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Physiology

Effect of Antistress Formula on Alleviation of Uninterrupted 900 Km Transport Stress Impacts in Japanese Quail (*Coturnix japonica*)

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ABSTRACT

Effects of long transportation (~ 900 km - Egypt) stress with or without the use of antistress formula on H/L ratio, stress hormones and plasma antioxidant enzymes were carried out in Japanese quail. The transported quail separated into two groups: G2 was the non-treated transported group, G3 was the treated group (given the antistress formula 48 h before and 24 h after transportation). The samples of blood collected before (G1) and after (G2 & G3) transportation. The G2 significantly had a higher heterophils %, H/L ratio, T4, malondialdehyde level, and corticosterone hormone concentration immediately on arrival and 24 h after transportation compared to G3. On the other hand, the results of the G3 significantly had a higher lymphocyte % and glutathione level immediately on arrival and after 24 h after transportation compared to the untreated group (G2). The total quail death throughout 24 h after 900 Km transportation were 21% (210 quail) for the G2 and 4% (40 quail) for the G3. The results indicate that the SH- antistress formula can help quail to better resist long-term transportation disorders which were reflected on the quail's physiological stability and avoid economic losses of poultry industry.

Keywords: Antioxidant, H/L ratio, Hormonal profiles, Mortality rate, Quail, Transportation stress.

1.INTRODUCTION

Concerns about the welfare issues linked with broiler chicken harvesting and transportation are developing. Broilers are subjected to a variety of stress and fear-inducing conditions before being slaughtered (Večerková et al., 2019). Stress is defined as a series of reactions to any external stimuli that result in the poultry flock's unexplained situations (Horváth and Babinszky, 2018). Because of the growing request for Japanese quail (*Coturnix japonica*) as laboratory and farm birds, the transportation of the birds to other research laboratory, ranches, or abattoirs has become extensive. However, there may be negative effects of transportation on the birds' wellbeing, production, meat quality, and investigational outcomes (Mehraei et al., 2016). Stress was found to be the cause of 40% of broilers on arrival deaths (Arikan et al., 2017) with the mortality rate increasing as the transit duration lengthened (Mousa-Balabel et al., 2017). The transport of living animals, which is an unavoidable part of animal husbandry, has been documented as one of the important sources of anxiety (Youssef et al., 2015). Transport stressors include management, lading, fasting, quarantine, spiral forces, sharp change in lighting conditions, inferior quality of air, and blending of foreign groups (Youssef et al., 2015). The

All procedures of birds' management as well as collection and discarding of samples applied according to the principles of Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, University of Sadat City, Egypt (Ethical approval number: vusc-006/1-21).

2.2. Diets

Quail fed with a starter diet (3030 kcal of ME/kg, 22% crude protein) from 1^{st} to 20^{th} day and finisher diet (3180 kcal of ME/kg, 19% crude protein) from 21^{th} to 35^{th} day. The experimental diets formulated to subtend the nutritious requirements for Japanese quail as suggested by the National Research Council (Bailey, 2020).

2.3. Antistress Formula

The present study was added the antistress formula (the commercial name is SH-Stress) to the drinking water (2 g L^{-1}) of group G3 for 48 h just before transportation and for 24 h after arrival at the endpoint of the journey (Aswan province). SH-Stress is an integrated product that contains a highly concentrated mixture of 7 ingredients; vitamin C (100g), betaine (69g), sorbitol (50g), ammonium chloride (300g), sodium sulfate (100g), potassium chloride (60g), and dextrose up to 1000g of SH Stress powder (Manufactured by Egyptian Veterinary Industries Company (EVICO- Sadat City- Menofia- Egypt) for Al Shorouk – Egypt Company for the drug trade, export, and import, Egypt).

2.4. Transportation stress and experimental grouping sampling

On day 35, the quail exposed to catching, handling, crating in plastic boxes (50 birds/crate), lading, and transporting for a 900 km journey (nearly 12 h) by a van cargo truck without a cargo box or any of active ventilation. The average speed of journey was 70 Km h^{-1} from Menofia province to Aswan provinces (900 km), Egypt. In Menofia and Aswan, the average high temperature in March (2021) was 24.0 & 29.5°C, respectively. The average low temperature is 12.0 &13.8°C, while the average relative humidity is 48 & 24 %, correspondingly. On arrival after transportation, quail kept in separated pens (2 m^2). The quail were divided into 3 groups:

G1: Negative control (before transportation and no treatment) sedentary group.

G2: Transport stress control (not given antistress formula) group.

increase of adrenal cortex activity, lower immune resistance, increased disease spread and death owing to contagious diseases, poor flesh quality, and emaciation are all physiological impacts of transportation stress (Samimi et al., 2019).

Numerous researches have been conducted to describe the behavioral and physiological responses of birds to transportation. For instance, measurements of tonic immobility, plasma corticosterone concentrations, heart rate, heterophils/lymphocytes (H/L) ratio, and changes in rectal temperature were used in certain research (Vahdatpour, 2018) via making a comparison of plasma corticosterone concentration and H/L ratio in poultry. Fidan et al. (2017) found that the H/L ratio is a more valuable measure of stress. Numerous studies on chickens have been conducted, with the majority of these studies focusing on the addition of antioxidant vitamins, electrolytes, probiotics, and sedatives in hens (Anoh et al., 2018) or the addition of betaine and vitamin C in quail (Egbuniwe et al., 2021).

The aim of this work was planned to investigate the effect of anti-stress formula "SH-Stress" water supplementation on some quail blood parameters and mortality rates when they were transported by road from cold (Menofia) to hot (Aswan) climatic zones of Egypt for uninterrupted long-term transportation (~900 km).

2. Materials and Methods

2.1. Birds and housing

Two thousand 1-day-old Japanese quail were obtained from local commercial hatcheries of Kafr El-Sheikh province (Egypt) and randomly divided into two groups (1000 quail/group); then, each group was separated into three replicates and each replicate was housed in separated pens (20 m^2). For making a deep litter system, we used wood shavings as the litter material. On the first day, the environmental temperature was 36°C, then and this was decreased to 25°C till the end of the study. Twenty-three h of light was provided for the quail in the first week and reduced to 20 h at the end of the experiment. The experimental birds were kept and maintained till marketing weight (35 days) under similar hygienic and environmental conditions. Feed and water were offered *ad libitum*.

fortified with Gen5 software at wave length of 450 nm (Biotek, Winooski, VT).

2.6. Determination of mortality rate percentage:

The percentage of mortality rate obtained from the following equation:

$$\text{Mortality rate \%} = \frac{A - B}{A} \times 100$$

Where, **A** = Number of the quail before transportation and **B** = Number of the live birds after transportation (0.0 h & 24 h).

2.7. Statistical Analysis

The results are provided as mean ± SE, and the statistical analysis of data was performed using the one-way analysis of variance (ANOVA) followed by Duncan test using the SPSS program. The results considered as statistically significant when the probability level less than 0.05 (Mishra et al., 2019).

3. Results

3.1. Heterophils / lymphocytes (H/L) ratio

As shown in Tables 1 and 2, the supplementation of quail of G3 with antistress formula (SH-Stress) 48 h before transportation and 24 h after arrival endpoint showed a significant decline ($p < 0.05$) in heterophils %, H/L ratio, T4, and corticosterone hormone concentrations compared to the untreated group (G2) and nearly returned to the pre-transport values of G1.

On the other hand, the supplementation with antistress formula treated group (G3) before and after transportation showed significant ($p<0.05$) higher lymphocyte % at both immediately on arrival endpoint 0.0 h and 24 h after transportation compared to the untreated group (G2). The plasma concentration of T3 before or after transportation (0.0 h and 24h) in both G2 and G3 recorded a non-significant ($p > 0.05$) changes as revealed in Table 2.

G3: Transport stress and treated group (given the antistress formula for 48 h before and 24 h of transportation at a rate of 2.0 g L^{-1} of drinking water as recommended by the supplier.

2.5. Blood sampling and analysis

Random blood samples of 10 birds of each group collected by venipuncture of the wing in tubes containing EDTA before (G1) treatment and transportation of quail. Also, blood samples were collected immediately at the time of arrival and 24 h after arrival for G2 and G3. Two blood drops obtained and two blood films prepared per sample. Blood films stained using Giemsa stain, and heterophils (H) and lymphocytes (L) count were recorded per 100 leucocytes (Scanes, 2016). The division of the heterophils number by the lymphocytes number recorded as H/L ratio. The means calculated per bird sample through counting of both slides. The collection of plasma samples carried out by centrifugation (3000 rpm/30 min) and stored at -80 ºC until analysis. It is recommended that the same individual collect all the samples and bird's excitation was prevented by all efforts.

The levels of malondialdehyde (MDA) in plasma were measured by spectrophotometer using the Bio-Diagnostics Kits (CAT. No. MD 2529) according to the procedure designated by Khoubnasabjafari et al. (2015). Also, the levels reduced glutathione (GSH) in plasma were measured by spectrophotometer using the kits of Bio-Diagnostics (CAT. No. GR 2511) according to Abdel-Daim et al. (2016). Plasma concentrations of T4 (µg/dl), T3 (ng/mL), were measured by radioimmunoassay (Abdel-Daim et al., 2016). The concentrations of corticosterone (ng/mL) were measured using an ELISA kit (Catalog No. ADI-900-097, Enzo life sciences, Farmingdale, NY) according to the directions of the manufacturer. The samples absorbance was read using a Biotek Synergy H1 plate reader

Table 1. Effects of the transport stress on heterophils/lymphocytes (H/L) ratio in quail (mean ± SE), *n*=10.

Parameters	Before Transportation (G1)	After Transportation			
		* 0.0 h after		24 h after	
		Untreated group (G2)	Treated group (G3)	Untreated group (G2)	Treated group (G3)
Heterophils %	20.48 ± 0.48^{c}	37.38 ± 0.62^{a}	31.26 ± 0.56^{b}	31.32 ± 0.63^{b}	21.16 ± 0.53^{c}
Lymphocytes %	76.82 ± 0.55^{a}	51.63 ± 0.71^{d}	57.54 ± 0.67^{b}	54.57 ± 0.70^{c}	77.28 ± 0.61^{a}

H/L ratio	00.27±0.01[c]	00.73±0.03[a]	00.54±0.01[b]	0.57± 0.01[b]	00.27 ± 0.01[c]

In the same row, means ± SE with different letters superscripts are significantly different at P < 0.05. * 0.0 h after transportation= immediately on the arrival endpoint of transportation.

3.2. Hormones profile

Table 2. Effects of the transport stress on some plasma stress hormones concentrations in quail (mean ± SE), n=10.

Parameters	Before Transportation (G1)	After Transportation			
		* 0.0 h after		24 h after	
		Untreated group (G2)	Treated group (G3)	Untreated group (G2)	Treated group (G3)
Corticosterone (ng/mL)	1.04±0.07[c]	1.96±0.09[a]	1.38±0.08[b]	1.22±0.08[bc]	1.07 ± 0.03[c]
T4 (µg/dL)	2.98±0.13[c]	4.17±0.15[a]	3.48±0.15[b]	3.52 ± 0.15[b]	3.32±0.16[bc]
T3 (ng/mL)	1.16±0.08[a]	1.09±0.05[a]	1.09± 0.06[a]	1.13± 0.07[a]	1.04±0.05[a]

In the same row, means ± SE with different letters superscripts are significantly different at P < 0.05. * 0.0 h after transportation= immediately on the arrival endpoint of transportation.

3.3. Antioxidant activity

Table 3 demonstrates the effects of antistress prescription (contains AA and betaine) supplementation on the antioxidant activities [glutathione and malondialdehyde - MDA "lipid peroxidation biomarker "] in long transportation stressed quail. Primarily, a rise ($p < 0.05$) in the MDA level in addition to a sharp decline ($p < 0.05$) in glutathione content were manifest in the control untreated quail either on endpoint arrival (13.32-30.49) or 24 h (11.66-31.11) of transportation journey relative to recorded values before (G1) transportation (9.77-36.20), respectively. Controversy, the levels of reduced glutathione were significantly increased ($p < 0.05$) in quail groups that were supplemented with antistress prescription either on endpoint arrival or 24h of transportation trip compared with those in control untreated group. In contrast, the MDA levels showed a significant ($p < 0.05$) decrease in antistress treated group relative to the untreated ones (G1) and returned to recorded values before transportation (G1).

Table 3. Effects of the transport stress on some plasma antioxidant enzymes concentrations in quail (mean ± SE), n=10.

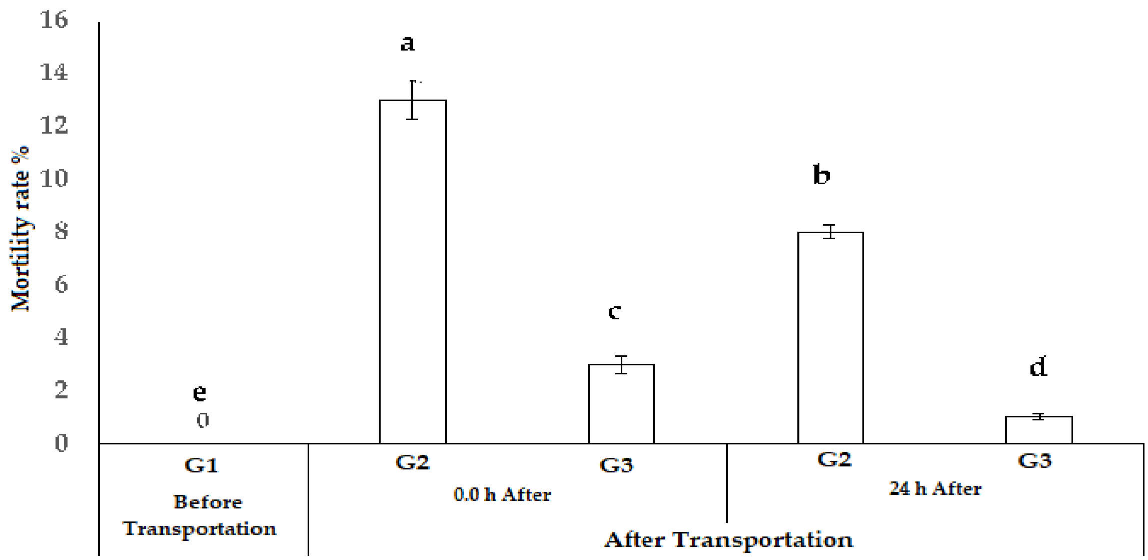
Parameters	Before Transportation (G1)	After Transportation			
		* 0.0 h after		24 h after	
		Untreated group (G2)	Treated group (G3)	Untreated group (G2)	Treated group (G3)
Malondialdehyde (mmol mL^{-1})	9.77±0.17[c]	13.32±0.43[a]	10.94±0.34[bc]	11.66±0.82[b]	10.18±0.44[c]
Reduced glutathione (mg dL^{-1})	36.27±1.43[a]	30.49±1.10[b]	35.42±0.94[a]	31.11±1.13[b]	36.26±1.13[a]

In the same row, means ± SE with different letters superscripts are significantly different at P < 0.05. * 0.0 h after transportation = immediately on the arrival endpoint of transportation.

3.4. Mortality rate percent

The data obtained in Figure 1 showed that the antistress formula supplemented group (G3) lower strikingly ($p<0.05$) the mortality rates % immediately on arrival (3.00 ± 0.34%) and 24 h (1.03 ± 0.11%) after transportation compared with the untreated transported (G2) one (13.00 ± 0.73% and 8.03 ± 0.27%, respectively). The total quail death throughout 24 h after 900 Km transportation was 21% (210 quail) for the non-treated group (G2) and 4% (40 quail) for the treated group (G3).

Figure 1. Effects of the transport stress on mortality rates % in quail (mean ± SE), * n=3.



Mean ± SE with different letters superscripts are significantly different at ($P < 0.05$). * Average number of dead chicks per replicate, i.e. 3 values per group (n=3), 0.0 h after transportation = immediately on the arrival endpoint of transportation. G2 = Untreated group, G3 = Treated group.

4. DISCUSSION

4.1. Heterophils/lymphocytes (H/L) ratio

The rapid H/L responses have been occurred in chickens after road journey (Habibian et al., 2014). Furthermore, Compendio et al. (2018) confirmed that H/L ratios can be increased within 24 - 48 h in fowls in reply to strains and reach its peak after 20 h. The results of the present study showed that the antistress formula-treated group (G3) showed a significant decrease ($P < 0.05$) in H/L ratios than the other group (G2) at both 0.0 h and 24 h post-transportation to the endpoint. The H/L ratio of the treated group after 24 h of transportation was reverted to the same value before transportation (0.27). Correspondingly, Compendio et al. (2018) reported that pretreatment of broiler chicks with vitamin C reduced H/L ratio responses to different types of stress (gathering, crating, and transport). There is a clear explanation of the vitamin C blocking effect on adrenal steroidogenesis (Gouda et al., 2020) which, was reflected in our research through lowering H/L ratios in quail supplemented with antistress formula when compared with control non-treated quail at both on arrival and after 24 h post-transportation to the endpoint (900 km).

Antistress formula contains a large amount of vitamin C (10%) with other synergistic ingredients as "betaine, sorbitol, and ammonium chloride……". Vitamin C (VC) is probably the most vitamin that have been studied for homeostasis distress in the poultry industry. There is considerable indication to recommend that the treatment of poultry with VC under field and laboratory conditions may augment yield, immunity, disease fighting, and livability under stressful circumstances (Njoku et al., 2017). Abo-Al-Ela et al. (2017) concluded that the avian species have the ability to synthesize VC, thus the VC supplementation generally thought to be needless. Nevertheless, environmental stress causes a visible rise in VC necessities, and the ability of the birds to produce adequate VC to substitute the significant losses of this vitamin during stress (Čolović et al., 2019). Consequently, VC supplementation might help overcoming any lack and augment resistance to stresses. Paredes et al. (2020) assumed that the adding of VC to drinking water of broiler chickens diminished the adrenal cortex response to the shocking practice of gathering and transport. Fidan et al. (2017) reported that the changes in plasma corticosterone concentration and H/L ratio responses is a good index of long-lasting stress in poultry when comparing plasma corticosterone concentration and H/L ratio responses to different stressors. Jaiswal et al. (2017) has reviewed the accuracy of the H/L ratio as a biological indicator of stress in avian species. Additionally, a solid connection between VC treatment and H/L ratio response has been reported (Shojadoost et al., 2021).

As an osmolyte, Betaine keep the cellular water equilibrium in birds to prevent cellular and tissues dehydration and osmotic inactivation through helping water holding in the body as well as maintaining both the energy balance and feed intake. The bird's maintenance energy requirement is then reduced, despite osmotic stress and more energy is available for growth and production. El-Moniary et al. (2019) described that the broiler diets which have been supplemented with betaine improved the broilers performance significantly during heat stress. Daudu et al. (2020) revealed that the increasing of betaine levels in duck diets linearly improved the average daily gain and feed: gain ratio. The quail diet that has been supplemented with betaine at 0.06 and 0.12% levels augmented the utilization of nutrients and ameliorate performance and production (Ratriyanto et al., 2017). Betaine supplementation increases nutrient digestibility owing to its osmoprotective characteristics, intestinal cells support and intestinal flora growth (Ratriyanto et al., 2017). So, betaine might be used as a valuable tool to improve bird's resistance to elevated temperatures and diminish the harmful impacts of heat stress.

4.2. Hormones profile

The stress responses include the endocrine response and the main indicator of stress in different species is cortisol (Dai et al., 2021). As stressful condition, transportation could stimulate the sympathetic nervous system and hypothalamo-pituitary-adrenal axis, which is recognized as stress response and leads to an increase in serum concentration of cortisol (Samimi et al., 2019). Cortisol is produced in the adrenal cortex and is considered as an index of stress in various animal species (Jama et al., 2016). Correspondingly, research by Compendio et al. (2018) concluded that pretreatment with vitamin C lowered the plasma corticosterone responses to different types of stress (harvesting, cooping, and transport) in broilers. The data of the current study revealed that the antistress formula-treated group (G3) had lower ($p<0.05$) plasma corticosterone and T4 hormones concentrations than the other group (G2) at both 0.0 h and 24 h after transportation. In contrast, the serum corticosterone concentration was not changed in response to acute and chronic heat stress in broiler breeders (Mosleh et al., 2018) relative to non-stressed ones. The current study was recorded a non-significant T3 values alteration of the transport stress in quail relative to pre-stressed ones. Contradictory, Mosleh et al. (2018) noted a reduced T3 and increase of plasma glucose of stressed birds which help in fighting acute heat stress in broiler breeders. There is a clear explanation of the vitamin C blocking effect on adrenal steroidogenesis (Gouda et al., 2020). The tested hormones profile has been improved through adding of antistress formula (which contains ammonium chloride and potassium chloride) and this effect may be attributed to the acidic properties of Cl^- ion, which maintains the electrolytes balance in the blood, improves the pH and some of the other blood parameters such as hemoglobin and red and white blood cells count (Saki et al., 2016). This allows the bird to survive under interior stability as through controlling internal biological processes, which is manifested in the thyroid gland function. So, the rise of T4 (to the pre-stressed birds) is a better indicator of the interior stability improvement in comparison with the control (Liang et al., 2016).

4.3. Antioxidant activity

In all types of poultry (including quail), stress has a high concern due to their susceptibility to elevated environmental temperature as result of sweat glands deficiency and high metabolic rates (Liao et al., 2018). Heat stress might also interrupt the oxidation-reduction stability and encourage oxidative stress through reactive oxygen species (ROS) production in broiler breeders (Mishra and Jha, 2018). The adverse effect of elevated environmental temperature and any types of stress on the broiler breeder's performance can be alleviated by several available methods. Due to the elevated costs of buildings cooling, the focus of these methods mostly has applied to the dietary and/or drinking water manipulations. The antioxidant character and the contribution in the antioxidant protection system considered as one of the most important functions of ascorbic acid (AA). Ascorbic acid is a vital micronutrient as it is needed for the normal body metabolic function (Dawood and Koshio, 2018). In particular, AA could guard against oxidative stress impairment via its scavenging activity to free-radical (Bai et al., 2016). Betaine (BET), is a naturally occurring compound with osmoprotective properties, which is vital in the nervous, immune, renal, and cardiovascular systems (Ghasemi and Nari, 2020). Betaine conserves osmotic balance and keeps the tertiary structure of the large molecules in the kidney and others (El-Moniem et al., 2016). Also, it is an important source for the methyl group that is needed for the methionine and S-adenosylmethionine (SAM) synthesis (El-Moniem et al., 2016). During exposure to heat stress, ample oxygen-derived free radicals are generated causing oxidative damage of macromolecules (Waiz et al., 2016). Additionally, catecholamines and corticosteroids are released in excess inducing lipid peroxidative damage (Chainy and Sahoo, 2020). In the current study, the tested anti-stress prescription was supplemented to improve transport stress impacts, antioxidants, and lipid peroxidation in quail. Because vitamin C is located at the membrane level, it contributes actively in the structure of organic compounds and diminishes the oxidative damage and fatty acids and phospholipid components peroxidation (El-Moniem et al., 2016). On the other hand, the methyl donor property of betaine could be partially responsible for its antioxidant activity (Jiang et al., 2019). The results of the present study agreed with the study of Jiang et al. (2019), who concluded that supplementation of betaine and VC were similarly powerful for fractional improvement of heat stress impacts on the performance of slow-growing chicks. Similarly, betaine showed a significant improvement in antioxidant status, digestibility, and growth performance with potency like that of vitamin C in heat-stressed rabbits (Zhang et al., 2016). Betaine might keep the thermos-neutral state of the animal through lowering the heat-induced inhibition of osmotic balance and maintaining the tertiary structure of the large molecules in the kidney and other tissues as result of its osmolyte properties and its property as methyl group donor (Van Every et al., 2021).

4.4. Mortality rate

Many studies have been done on transport stress in broiler to determine the chief factors influencing the rate of mortality and the losses in live weight during transportation between the farms and/or the abattoirs. These factors include catching method (Jacobs et al., 2017), transportation distance and time (Vecerek et al., 2016), crate stocking density (Arikan et al., 2017), and daily average temperature (Arikan et al., 2017). The antistress formula-treated group (G3) of the current study were recorded a lower mortality rate (4%) than the untreated group (G2-21%) at both immediately on arrival and 24 h after 900km transportation. The cause of elevated mortality rate in the untreated transported stressed group (G2) is the long duration of transportation of the birds by road at which the birds may be exposed to many stressors such as temperature changes, movements, trembling, food deprivation, water removal, social disruption, noise, and internal vehicle thermal microenvironment (Jayaprakash et al., 2016). Similarly, Kettlewell et al. (2000) established that the post-mortem investigation of on arrival birds deaths at the abattoir revealed that up to 40% of the birds died from heat stress caused by irregular truck aeration and avian thermoregulation failures. The high occurrence of deaths during transportation is related to bad welfare and it is responsible for a significant loss in the poultry industry (Vecerek et al., 2016). In addition to its effect on meat quality parameters (color, texture, palatability, preservation time …etc.) that could have negative effects on consumer satisfactoriness and poultry products manufacturing (Schwartzkopf-Genswein et al., 2012). The current results indicate that the transport stressed treated group (G3) by the valuable harmonized antistress formula can efficiently aid Japanese quail to tolerate transport stress, which was reflected on the noticeable lower mortality rates.

5. Conclusions

The synergistic constituents of SH-Antistress formula (vitamin C, betaine, ammonium chloride……etc.) offers a better handling practice to diminish transport and heat stress related disorders in Japanese quail, which were reflected on the pathophysiological stability of hematological, endocrine, antioxidant, and mortality rates status, especially during long-distance transportation (900 km) and reduce the poultry industry's economic losses.

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REFERENCES

Abdel-Daim, M., El-Bialy, B.E., Rahman, H.G.A., Radi, A.M., Hefny, H.A., and Hassan, A.M. 2016. Antagonistic effects of Spirulina platensis against sub-acute deltamethrin toxicity in mice: biochemical and histopathological studies. Biomed. Pharmacother., 77: 79-85.

Abo-Al-Ela, H.G., El-Nahas, A.F., Mahmoud, S., and Ibrahim, E.M. 2017. Vitamin C modulates the immunotoxic effect of 17α-methyltestosterone in Nile tilapia. Biochemistry, 56: 2042-2050.

Anoh, K., Atte, P., Olayinka, O., and Abdulahi, I. 2018. Effect of transportation stress on the thermoregulatory response of young and adult rabbits. Niger. J. Anim. Prod., 20, 490-495.

Arikan, M., Akin, A., Akcay, A., Aral, Y., Sariozkan, S., Cevrimli, M., and Polat, M. 2017. Effects of transportation distance, slaughter age, and seasonal factors on total losses in broiler chickens. Braz. J. Poult. Sci., 19: 421-428.

Bai, K., Xu, W., Zhang, J., Kou, T., Niu, Y., Wan, X., Zhang, L., Wang, C., and Wan, T. 2016. Assessment of free radical scavenging activity of dimethylglycine sodium salt and its role in providing protection against lipopolysaccharide-induced oxidative stress in mice. PLoS ONE, 11(5): 1-17.

Bailey, C.A. 2020. Precision poultry nutrition and feed formulation. In Animal Agriculture; Elsevier; pp. 367-378.

Chainy, G.B., and Sahoo, D.K. 2020. Hormones and oxidative stress: an overview. Free Radic. Res., 54: 1-26.

Čolović, R., Puvača, N., Cheli, F., Avantaggiato, G., Greco, D., Đuragić, O., Kos, J., and Pinotti, L. 2019. Decontamination of mycotoxin-contaminated feedstuffs and compound feed. Toxins, 11 (617): 1-18.

Compendio, J.D.Z., Espina, D.M., and Ybañez, A.P. 2018. Simulated transportation stress: Its effect on the productivity and selected biochemical and haematological indices of laying hens. Philipp. J. Vet. Med., 42: 91-101.

Dai, W., Wagh, S.A., Chettiar, S., Zhou, G.D., Roy, R., Qiao, X., Visich, P.S., and Hoffman, E.P. 2021. Blunted circadian cortisol in children is associated with poor cardiovascular health and may reflect circadian misalignment. Psychoneuroendocrinology, 129: 105252, 1-9.

Daudu, O., Kpachi, J., Clement, N., Odegbile, O., Salihu, E. and Ademu, L. 2020. Thermoregulatory, growth and blood indices of broiler chicks fed betaine hydrochloride supplemented diets under high ambient temperature. Niger. J. Anim. Prod., 47, 58-63.

Dawood, M.A., and Koshio, S. 2018. Vitamin C supplementation to optimize growth, health and stress resistance in aquatic animals. Rev. Aquac., 10: 334-350.

Egbuniwe, I.C., Uchendu, C.N., and Obidike, I.R. 2021. Dietary ascorbic acid and betaine improve stress responses, testosterone Levels and some sexual traits in male Japanese quail during the dry season. Exp. Results., 2 (e23): 1–11.

El-Moniary, M.M., Goud, A., Hamouda, Y., Youssef, A.W., and Hassan, H.M. 2019. Research Article Influence of Early Heat Conditioning and Betaine or Ammonium Chloride Supplementation on Performance and Physiological Parameters of Quail Chicks in Hot Climate. Int. J. Poult. Sci., 18: 562-569.

El-Moniem, A., Elham, A., Daader, A., Al-Sagheer, A., and Gabr, H. 2016. Effect of vitamin C, vitamin E or betaine addition on alleviation of heat stress impacts on growing rabbits. Zagazig J. Agric. Res., 43: 1601-1613.

Fidan, E.D., Nazligül, A., Türkyilmaz, M.K., Karaarslan, S., and Kaya, M. 2017. Effects of photoperiod length and light intensity on performance, carcass characteristics and heterophil to lymphocyte ratio in broilers. Kafkas Univ. Vet. Fak. Derg., 23: 39-45.

Ghasemi, H., and Nari, N. 2020. Effect of supplementary betaine on growth performance, blood biochemical profile, and immune response in heat-stressed broilers fed different dietary protein levels. J. Appl. Poult. Res., 29: 301-313.

Gouda, A., Amer, S.A., Gabr, S., and Tolba, S.A. 2020. Effect of dietary supplemental ascorbic acid and folic acid on the growth performance, redox status, and immune status of broiler chickens under heat stress. Trop. Anim. Health pro., 52: 2987-2996.

Habibian, M., Ghazi, S., Moeini, M.M., and Abdolmohammadi, A. 2014. Effects of dietary selenium and vitamin E on immune response and biological blood parameters of broilers reared under thermoneutral or heat stress conditions. Int. J. Biometeorol., 58: 741-752.

Horváth, M., and Babinszky, L. 2018. Impact of selected antioxidant vitamins (Vitamin A, E and

C) and micro minerals (Zn, Se) on the antioxidant status and performance under high environmental temperature in poultry. A review. Acta Agric. Scand. - A: Anim. Sci., 68: 152-160.

Jacobs, L., Delezie, E., Duchateau, L., Goethals, K., and Tuyttens, F.A. 2017. Broiler chickens dead on arrival: associated risk factors and welfare indicators. Poul. Sci., 96: 259-265.

Jaiswal, S.K., Raza, M., and Chaturvedani, A. 2017. Effect of thermal stress on serum biochemical and haematological parameters in broiler chickens. Ind. J. Vet. Sci. Biotechnol., 12: 19-22.

Jama, N., Maphosa, V., Hoffman, L., and Muchenje, V. 2016. Effect of sex and time to slaughter (transportation and lairage duration) on the levels of cortisol, creatine kinase and subsequent relationship with pork quality. Meat Sci., 116: 43-49.

Jayaprakash, G., Sathiyabarathi, M., Robert, M.A., and Tamilmani, T. 2016. Transportation stress in broiler chicken. Int. J. Sci. Environ. Technol., 5: 806-809.

Jiang, Y.-P., Yang, J.-M., Ye, R.-J., Liu, N., Zhang, W.-J., Ma, L., Zheng, P., Niu, J.-G., Liu, P., and Yu, J.-Q. 2019. Protective effects of betaine on diabetic induced disruption of the male mice blood-testis barrier by regulating oxidative stress-mediated p38 MAPK pathways. Biomed. Pharmacother., 120: 109-474.

Kettlewell, P.J., Hoxey, R.P., and Mitchell, M.A. 2000. Heat produced by broiler chickens in a commercial transport vehicle. J. Agric. Eng. Res., 75: 315-326.

Khoubnasabjafari, M., Ansarin, K., and Jouyban, A. 2015. Reliability of malondialdehyde as a biomarker of oxidative stress in psychological disorders. BioImpacts: BI, 5 (3): 123-127.

Liang, H.-M., Lin, D.-Y., Hsuuw, Y.-D., Huang, T.-P., Chang, H.-L., Lin, C.-Y., Wu, H.-H., and Hung, K.-H. 2016. Association of heat shock protein 70 gene polymorphisms with acute thermal tolerance, growth, and egg production traits of native chickens in Taiwan. Arch. Anim. Breed., 59: 173-181.

Liao, X., Li, W., Zhu, Y., Zhang, L., Lu, L., Lin, X., and Luo, X. 2018. Effects of environmental temperature and dietary zinc on egg production performance, egg quality and antioxidant status and expression of heat-shock proteins in tissues of broiler breeders. Br. J. Nutr., 120: 3-12.

Mehraei Hamzekolaei, M., Zamani Moghaddam, A., Tohidifar, S., Dehghani Samani, A., and Heydari, A. 2016. The effects of transportation stress on J apanese quail (C oturnix Coturnix japonica) fed corn-based diet in comparison with wheat-based diet supplemented with xylanase and phytase. J. Anim. Physiol. Anim. Nutr., 100: 618-622.

Mishra, B., and Jha, R. 2019. Oxidative stress in the poultry gut: potential challenges and interventions. Front. Vet. Sci., 6: 60.

Mishra, P., Singh, U., Pandey, C.M., Mishra, P., and Pandey, G. 2019. Application of student's t-test, analysis of variance, and covariance. Ann. Card. Anaesth., 22 (4): 407-411.

Mosleh, N., Shomali, T., Nematollahi, F., Ghahramani, Z., Ahrari Khafi, M.S., and Namazi, F. 2018. Effect of different periods of chronic heat stress with or without vitamin C supplementation on bone and selected serum parameters of broiler chickens. Avian Pathol., 47: 197-205.

Mousa-Balabel, T.M., El-Sheikh, R.A., and Senna, S.E.-S. 2017. Useful managemental practices for mitigating the effect of transportation stress on broiler under Egyptian conditions. Life Sci. J., 14 (11): 57-63.

Njoku, C., Okunniyi, T., Omidiran, I., Adeyemi, O., Sogunle, O., and Oluwatosin, O. 2017. Interactive compensatory response of growing pigs to feed withdrawal periods and ascorbic acid supplementation. Niger. J. Anim. Prod., 19: 173-181.

Paredes, M., Terrones, A., Hoban, C., and Ortiz, P. 2020. Efecto de la suplementación dietaria con vitamina C sobre el rendimiento productivo, estrés y respuesta inmunitaria del pavo criado en un ambiente hipóxico natural. Sci. Agropecu., 11(3): 357– 364.

Ratriyanto, A., Indreswari, R., and Nuhriawangsa, A. 2017. Effects of dietary protein level and betaine supplementation on nutrient digestibility and performance of Japanese quail. Braz. J. Poult. Sci., 19: 445-454.

Saki, A.A., Maleckey, M., Johari, R., Goudarzi, S.M., and Abdolmaleki, M. 2016. The effects of protein, amino acid, and dietary electrolyte balance on broiler chicken performance and blood parameters under heat stress. Acta Sci. - Anim. Sci., 38: 285-292.

Samimi, A.S., Nazifi, S., Tajik, J., Sahebi, B., and Eshteraki, R. 2019. Influence of starvation and transportation on serum galanin and hematobiochemical parameters in goats. Comp. Clin. Path., 28: 137-142.

Scanes, C.G. 2016. Biology of stress in poultry with emphasis on glucocorticoids and the heterophil to lymphocyte ratio. Poul. Sci., 95: 2208-2215.

Schwartzkopf-Genswein, K.S., Faucitano L., Dadgar, S., Shand, P., González, L.A., and Crowe, T.G. 2012. Road transport of cattle, swine and poultry in North America and its impact on animal welfare, carcass and meat quality: A review. Meat Sci., 92: 227- 243.

Shojadoost, B., Yitbarek, A., Alizadeh, M., Kulkarni, R.R., Astill, J., Boodhoo, N., and Sharif, S. 2021. Centennial Review: Effects of vitamins A, D, E, and C on the chicken immune system. Poul. Sci., 100 (100930): 1-17.

Vahdatpour, T. 2018. Effects of feed additives on biochemical and immunological indices of blood and performance of Japanese quail (Coturnix coturnix Japonica). Anim. Prod. Sci., 9: 40-51.

Van Every, D.W., Plotkin, D.L., Delcastillo, K., Cholewa, J., and Schoenfeld, B.J. 2021. Betaine Supplementation: A Critical Review of Its Efficacy for Improving Muscle Strength, Power, and Body Composition. Strength Cond. J., 43: 53-61.

Vecerek, V., Voslarova, E., Conte, F., Vecerkova, L., and Bedanova, I. 2016. Negative trends in transport-related mortality rates in broiler chickens. Asian-australas. J. Anim. Sci., 29: 1796-1804.

Vecerek, V., Voslarova, E., Francesca Conte, F., Vecerkova, L., and Bedanova, I. 2016. Negative trends in transport-related mortality rates in broiler chickens. Asian Australas. J. Anim. Sci., 29 (12): 1796-1804.

Večerková, L., Voslářová, E., and Večerek, V. 2019. Comparison of the welfare of laying hens, broiler chickens and turkeys in terms of bird health as surveyed during inspection in slaughterhouses. Acta Vet. Brno., 88: 243-248.

Waiz, S.A., Raies-ul-Haq, M., Dhanda, S., Kumar, A., Goud, T.S., Chauhan, M., and Upadhyay, R. 2016. Heat stress and antioxidant enzyme activity in bubaline (Bubalus bubalis) oocytes during in vitro maturation. Int. J. Biometeorol., 60: 1357-1366.

Youssef, C., Rita, B., Kaoutar, B., Islah, L., Abdarrahmane, B., and Bernard, F. 2015. Impact of transport distance on stress biomarkers levels in dromedary camel (Camelus dromedarius). Emir. J. Food Agric., 27 (6): 507-512.

Zhang, M., Zhang, H., Li, H., Lai, F., Li, X., Tang, Y., Min, T., and Wu, H. 2016. Antioxidant mechanism of betaine without free radical scavenging ability. J. Agric. Food Chem., 64: 7921-7930.