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COMPARATIVE EFFECT OF VITAMIN COMPLEX AND ORANGE EXTRACT ON PHYSIOLOGICAL AND BLOOD PARAMETERS OF TRANSPORTED PULLETS IN HUMID TROPICS

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ABSTRACT: The comparative modulating effects of synthetic and natural source of ascorbic acid (AA) were investigated on transporting pullet birds in the hot-dry season of humid tropics. Ninety-six 16 weeks Isa-brown pullets were randomly allotted in a completely randomized design into four treatments of oral vitamin supplementation 5 days before transportation i.e; T_1 (ordinary water), T_2 (synthetic vitamin), T_3 (30% citrus-sweet orange), T_4 (50% citrus-sweet orange). Birds were crated and transported for 3 hrs covering 135km at 45km/hr. Meteorological values were monitored during the journey and no mortality was recorded. The results revealed that treatments had a significant effect ($p < 0.05$) on measured physiological parameters [body temperature (BTC), rectal temperature (RTC), respiratory rate (RR) and panting rate (PR)], hematological parameters and measured serum biochemical parameters as compared to the control group (T_1). The treatments group of orange at different inclusion ratios (T_3 and T_4) compared well with pullet birds on oral supplementation of synthetic vitamin (T_2) and were significantly different ($p < 0.05$) from birds on control water treatment (T_1). Birds on control (T_1) had the highest values for all measured physiological parameters which were significantly different from other groups ($p<0.05$). Birds in the control treatment (T_1) were more stressed as compared to other treatment groups, indicated by increased hematological and serum biochemical parameters except for a decrease in hemoglobin (Hb) as compared to other treatments. It can be deduced from this study that the oral supplementation of natural source of ascorbic acid (*Citrus sinensis)* and synthetic vitamin supplement helps to ameliorate the effect of transportation stress. *Citrus sinensis* extract can be a suitable alternative that is readily available for farmers and stakeholders.

Keywords: Ascorbic acid, Physiological measures, Pullets, Synthetic vitamin, Transportation stress.

INTRODUCTION

Livestock transportation is an important husbandry protocol during their lifecycle. Livestock are transported for different reasons such as reproduction, feeding, slaughtering, restocking, etc. (Shearer, 2021). The increase in demand for animal proteins to meet the growing global population and industrialization necessitated transportation across different ecological/climatic zones. Road transportation presents as the most popular means of transportation, which is capable of inducing stress directly affecting production in terms of both economy and welfare (Minka and Ayo, 2009, Bhatt et al., 2021)

The transportation of food animals on road can impair the optimum physiological condition through the changes in blood biochemical composition, muscles biochemical reactions, adverse effect on meat quality, loss in body weight, the spread of infectious diseases, increased morbidity, and subsequently mortality (Minka and Ayo, 2007; Minka and Ayo, 2009). Transportation-related stressors such as; handling, restraining, feed withdrawal, loading, unloading, vehicle vibration, ventilation, and transportation time, can also contribute to an adverse health condition which can increase the degree of pathogen infection/physiological disruption in subclinically infected animals (Ritter et al., 2009; Adenkola and Ayo, 2009a, Bhatt et al., 2021)

The prevailing climatic conditions of the immediate environment during transportation are an important factor capable of contributing to transportation stress (EFSA Panel on Animal Health and Welfare, 2022). Prolonged heat or cold stress is a stressor detrimental to the health of transported animals (Avero et al., 2008, Nielsen et al., 2011). Young and adult animals are likely to be more susceptible to extreme climatic conditions i.e. temperature (Lewis and Berry, 2006). The Onboard climatic control with appropriate densities during the transportation is a tool to achieve age-species specific transportation regime for effective animal welfare (Nielson et al., 2011, Ayoola et al., 2020).

The poultry industry is contributing immensely to the World economy, and a high in demand for major poultry products has led to the transportation of live birds (Minka and Ayo 2011, Lalonde et al., 2021). Although the awareness and establishment of standard regulation for transportation are in many countries (FAWC, 1992, EU, 2004), its strict

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compliance by farmers and relevant stakeholders is yet to be effective (Minka and Ayo, 2009, Jayaprakash et al., 2016). The bulk of previous studies on transportation stress are on broiler chickens, they are typically transported once in a production cycle for slaughter. The stress factor most often studied with transportation is thermal stress. Chickens are homeotherms, as a primary response to thermal stress, birds can modify their behavior to conserve or dissipate heat (Gou et al., 2021). If the bird continues to be subjected to heat stress, then physiological changes may probably be initiated.

Applications of amino acids, tranquilizers, and electrolytes have been reported to suppress stress in food animals (Ayo et al., 2005, Ahmadu, et al., 2016). However, the residual effects of a few of these substances contradict legislative regulations (Jacobs et al., 2017, Hussnain et al., 2019). Therefore, a need to source readily available, relatively cost-effective, reliable and non-toxic prophylactic material to ameliorate the adverse effect of road transportation stress is imminent.

Organic agriculture involves utilization of natural materials such as orange pulp, honey, *Telfairia occidentalis* leave extract etc. contribute to animal welfare as against the use of inorganic products in livestock production. *Citrus Sinensis* (sweet orange) is said to have a good content of Vitamin C (ascorbic acid) (Etebu and Nwauzoma, 2014). Vitamin C an active ingredient in orange extract is responsible for the biosynthesis of Corticosterone, a hormone that enhances energy supply during transportation stress. The ameliorating effect of Vitamin C is evident when the body's supply of ascorbic acid is depleted due to stress factors (Deters and Hansen, 2020).

The evaluation of sweet orange extract as oral supplements, a suitable alternative for synthetic vitamin C during road transportation of point of lay pullet birds belonging to Isa brown breed will be investigated. The research is aimed at evaluating the effect of transportation stress on physiological parameters, hematological, and serum indices with or without conventional vitamin and supplements, and orange juice at the varied concentration on pullet birds.

MATERIALS AND METHODS

Ethical approval

This study was carried out in accordance with the ethical regulation and approval of Bowen University research and ethical committee BUREC/2022/010. All animals used are in strict adherence to the guidelines.

Study area

The study was carried out at wonderful springboard farms, a commercial poultry farm, situated in Ibadan, Oyo State, southwest of Nigeria. The study area is on coordinate latitude 7°3047.7"N3°59'06.3"E.

Birds and management

One hundred and fifty pullets of Isa brown strain were obtained as day-old chicks from a reputable hatchery. They were raised following strict management practices and fed *ad libitum* with feed and water until they attained 12 weeks old. A total of 96 healthy pullet birds of body weight range (1.0 ± 0.1 kg) were selected and randomly allocated into oral supplementation treatment groups. Birds were allotted to four treatment groups; T_1 (ordinary water), T_2 (synthetic vitamin), T_3 (30% citrus-sweet orange), T_4 (50% citrus-sweet orange) for five days before loading and transportation. On the day of transportation, loading and crating were done carefully. Birds were crated according to treatment groups with 8 birds allotted to each crate at 0.25 m^2 / birds and replicated thrice for each treatment. Management practices such as vaccination, medication etc., were done according to (NVRI), Jos, Nigeria.

Experimental Unit

The experimental unit comprised of 16 weeks old Isa Brown birds. Birds were arranged into four oral treatments; water, conventional vitamin supplement, orange juice (30 ml/l of water), orange juice (50ml/l of water) with treatments administered for five days before transportation. The experimental birds were fed ad libitum and each treatment was replicated thrice.

Vehicle design and journey duration

A Toyota Hiace bus was used for the journey. The vehicle has an inner floor dimension of 4 × 1.2 m, with an iron roof top pilled with insulated foam and rug material. The vehicle floor was of metal, covered by a rug and foam underlay. The vehicle windows made of glass, located on both sides, with a sliding design for ventilation. The windows were opposite each other, placed at 800 cm measured from the inner floor, at (50×35 cm each) which allows adequate ventilation. Transportation crates containing the birds were carefully loaded to ensure similar conditions within the bus for all treatments and replicates (Buckham et al., 2008, Minka and Ayo 2011). The vehicle traveled on a mixture of un-tarred (rough) tarred roads for 3 hours, travelled through for 135 km with an average speed of 45 km/h.

Meteorological data

The ambient temperature (AT) and relative humidity (RH) were recorded at intervals for both inside and outside of the vehicle using a wet- and dry–bulb thermometer (Brannan, England). The average AT and RH outside the vehicle was 37.5^0 C and 54.7%, while inside the vehicle were 38.8^0C and 63.4%. The average wind speed during the journey was 37^0C.

The temperature humidity index (THI) was calculated using:

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THI = 0.8*T + RH*(T-14.4) + 46.4 (Scope et al., 2002)

Where T = ambient or dry-bulb temperature in °C

RH=relative humidity expressed as a proportion i.e. 75% humidity is expressed as 0.75

THI (Outside) the vehicle = 0.8*37.5 + 0.547* (37.5 – 14.4) + 46.4 = 89.04

THI (Inside) the vehicle = 0.8*38.8 + 0.634* (38.8 – 14.4) + 46.4= 92.91

Physiological data

The respiratory rate (RR), panting rate (PR), Body temperature (BR), and rectal temperature (RT) were measured for initial and final periods; before loading and transportation (pre-transportation) and immediately after the end of the journey (post-transportation). The physiological parameters were described as adapted from Oguntunji et al. (2019).

Hematological and serum biochemical data

Blood samples were collected before loading and transportation (pre-transportation) and immediately after the journey (post-transportation). 4 ml of the blood sample was collected via the wing vein. 2 ml of blood sample was collected in a heparinized bottle for hematology parameters while a plain container, left to clot was used for serum biochemical analysis. All blood samples were analyzed for hematology and serum biochemical according to Odere et al., (2004).

Statistical analysis

The physiological, hematological, and biochemical parameters were analyzed with the general linear model as follows: Yij =μ + tTi +Pj + eij

Yij = individual observation; μ = fixed effect of genotype; eij = experimental error

Statistical differences between means were determined using new Duncan multiple tests at a 5% probability level

RESULTS

The results of the effect of the treatments on physiological parameters as presented in Table 1. The treatment effect had ($p<0.05$) effect on the investigated physiological parameters. The treatments group of orange extract at different inclusion ratios (T_3 and T_4) compared well with pullet birds on oral supplementation of synthetic vitamin (T_2) and were significantly different ($p < 0.05$) from birds on control water treatment (T_1). Birds on control (T_1) had the highest values for all measured physiological parameters which are ($p<0.05$) different from other treatment groups. The period has an effect ($P< 0.05$) on measured physiological parameters. Initial measured parameters were ($p<0.05$) lower as compared to final measurements.

The results of treatment effect on hematological parameters are presented in table 2. The treatment has an effect ($p<0.05$) on measured blood parameters. The packed cell volume and red blood cells of birds on T_1 are significantly higher ($p<0.05$) as compared to other treatments. T_2 – T_4 is not significantly ($p>0.05$) different. T_1 had the lowest hemoglobin value (Hb) which is significantly ($p<0.05$) different from other treatments. Birds transported on water treatment alone (T_1) had the highest value for white blood cell (WBC) and its constituents, which is significantly ($p<0.05$) different from other treatments. The periods had a significant ($p<0.05$) effect on the treatments. Initial measured blood parameters are significantly ($p<0.05$) lower as compared to final values except for hemoglobin where the value is significantly higher ($p<0.05$) as compared to final measured blood parameters.

The results of treatment effects on serum biochemistry of transported birds as presented in table 3 revealed significant ($p<0.05$) effects of treatment on measured parameters. The glucose, aspartate amino transaminase, alanine amino transaminase, and creatinine level of birds on T_1 is higher ($p<0.05$) as across the treatments. The period has significant ($p<0.05$) effects on transported birds irrespective of the treatments. The initial period was significantly ($p<0.05$) lower for all measured serum blood biochemical parameters as compared to the final period.

DISCUSSION

Effect of treatments and period on physiological parameters of transported pullets

The fluctuations in measured physiological parameters have been attributed to variations in ambient temperature, duration of transportation, and vehicle vibration/mode of transportation (Piccione and Caola, 2002, Ayo and Minka, 2005). The measured panting rate (PR), rectal temperature (RT), body temperature (BT), and respiratory rate (RR) are described as the relevant on-spot diagnostic parameters to evaluate the welfare and adaptability of an animal subjected to stressors (Minka and Ayo, 2009, Hussnain et al., 2019, Gou et al., 2021). As reported in table 1, transported pullets with oral supplementation of fresh orange extract at different inclusion ratios (T_3 and T_4) compared well with pullet birds on oral supplementation of synthetic-vitamin (T_2) and were significantly different ($p<0.05$) from birds on control water treatment (T_1). The period also has a significant ($p<0.05$) effect on transported animals for measured parameters, as final measured parameters, are significantly ($p<0.05$) higher than initial.

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Table 1- Effect of treatments and periods on physiological parameter

Parameter	Treatment				Period	
	T_1 Water	T_2 Synthetic vitamin	T_3 30ml/L	T_4 50ml/L	Initial	Final
BTC ^{0}C	37.08±0.25[a]	34.91±0.10[b]	35.85±0.11[b]	34.89±0.13[b]	35.09±0.11[b]	37.77±0.09[a]
RTC ^{0}C	42.69±0.25 [a]	39.59±0.23[b]	40.76±0.23[b]	39.61±0.23[b]	39.79±0.16[b]	42.83±0.19[a]
RR breath/min	26.00±13.09[a]	20.63±14.16[b]	19.75±12.95[b]	19.38±12.68[b]	0.00±0.00[b]	40.38±10.45[a]
PR breath/min	45.50±17.32[a]	40.12±16.30[b]	35.75±15.55[bc]	33.88±14.98[bc]	0.00±0.00[b]	67.63±10.16[a]

[abc] values along the same row with different superscripts are significantly different ($p<0.05$). Where: BTC – Body temperature, RTC- rectal temperature, RR- Respiratory rate, PR- panting rate

Table 2- Effect of treatments and periods on hematological parameters

Parameters	Treatment				Period	
	T_1 Water	T_2 Synthetic-vitamin	T_3 30ml/L	T_4 50ml/L	Initial	Final
PCV (%)	29.25±4.21[a]	25.20±2.13[b]	20.75±1.65[b]	24.20±2.22[b]	23.10±1.99[b]	28.38±1.28[a]
HB (g/dl)	6.50±1.51[b]	8.72±0.58[a]	8.80±0.66[a]	9.36±0.81[a]	14.39±4.77[a]	7.21±0.50[b]
RBC (x10^6uL)	2.69±0.47[a]	2.42±0.24[b]	2.31±0.13[b]	2.46±0.36[b]	1.22±0.23[a]	2.91±0.14[b]
WBC X10^3UL	13925.00±314.58[a]	13450.00±393.07[a]	13325.00±178.54[a]	13500.00±252.98[a]	13355.00±177.865[b]	13900.00±277.10[a]
Platelet	12225.00±2015.56[a]	134600.00±3187.48[a]	132000.00±8755.95[a]	127600.00±4643.27[a]	125700.00±3907.40[a]	13605.00±3190.04[a]
LYM %	69.75±0.63[a]	62.40±1.29[b]	62.25±1.49[b]	64.60±2.23[b]	64.50±1.42[b]	67.75±1.49[a]
HET %	33.75±0.85[a]	28.00±1.38[b]	27.50±1.55[b]	25.60±2.38[a]	27.20±1.47[b]	30.88±1.41[a]
HET/LYM%	0.47±1.34[a]	0.45±1.06[b]	0.43±1.06[b]	0.39±1.06[bc]	0.42±1.03	0.45±0.94
MON %	3.50±0.75[a]	2.80±0.37[a]	3.20±0.65[a]	3.30±0.40[a]	2.90±1.47[b]	3.50±0.44[a]
EOS %	4.75±0.48[a]	4.20±0.37[a]	3.75±1.31[a]	3.80±0.66[a]	3.30±0.54[b]	4.13±0.44[a]
BAS %	0.55±0.25[a]	0.60±0.241[a]	0.00±0.00[a]	0.40±0.24[a]	0.40±0.15[b]	0.41±0.18[a]

[ab] values along the same row with different superscript are significantly different ($p<0.05$)

Table 3. Effect of treatments and periods on serum biochemical indices

Parameters	Treatment				Period	
	T_1 Water	T_2 Synthetic-vitamin	T_3 30ml/L	T_4 50ml/L	Initial	Final
Glucose (mg/dl)	172.00±4.28[a]	141.23±21.43[b]	148.18±11.00[b]	140.03±31.17[b]	139.39±11.67[b]	191.36±8.10[a]
AST (i.u/L)	68.82±4.66[a]	53.99±4.74[b]	55.13±3.22[b]	54.83±4.75[b]	60.40±8.84[b]	66.47±2.76[a]
ALT (i.u/L)	2.50±1.13[a]	1.80±0.55[b]	1.32±0.40[b]	1.25±1.04[b]	1.35±2.17[b]	4.38±0.34[a]
Creatinine (mg/dl)	1.88±0.10[a]	1.08±0.05[b]	0.90±0.04[b]	1.00±0.07[b]	0.95±0.04[b]	1.98±0.06[a]

[ab] values along the same row with different superscript are significantly different ($p<0.05$)

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This result agreed with other authors who reported that road transportation is generally considered a stressor for food animals. In addition, ambient temperature, length of travel, feed/water deprivation; density, and age of birds are factors to be considered (Ritter et al., 2004; Adenkola et al., 2008; Adenkola et al., 2009, Ayoola et al., 2020). Poultry lacks sweat glands, which other mammals use to mitigate thermal load during unfavorable conditions. Therefore, birds adopts panting and increased respiratory rate in an attempt to balance the thermal-induced stress as found in birds under investigation. The report of Oguntunji et al (2019) and Ayoola et al (2020) are per with this study, they reported that when the body temperature of birds rises above the thermal-optimum range due to metabolic activities or environmental conditions, birds' physiology is adjusted to ensure heat dissipation through increased respiratory rate, and panting rate.

The oral supplementation with synthetic-vitamin in this study was found to have an effect ($p<0.05$) on birds, as it suppress the effect of transportation stress on measured physiological parameters. This study was in line with the report of Adenkola et al. (2008) who found that administration of ascorbic acid which is the main component of synthetic-vitamin ameliorates the adverse effect of stress on transported pigs during 8 hours and 4 hours as evident in measured rectal temperature. This study also corroborates Jacobs et al (2017) who observed a reduction in rectal and body temperature of broilers and layers administered ascorbic acid after being subjected to heat stress. Citrus (sweet orange) used in this study compared well as an organic source of Vit C with synthetic- Vit C as observed for T_3 and T_4 with no significant difference ($p>0.05$) as compared to T_2 for all measured parameters. *Citrus sinensis* are rich sources of bioactive compounds, provide about 51% vitamin C and large quantities of carotenoids (Tiwari et al., 2009). To the best of our knowledge, previous reports on the comparative application of orange extract with synthetic vitamin C to reduce transportation stress in livestock are scarce, therefore, this study serves as one of the leading reports.

Effect of treatments and period on hematological parameters of transported pullets

In the report of Scope et al. (2002) on pigeons, it was reported that measured total leucocyte value had a progressive increase with length of travelling and peak at the end of transportation. Ayo et al (2006) and Minka and Ayo (2008), reported that transportation stress increases the H/L ratio, erythrocytes, hematocrit, and leucocytes as resulted from transportation stress, especially thermal stress induced by transportation in humid tropics.. In table 2, the measured value of packed cell volume (PCV), red blood cell (RBC), and white blood cell (WBC) were significantly increased ($p<0.05$) across the treatments post transportation. T_1 had the highest value which is significantly ($p<0.05$) different as compared to other treatments. Hemoglobin (Hb) value decreased significantly ($p<0.05$) across the treatments with T_1 having the least value post transportation.

The results of this study agreed with Minka and Ayo (2008) who found a significant ($p<0.05$) increase in hematocrit and total protein values of adult ostrich and attributed the changes to dehydration. Similarly, Adenkola and Ayo (2009) reported that the value of PCV can increase due to dehydration or splenic contraction due to transportation stress. However, our report differs from Tadich et al. (2005) and Deepanshu et al (2020) reported that PCV decreased after loading and subsequent transportation. Also, Cockram (2022) reported a low PCV for transported animals, they concluded that stressors can cause actual, than apparent reduction in hematocrit values. The observation on measured Hb in this study can be attributed to a reduction in the oxygen-carrying capacity of the blood due to transportation stress and dehydration during the journey (Ayoola et al., 2019).

Birds in the control treatment (T_1) were more stressed as compared to other treatment groups, indicated by increased WBC and its constituents. Lin et al. (2006) reported that H/L ratio is an indicator of chronic stress in food animals. Our study disagree with Lalonde et al. (2021), who found no impacts of either the temperature/relative humidity or duration of transportation on pullet H/L ratio, these differences can be attributed to the simulated transportation system used for their experiment, differences in atmospheric condition, and strain of pullet under investigation.

However, the results of this study were in agreement with the report of Borges et al. (2004) and Lalonde et al. (2021) that supplementation of vitamin C lowered the H/L ratio. Inclusion of natural source of vitamin C- citrus (sweet orange) at the two inclusion levels T_3 (30%) and T_4 (50%) compared well with the synthetic vitamin source (T_2). The present results for hematological parameters were in agreement with Guardia et al. (2009), Alam et al. (2018), and Cockram (2022).

Effect of treatments and period on serum biochemical parameters of transported pullets

Transportation stress, especially within the hot humid agro-ecological zone, causes physical stress which disrupted the thermal homeostasis, behavioral adaptation, and consequently physiological adaptation in livestock animals (Averos et al., 2008, Minka and Ayo, 2009, Bhatt et al., 2020). Notable physiological disruption includes an increase in the blood level of hormones and enzymes like aspartate aminotransferase (AST), alanine aminotransferase (ALT), plasma glucose, creatine phosphate kinase (CPK), cortisol, nitrogen urea, lactic acid, and uric (Parker et al., 2003, 2007; Ferguson and Warner, 2008, Minka and Ayo, 2009).

As reported in table 3; AST, ALT, creatine, and glucose were affected ($p<0.05$) by treatments and transportation stress. The control group (T_1) had the highest values for measured parameters which were significantly ($p<0.05$) different from other treatments. T_3 and T_4 compared well with T_2 with no significant ($p>0.05$) difference between the treatment groups. The rise in the value of measured liver function enzymes which include alanine amino transferase, aspartate

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amino transaminase, CPK occurs due to rough handling during pre-loading, loading and transportation. (Averos et al., 2008, Deters and Hansen 2020, Deepanshu et al., 2020).

Transportation over long distance is a stressor for animals. Animals are require to ensure homeostasis, while contact between animals' during transportation can produce fatigue and bruising, touching the permeable membranes and led to the extrusion of enzymes into the blood (Lopez et al., 2006, Deepanshu et al., 2020, Gou et al., 2021). Plasma glucose level was considerably high (p concentration), primarily due to the breakdown of glycogen in the liver (Jacobs et al., 2017).

The significant difference between the control (T_1) and other treatment groups (T_2, T_3, and T_4) can be attributed to oral supplementation of ascorbic acid sources in the form of synthetic vitamins and citrus. The oral supplementation of these ascorbic sources ameliorates the effect of transportation on pullets as evident in measured serum blood parameters. The findings were in line with goats administered ascorbic acid (Minka and Ayo, 2007), in the broiler (Lin et al., 2006).

CONCLUSION

The oral supplementation of a natural source of ascorbic acid (Citrus-sweet orange) and synthetic Vitamin c supplement helps to reduce the effect of transportation stress during the hot humid season in the tropics. Their ameliorating effect improves the health status of the birds during transportation. In addition, the oral supplementation of citrus-sweet orange compared effectively with the synthetic vitamin, hence a suitable alternative that is readily available for farmers and stakeholders. Further studies are hereby recommended to evaluate other associated effects of *Citrus sinensis* on birds' welfare.

DECLARATION

Consent
Not Applicable

Competing Interest
The authors declare that there are no competing interest during the data collection or writing up of this article

Author's Contribution
M. O. contributed to data collection and article write up, F. A. contributed to writing of manuscript, O.M. Alabi contributed to research design and data collection, A. O. contributed to statistical analysis and result interpretation, O. A. Oladejo :Contributed to statistical analysis, M. A contributed to data collection and laboratory analysis.

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REFERENCES

Adenkola AY and Ayo JO (2009a). Effect of road transportation on erythrocyte osmotic fragility of pigs administered ascorbic acid during the harmattan season in Zaria, Nigeria. Journal of Cell and Animal Biology, 3(1): 004-008. https://academicjournals.org/journal/JCAB/article-full-text-pdf/84A6C0912499.pdf

Adenkola AY and Ayo JO (2009b). Effect of ascorbic acid on rectal temperature fluctuations in indigenous turkeys during the hot-dry season. International Journal of Poultry Science, 8(5): 457-461. https://www.cabdirect.org/cabdirect/abstract/20093175568

Adenkola AY, and Anugwa FOI (2007). Effects of dietary supplement of ascorbic acid on performance of piglets in Makurdi, Benue State of Nigeria. Tropical Veterinarian, 25(1): 15-17. http://www.sdiarticle3.com/wp-content/uploads/2019/07/Ms_AJRAVS_49913.pdf

Adenkola AY, and Ayo JO (2009). Effect of road transportation on erythrocyte osmotic fragility of pigs administered ascorbic acid during the harmattan season in Zaria Nigeria. Journal of Cell and Animal Biology, 3(1): 4-8. https://academicjournals.org/journal/JCAB/article-full-text-pdf/84A6C0912499

Ahmadu S, Mohammed A, Buhari H, and Auwal A (2016). An overview of vitamin c as an anti-stress in poultry. Malaysian Journal of Veterinary Research, 7 (2):9-22. https://www.dvs.gov.my/dvs/resources/user_14/MJVR_V7N2/MJVR-V7N2-p9-22.pdf

Alam M, Hasanuzzaman M, Hassan MM, Rakib TM, Hossain ME, Rashid MH, and Sayeed MA (2018). Assessment of transport stress on cattle travelling a long distance (≈ 648 km), from Jessore (Indian border) to Chittagong, Bangladesh. Veterinary Record Open, 5(1): 240-248. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6018847/

Averos X, Martin S, Riu M, Serratosa J and Gosalvez LF (2008). Stress response of extensively reared young bulls being transported to growing-finishing farm under Spanish summer commercial conditions. Life Science, 119(1-3): 174-182. https://www.sciencedirect.com/science/article/pii/S1871141308001121

Citation: Mathew O. A, Foluke A, Olufemi M. A, Opeyemi A and Micheal A (2023). Comparative effect of vitamin complex and orange extract on physiological and blood parameters of transported pullets in humid tropics. *Online J. Anim. Feed Res.*, 13(2): 97-104. DOI: https://dx.doi.org/10.51227/ojafr.2023.15

Ayo JO, Minka NS, and Fayomi A (2005). Effects of ascorbic acid on rectal temperature of pullets transported by road during the hot-dry season in Northern Nigeria. Proc. 10th Annual Conference of Animal Science Association of Nigeria, Ado Eketi, Nigeria, 10: 58-60.

Ayoola MO, Oguntunji, AO, and Babalola AT (2020). Variation in hematological, biochemical parameters and physiological adaptation of cockerel strains to transportation density in a hot tropical environment. Journal of Animal Science and Veterinary Medicine, 5(2): 72-79. https://integrityresjournals.org/journal/JASVM/article-abstract/CD0FD83D7

Bhatt N, Singh NP, Mishra AK, Kandpal D, Rajneesh D and Jamwal S (2021). A detailed review of transportation stress in livestock and its management techniques. International Journal of Livestock Research, 11(1):30-41. http://dx.doi.org/10.5455/ijlr.20201109 102902

Borges, SA, Silva AVF, Majorka A, Hooge DM, and Cummings KR. (2004). Physiological responses of broiler chickens to heat stress and dietary electrolyte balance (sodium plus potassium minus chloride, milliequivalents per kilogram). Poultry Science 83:1551–1558. https://doi.org/10.1093/ps/83.9.1551

Buckham Sporer KR, Xiao L, Tempelman RJ, Burton JL, Early B, and Crowe MA (2008). Transportation stress alters the circulating steroids environment and neutrophil gene expression in beef bulls. Veterinary Immunology and Immunopathology. 121: 300-320. https://doi.org/10.1016/j.vetimm.2007.10.010

Cockram MS (2022). Cattle handling stress and transportation. Reference Module in Food Science 2022, Elsevier Inc. https://www.sciencedirect.com/science/article/pii/B9780323851251000338

Cockram MS, Kent JE, Goddard PJ, Warans NK, Jackson RE, Mccilp IM, and Southalls EI (2000). Behavioral and physiological responses of sheep to 16 h transport and a novel environment post-transport. Veterinary Journal, 159: 139-146. https://doi.org/10.1053/tvjl.1999.0411

Deters. EL and Hansen. SL (2020): Linking road transportation with oxidative stress in cattle and other species. Applied Animal Science 36 (2), 183-200. https://www.sciencedirect.com/science/article/pii/S2590286520300343

EFSA Panel on Animal Health and Welfare (AHAW) (2022).Welfare of cattle during transport. EFSA Journal, 20(9): e07442. https://doi.org/10.2903/j.efsa.2022.7442

Etebu E, and Nwauzoma AB. (2014). A review on sweet orange (Citrus Sinensis Osbeck): health, diseases, and management. American Journal of Research Communication, 2(2): 33-70. http://www.usa-journals.com/wp-content/uploads/2014/01/Etebu_Vol22.pdf

European Council. Council Regulation (EC) (No 1/2005 of 22 December 2004) on the protection of animals during transport and related operations and amending Directives 64/432/EEC and 93/119/EC and regulation (EC) No 1255/97. Official Journal of European Union, 3:1–44

Farm Animal Welfare Council (FAWC) (1992). Report: The Welfare of Poultry at the Time of Slaughter, Tolworth, 1992.

Ferguson DM, and Warner RD (2008). Have we underestimated the impact of pre-slaughter stress on meat quality in ruminants? Meat Science, 80:12-19. https://pubmed.ncbi.nlm.nih.gov/22063165/

Gou Z, Abouelezz KF, Fan Q, Li L, Lin X, and Wang Y (2021). Physiological effects of transport duration on stress biomarkers and meat quality of medium-growing yellow broiler chickens. Animal, 15(2): 100079. https://doi.org/10.1016/j.animal.2020.100079

Guardia MD, Estany J, Balasch S, Oliver MA, Gispert M, and Diestre A (2009). Risk assessment of skin damage due to pre-slaughter conditions and RYRI gene in pigs. Meat Science. 81: 745-751. https://pubmed.ncbi.nlm.nih.gov/20416560/.

Gupta D, Kashyap G, and Ashutosh M (2020). Ameliorative effect of vitamin C, electrolyte and Jaggery on transportation stress at different flocking densities in hot humid and winter seasons on hormonal parameters of goats. Livestock Science, 242 (12):104271. https://www.sciencedirect.com/science/article/abs/pii/S1871141320302080

Hussnain F, Mahmud A, Mehmood S, and Jaspal MH. (2019). Effect of broiler crating density and transportation distance on preslaughter losses and physiological response during the winter season in punjab, Pakistan. Brazilian Journal of Poultry, 22(2): 001-010. https://doi.org/10.1590/1806-9061-2019-1169

Jacobs L, Delezie E, Duchateau L, Goethals K, Ampe B, Buyse J, and Tuyttens FA (2017). Impact of transportation duration on stress responses in day-old chicks from young and old breeders. Research in Veterinary Science, 112 (06): 172-176. https://doi.org/10.1016/j.rvsc.2017.04.015

Jayaprakash G, Sathiyabarathi M, Robert MA, and Tamilmani T (2016). Transportation stress in broiler chicken. International Journal of Science, Environment and Technology 5(2):806-809. https://www.ijset.net/journal/936.pdfhttps://www.researchgate.net/profile/Sathiyabarathi-

Lalonde S, Beaulac K, Crowe TG, and Schwean-Lardner K (2021). The effects of simulated transportation conditions on the core body and extremity temperature, blood physiology, and behavior of white-strain layer pullets. Poultry Science, 100:697–706 https://doi.org/10.1016/j.psj.2020.10.077

Lewis NJ and Berry RJ (2006). Effects of season on the behavior of early-weaned piglets during and immediately following transport. Applied Animal Behavior Science, 100: 182 – 192. https://www.researchgate.net/publication/248335885_Effects_of_season_on_the_behavior_of_early-weaned_piglets_during_and_immediately_following_transport

Lin H, Jiao HC, Buyse J, and Decuypere E (2006). Strategies for preventing heat stress in poultry. World's Poultry Science Journal, 62(1):71-86. https://www.cambridge.org/core/journals/world-s-poultry-science-journal/article/strategies-for-preventing-heat-stress-in-poultry/114CAADF22599E20B2E1192AEE49FE69

Lopez O, Marco I, Montane J, and Lavin S (2006). Transport stress in Southern chamois (*Rupicapra pyrenaica*) and its modulation by acepromazine. Veterinary Journal. 1720: 347-355. https://pubmed.ncbi.nlm.nih.gov/16154370/

Minka NS and Ayo JO (2009). Physiological responses of food animals to road transportation stress. African Journal of Biotechnology, 8 (25):7415-7427. https://www.ajol.info/index.php/ajb/article/view/77711

Citation: Mathew O. A, Foluke A, Olufemi M. A, Opeyemi A and Micheal A (2023). Comparative effect of vitamin complex and orange extract on physiological and blood parameters of transported pullets in humid tropics. *Online J. Anim. Feed Res.*, 13(2): 97-104. DOI: https://dx.doi.org/10.51227/ojafr.2023.15

Minka NS and Ayo JO (2011). Modulating role of vitamins C and E against transport-induced stress in pullets during the hot-dry conditions. International Scholarly Research Network ISRN Veterinary Science, 2011: Article ID 497138. https://www.hindawi.com/journals/isrn/2011/497138/

Minka SN and Ayo JO (2007). Effect of loading behaviour and road transport stress on traumatic injuries in cattle transported by road during the hot-dry season. Livestock Science, 107: 91-95. https://www.sciencedirect.com/science/article/abs/pii/S1871141306004021

Nielsen BL, Dybkjær L, and Herskin MS (2011). Road transport of farm animals: effects of journey duration on animal welfare. Animal, 5(03), 415–427. https://pubmed.ncbi.nlm.nih.gov/22445408/

Odere R, D'Angelo A, Badino P, Bellino C, Pagliasso S, and Re G (2004). Road transportation effects blood hormone levels and lymphocyte glucocorticoid and B-adrenergic receptor concentrations in calves. The Veterinary Journal, 168: 297-303. https://doi.org/10.1016/j.tvjl.2003.09.008

Oguntunji AB, Oladejo AD, Ayoola OL, Oluwatomini IF, Oriye OP, and Egunjobi MO (2019). Genetic variation in physiological adaptation of local, exotic and crossbred ducks to heat stress in a tropical environment. Genetics & Biodiversity Journal, 3(1):39-49. https://journals.univ-tlemcen.dz/GABJ/index.php/GABJ/article/view/48

Parker AJ, Dobson GP, and Fitzpatrick LA (2007). Physiological and metabolic effects of prophylactic treatment with the osmolytes glycerol and betaine on Bos indicus steers during long duration transportation. Journal of Animal Science. 85: 2916-2923. https://pubmed.ncbi.nlm.nih.gov/17644781/

Parker AJ, Hamlin GP, Coleman CJ, and Fitzpatrick LA (2003). Quantitative analysis of acid-base balance in *Bos indicus* steers subjected to transportation of long duration. Journal of Animal Science, 81: 1434-1439. https://pubmed.ncbi.nlm.nih.gov/12817490/

Piccione G, and Caola G (2002). Biological rhythm in livestock. Journal of Veterinary Science, 3: 145-157. https://pubmed.ncbi.nlm.nih.gov/12514324/

Ritter MJ, Ellis M, Anderson DB, Curtis SE, Keffaber KK, Killefer J, Mckeith FK., Murphy CM and Peterson BA (2009). Effect of multiple concurrent stressors on rectal temperature, blood acid-base status, and longissimus muscle glycolytic potential in market-weight pigs. Journal of Animal Science, 87: 351-362. https://pubmed.ncbi.nlm.nih.gov/18676725/

Scope FT, Gabler C, and Resch F (2002). The influence of stress from transport and handling hematological and clinical chemistry blood parameters of racing pigeon (columbia liva domestica). Avian Diseases, 46: 224-229. https://doi.org/10.1637/0005-2086(2002)046[0224:tiosft]2.0.co;2

Shearer J (2021). Optimizing welfare in transport and slaughter of cattle. InUnderstanding the behaviour and improving the welfare of dairy cattle. Burleigh Dodds Science Publishing, UK, pp. 219-249. https://www.taylorfrancis.com/chapters/edit/10.1201/9781003180630-13/optimizing-welfare-transport-slaughter-cattle-jan-shearer

Tadich N, Gallo C, Brito ML and Broom DM (2009). Effects of weaning and 48 h transport by road and ferry on some blood indicators of welfare in lambs. Livestock Science, 121:132–136.

Tadich N, Gallo H, Bustamante H, Schwerter M, and van Schaik G (2005).Effects of transport and lairage time on some blood constituents of Friesian-Cross steers in Chile. Livestock Production Science, 93: 223-233. https://doi.org/10.1016/j.livsci.2008.06.001 https://www.sciencedirect.com/science/article/pii/S0301622604002398

Tiwari BK, O' Donnell CP, Muthukumarappan K, and Cullen PJ. (2009) Ascorbic acid degradation kinetics of sonicated orange juice during storage and comparison with thermally pasteurized juice. LWT - Food Science and Technology, 42:700-704. https://www.sciencedirect.com/science/article/abs/pii/S0023643808002594