields **(Veerkamp, 1986).** Under this experiment, the blood glucose and total protein levels were reported to increase slightly in birds that transported without treatment if compared with treated groups. Deprivation of feed and water has been shown to cause depletion of glycogen in the liver, which is the primary store available for maintaining blood glucose levels, as early as after three hours of fasting **(Warriss *et al.,* 1999 & 2005).** These results are in agreement with that recorded by **Wal *et al.* (1999)** who proved that the breakdown of liver glycogen prevents large changes in blood glucose levels. As, feed deprivation for 5 h before transport resulted in depletion of glycogen stores in the liver as indicated by liver pH at time of slaughter. This subsequently resulted in a 15% lower blood glucose level at the time of slaughter on day 1 in chickens that had fasted. This reduction is consistent with previous studies and explained by prolonged deprivation of feed and water exhausts the energy stores of the animal, and thereby its capability to cope with stressful situations **(Nicol and Scott, 1990).** The results showed that H/L ratio, T_3 and T_4 levels were increased in the control group in comparison with other treated groups. The heterophil-lymphocyte ratio has often been used as an indicator of stress **(Zhang *et al.,* 2009).**

Table 2. Effect of transportation on biochemical indices and H/L ratio in broilers.

	C	Treatment			
		V	FW	BL	D
Glucose (mg/dl)	45± 0.023[b]	41± 0.022[c]	50± 0.021[a]	38± 0.034[c]	36± 0.022[c]
Total Protein (mg/dl)	3.30± 0.023[b]	2.33± 0.015[c]	3.40± 0.019[a]	2.01± 0.022[c]	1.77± 0.015[d]
H/L ratio	0.94± 0.014[b]	0.78± 0.013[c]	0.96± 0.018[a]	0.68± 0.025[c]	0.51± 0.026[d]
T3 (ng/ml)	0.74± 0.016[b]	0.55± 0.022[c]	0.84± 0.014[a]	0.52± 0.019[c]	0.28± 0.013[d]
T4 (mg/dl)	1.21± 0.024[a]	0.80± 0.019[c]	1.25± 0.017[b]	0.70± 0.018	0. 61± 0.017[c]

*Means, in the same row, which superscript with different small letters (a, b, c,…..) differ significantly at (P<0.05)

Physiological and behavioral responses to transport indicate that this is probably stressful to the animal. Dehydration may be harmful to the bird, but does not seem to induce a stress response. Respiratory and heart rates, which are homeostatically regulated in avian species, have also been demonstrated to be affected by transportation **(Strawford *et al.,* 2011).** The obtained data for RR and heart rate were increased by transporting. On the other hand, the broiler in the treated groups recorded the lowest values for these parameters. These findings indicate that the physiological mechanisms are insufficient to cope with transport stress. While this may represent a welfare issue, it is also related to the additional energy demand imposed on poultry transported in adverse climatic conditions which associated with decreases in blood glucose and muscle glycogen reserve, both indicators of energy availability **(Dadgar *et al.,* 2012).** Besides, tonic immobility duration (sec) was increased after transportation in the control group if compared with treated groups (Table 3). This may be due to transport stress and rising microclimate elements during transportation have reliably resulted in an increased H/L ratio, via the effects of adrenocorticotropic hormone **(Altan *et al.,* 2003).**

Table 3. Effect of transportation on TI and some physiological parameters in broilers.

	C	Treatment			
		V	FW	BL	D
Respiratory rate (Cycle/min)	77± 0.017[a]	67± 0.015[b]	78± 0.023[a]	56± 0.019[c]	46± 0.016[d]
Heart rate (Beat/min)	164± 0.016[a]	142± 0.018[b]	165± 0.014[a]	131± 0.019[c]	128± 0.015[c]
TI Duration (Sec)	94.3± 0.013[b]	71.6± 0.021[c]	99.0± 0.014[a]	62.3± 0.017[c]	47.0± 0.020[d]

*Means, in the same row, which superscript with different small letters (a, b, c,…..) differ significantly at (P<0.05)

In conclusion, under this experiment, using of darkness, blue light and adding vitamin E & selenium in the drinking water before the transportation process, help in improving from the harm effects of transportation stress on broiler in comparison with feed withdrawing**.**

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