

Evaluation of zinc oxide nanocomposites and L-ascorbic acid on meat quality during transport induced stress in birds of Sonali breed

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ABSTRACT

The study assessed the efficacy of phytofabricated zinc oxide nanoparticles (CF-ZnONPs) and L-ascorbic acid in alleviating pre-slaughter transport induced stress in birds of Sonali breed (Cross bred of Rhode Island Red cocks and Fayoumi hens). A total of 120 birds were divided into four groups: negative control (T_1) which were loaded in vehicle but not transported, Positive control (T_2) birds were transported without any prior supplementation, T_3 and T_4 birds were transported with prior supplementation (a day before transportation) of CF-ZnONPs @100 µg/ml and L-ascorbic acid @82 µg/ml in drinking water. Results obtained observed significant reduction in live weight, meat colour and pH but increased drip loss percentage in T_2 group. Significantly elevated H/L ratio was recorded in all transported groups. Increased cortisol and TSH levels and decreased thyroid hormones, Triiodothyronine and thyroxine (T_3 and T_4) levels were recorded in T_2 group. However, supplementation with CF-ZnONPs and L-ascorbic acid notably reversed these adverse effects, restoring meat quality and other metabolic profiles. Notably, ascorbic acid exhibited greater efficacy, suggesting its superior role in mitigating transportation-induced stress. Overall, these findings highlight the importance of antioxidant supplementation in alleviating transport induced stress in birds.

Keywords: Broilers, Hormone, Meat quality, Metabolite, Transportation

World meat consumption is outpacing the sale of other major agricultural commodities, especially in developing countries. The amount of meat produced in 2014–15 was 6.69 million tons, and in 2021–2022 it reached 9.29 million tons. Meat production has shown positive growth of 5.62% during 2021-23 (OECD 2021). Meat consumption has been shifting towards poultry. Global production of chicken meat reached 139.68 million metric tons in 2023, 17.61 million metric tons more than that of pork (Kleyn and Ciacciariello 2021).

Transportation in poultry from farms to processing plants or market poses stress on rapidly growing broilers which cause major economic losses in poultry enterprises and deteriorates consumer (Gou *et al.* 2021). The stressors of transport are categorized into mental, physical, and mixed factors; the physical stressors include holding, catching, crating, and the environment temperature and other conditions during transport, which may cause physical injuries promoting exhaustion of the natural antioxidant capacity of the bird and exposing the cells to harmful reactive oxygen species. Moreover, it was established that pre-slaughter transport stress in broilers alters the flavour, texture and tenderness of meat (Arikan *et al.* 2017).

Antioxidants are crucial for life and are required for good health due to their significant impact on the control of oxidative stress by maintaining the equilibrium between the antioxidant capacity and the activity of free radicals (Zhao *et al.* 2023). Meat quality can primarily be shielded from oxidative stress-induced degradation by combining antioxidant micronutrients like zinc, magnesium, and selenium with vitamins (Guo and Dalrymple 2017). Zinc nanoparticles and vitamin C are known for their antioxidant properties, which include scavenging free radicals and reducing oxidative damage in biological tissues (Gegotek *et al.* 2024). Consequently, strategies like supplementation of antioxidants aimed at combating excess free radicals, restoring energy homeostasis not only enhance the health and welfare of broilers but also improve chicken meat quality (Ma *et al.* 2024).

In general, there is a paucity of information on the biochemical alterations in broiler birds during transportation and prophylactics which can combat the adverse effects due to transport stress. Therefore, this study aimed to evaluate the negative effects of transport stress and the development of nutritional strategies to improve meat quality.

MATERIALS AND METHODS

The present study was conducted at the lies between 20°54' and 21°32' north latitude & 81°10' and 81°36' east longitude, and Commercial dairy farm Rajnandgaon, Chhattisgarh during August-October, 2023

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Preparation of plant extract: Zinc oxide nanoparticles were synthesized utilizing aqueous extract of *Cassia fistula* (Amaltas) leaves by the methodology outlined by Bala *et al.* (2015). Fresh and healthy leaves of *Cassia fistula* were collected from nearby areas of the College of Veterinary Science and Animal Husbandry, Anjora, Durg, Chhattisgarh washed thoroughly twice with distilled water and shade dried. The leaves were ground in a mixer grinder (30 g) and transferred to a sterile 250 ml conical flask. Milli-Q water 100 ml was added to the flask and heated at 60°C for 4 h in a hot water bath with intermittent shaking then kept overnight (12 h) at room temperature (25°C). This extract was then boiled at 60°C for 15 m, filtered with Whatman filter paper No. 1 and stored at 4°C for further use.

Phytofabrication of zinc oxide nanoparticles: Zinc nitrate [$\text{Zn}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$] (Merck Life Science Pvt. Ltd.) (0.1 M) 100 ml solution was prepared and mixed with 20 ml of *Cassia fistula* leaf extract solution under constant stirring. Then 0.2 M of sodium hydroxide solution (10 ml) was added dropwise (1 drop / 5 s) till pH 12 was achieved. After the completion of the reaction, the solution was centrifuged at $10,000 \times g$ for 10 min and the supernatant was discarded. The sediment obtained was washed, dried and calcined at 400°C for 5 h. *Cassia fistula* induced zinc oxide nanoparticles (CF-ZnONPs) formed was characterized by field emission scanning electron microscopy (FE-SEM) images using a Zeiss EVO18 Scanning Electron Microscope

Experimental animals: The study protocol was approved by the Institutional Animal Ethics Committee (VCA/VPB/1/3/2023).

A total of 120, 6-7 weeks old (either sex) of Sonali breed with body weight ranging from 980 ± 50 g, raised in a deep litter system fed with standard feed and water (*ad lib.*), were randomly allocated to four experimental treatments with three replicates of 10 birds each. Birds in the first treatment (T_1) were loaded in open vehicle in cages but not transported and were considered as negative control. All the birds of T_2 , T_3 and T_4 groups were marked and transported in cages (3 birds/cage) each of dimension $74 \times 55 \times 27$ cm (length $\times$ width $\times$ height), in one vehicle, for a distance of 90 km @35-40 km/h. All shipments occurred at 0600 h and digital thermohygrometer (Model: HTC 288-ATH) was installed in vehicle to record temperature and relative humidity. Birds of T_2 group did not receive any treatment whereas T_3 and T_4 groups were treated orally with CF-ZnONPs @100 $\mu\text{g}/\text{ml}$ and L-ascorbic acid @82 $\mu\text{g}/\text{ml}$ dissolved in double sterile drinking water, supplemented a night before transportation.

The individual body weight and temperature was recorded before and after transport. Drinking water was made available until the shipping time, but the feed was removed 10 h before shipment. Throughout the study's transportation period, drinking water was not provided. At the same time, every bird boarded the truck, and once the designated transportation was complete, the cages were

promptly removed, and samples were collected 30 min after the completion of transport.

Meteorological data: The meteorological data, ambient temperature (AT) and relative humidity (RH) were recorded with the aid of a wet- and dry-bulb thermometer and temperature humidity index (THI) was calculated.

Sample collection and analysis

Meat quality: Two birds with the greatest weight loss from each replicate were slaughtered and thigh muscle samples (15 g) were used for the determination of muscle pigments (Hrynets *et al.* (2011), drip loss percentage (Jiang *et al.* 2007) and pH was determined post-slaughter, (pH_{30} , pH_{60} , $\text{pH}_{24\text{h}}$) using a portable pH meter (Lab equipment supplier, B099DX4GBT) by thrusting the probe into the minced muscle.

Heterophil to lymphocyte ratio: About 2 ml blood sample was drawn to determine heterophil to lymphocyte (H/L) ratio using the straight edge method (Gross and Siegel 1983).

Blood metabolites: Blood samples were also collected for the extraction of serum for cortisol, T3, T4, and TSH hormone estimation using commercially available kit (Gamma B T4; IDS, Tyne and Wear, UK).

Inflammatory markers: Interleukin 6 (IL6) and C-reactive protein (CRP) was determined using Sandwich Electrochemiluminescence Immunoassay (Agilus diagnostics, Mumbai).

Statistical analysis: The general linear model of one-way analysis of variance, or ANOVA, was used to statistically analyze the gathered data. Tukey's test was used to assess the significance of the group differences (IBM SPSS Statistics version 26.0). To simplify the presentation of these findings, interleaved bars $\pm$ SEM are shown.

RESULTS AND DISCUSSION

Synthesis and characterization of phytofabricated CF-ZnONPs: The FE-SEM morphology of CF-ZnONPs depicted spherical surface morphology with sizes ranging from 22 to 37 nm (Fig. 1).

Temperature humidity and temperature humidity Index

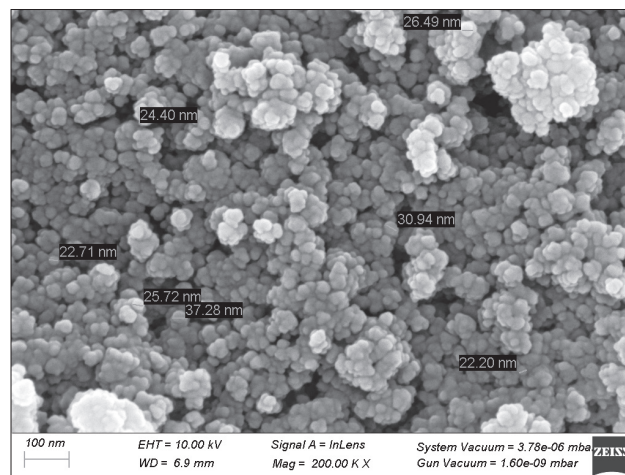


Fig. 1. FESEM analysis of phytofabricated CF-ZnONP.

Table 1. Meteorological data during road transportation of vehicles containing crates of broiler birds of the Sonali breed

Parameter	At 6:00 AM	At 7:00 AM	At 8:00 AM	At 9:00 AM
Environmental temperature surrounding the vehicle (°C)	25	26.8	28	30
The temperature inside the vehicle loaded broiler bird (°C)	26.2	27.7	28.4	30.2
The temperature of a crate loaded with birds inside the vehicle (°C)	26.6	27.4	29.2	31.9
Relative humidity surrounding the vehicle (%)	52	49	48	45
Wind velocity during vehicle transport (km/h)	30-35	30-35	30-35	30-35
Temperature humidity index (THI)	71	71.56	75	79

No. of animals, n=6. Values presented as Standard error of the mean. ^{abc}, Average values within a row bearing different superscripts differ significantly (p<0.01).

(THI): The environmental temperature varies between 25°C to 30°C and relative humidity was 45% to 52%. The average temperature inside the loaded cage was 28.77°C, and the ambient temperature was 27.4°C. During the transportation, relative humidity decreased whereas THI increased with increasing duration of transport (from 24.35 to 29.30) at different time intervals (Table 1).

In the present study, no mortality was recorded during or after transportation. The birds of T₂ group showed significant (p<0.01) reduction in body weight (927.27±3.69 g) as compared to the birds of the negative control group (995.36±2.34 g). However, supplementation of CF-ZnONPs (T₃) and L-ascorbic acid (T₄) in drinking water before the transport of birds had no significant change in body weight after transportation. The temperature recorded in birds before transportation was 107±1.1°F and no significant change in body temperature was recorded after transportation in any of the studied group. Our observations are consistent with the findings of Gou *et al.* (2021). The present study recorded significant (p<0.01) decreased body weight after transportation in birds of positive control group. The body loss may be due to the off-fed condition of birds before transport which was 10 h in the present study.

Meat quality parameters: Present study recorded meat quality parameters in experimental birds during transportation induced stress (Table 2). The colour of meat was reduced significantly (p<0.01) in birds transported without any treatment (T₂) compared to negative control birds (T₁). Fehri *et al.* (2022) recorded the similar findings and found that oxidation of myoglobin leads to decreased redness and increased yellowness of meat. The present study recorded an improvement in the meat colour by the supplementation of CF-ZnONPs and L-ascorbic acid in birds before pre-slaughter transportation.

The changes in thigh muscle pH were recorded after 30mts, 60mts and 24 hours (pH_{30min} and pH_{60min} and pH_{24h}) in all the studied groups. Transportation of T₂, T₃, and T₄ broilers depicted a significant decrease (p<0.01) in pH value in different time interval as compared to the negative control group. Significant recovery (p<0.01) of pH values were observed in T₃ and T₄ groups as compared to the positive control group (T₁). The pH of muscle tissue serves as a crucial indicator of meat quality, with deviations from optimal pH levels reflecting changes in cellular metabolism and physiological stress. Present observations are further supported by studies conducted by Ramkrishna *et al.* (2022). Notably, zinc is required for the activity of carbonic anhydrase, influencing the generation and buffering of protons within muscle cells, thereby regulating intracellular pH levels (Fatima *et al.* 2024). Consequently, the observed attenuation of pH decline in the L-ascorbic acid-treated group suggests a protective effect against oxidative stress.

The drip loss percentage (3.22%) in the thigh muscle samples of the positive control group (T₂) was significantly elevated (p=0.001) compared to the birds of the negative control group (T₁) (2.55%). birds supplemented with CF-ZnONPs and L-ascorbic acid showed significant recovery in drip loss percentage as compared to birds of the positive control group. No significant changes were observed in between the two treatment groups (T₃ and T₄). High drip loss in meat indicates poor quality meat due to loss in nutrients, flavour and tenderness (Carrasco-Garcia *et al.* 2020). The present study recorded an increase in temperature during transport that might harm water-holding capacity and increase drip loss in poultry meat. The loss of integrity in muscle cell membranes due to oxidation stress during transport leads to exudative loss from muscle cells, which results in nutrient loss, increased drip loss, and reduced muscle pigment (Warner 2023).

Table 2. Effect of transport stress and alleviation potential of CF-ZnONPs and L-ascorbic acid supplementation on meat quality parameters in broilers of Sonali breed

Parameter	T ₁	T ₂	T ₃	T ₄
Meat colour (mg of myoglobin/ g of meat)	1.832±0.001 ^c	1.675±0.001 ^a	1.69±0.001 ^a	1.702±0.0212 ^b
pH _{30 min}	6.4233±0.01 ^d	5.7833±0.16 ^a	5.96±0.02 ^b	6.11±0.01 ^c
pH _{60 min}	6.480±0.02 ^d	5.700±0.02 ^a	5.90±0.025 ^b	6.00±0.02 ^c
pH _{24h}	6.50±0.025 ^d	6.0167±0.01 ^a	6.00±0.02 ^b	6.20±0.025 ^c
Drip loss %	2.35±0.005 ^a	3.22±0.006 ^c	2.95±0.002 ^b	2.90±0.003 ^b

No. of animals, n=6 in each group. Values presented as Standard error of the mean. ^{abc}, Average values within a row bearing different superscripts differ significantly (p<0.01).

Table 3. Effect of transport stress and alleviation potential of CF-ZnONPs and L-ascorbic acid supplementation on cortisol, T3, T4, and TSH hormones in broilers of Sonali breed

Parameter	T ₁	T ₂	T ₃	T ₄
Cortisol (ng/ml)	1.12±0.415 ^a	1.89±0.006 ^c	1.84±0.013 ^c	1.74±0.018 ^b
T3 (ng/ml)	2.82±0.17 ^c	1.95±0.042 ^a	2.24±0.042 ^b	2.32±0.047 ^b
T4 (mg/ml)	3.21±0.60 ^c	2.03±0.04 ^a	2.45±0.021 ^b	2.40±0.042 ^b
TSH (μIU/ml)	1.82±0.014 ^a	2.07±0.026 ^c	1.93±0.005 ^b	1.91±0.042 ^b

No. of animals, n=6. Values presented as Standard error of the mean. ^{abc}, Average values within a row bearing different superscripts differ significantly (p<0.01).

Table 4. Effect of transport stress and alleviation potential of CF-ZnONPs and L-ascorbic acid supplementation on inflammatory markers in broilers of Sonali breed

Parameters	T ₁	T ₂	T ₃	T ₄	p-value
IL-6 (pg/ml)	2.65±0.022 ^a	2.9±0.036 ^b	2.885±0.003 ^b	2.881±0.003 ^b	0.00
C-reactive protein (mg/L)	0.35±0.002 ^a	0.358±0.003 ^a	0.355±0.0021 ^a	0.355±0.0022 ^a	0.142

No. of animals, n=6 in each group. Values presented as Standard error of the mean. ^{abc}, Average values within a row bearing different superscripts differ significantly (p<0.01).

Immune response: Present study recorded a significant (p<0.01) elevated H/L ratio in T₂ (0.89±0.03), T₃ (0.89±0.04), and T₄ (0.87±0.01), group as compared to T₁ (0.85±0.02). Studies demonstrated increased HL ratio in stress is due to the circulating concentrations of corticosterone (Maness *et al.* 2023). Dietary supplementations of CF-ZnONPs and L-ascorbic acid boosted the immune response which might be either due to lower cortisol concentration in the T₃ and T₄ group.

Metabolic hormones

Serum cortisol, T3, T4 and TSH: Present study recorded a significant increase (p=0.001) in serum cortisol and TSH levels in chicken of the T₂ group as compared to birds of the negative control group (T₁). Supplementation of CF-ZnONPs and L-ascorbic acid recorded a decreased cortisol and TSH levels. Serum thyroxine (T4) and triiodothyronine (T3) levels of the positive control group were significantly lower (p<0.01) as compared to the negative control group. Birds supplemented with CF-ZnONPs and L-ascorbic acid recorded significant recovery in levels of hormones T3 and T4 hormones (Table 3).

Similarly, Hussnain *et al.* (2020) also observed elevated cortisol levels in similar circumstances, supporting a recurring trend in response to transportation stress among avian populations. ZnONPs modulating inflammatory signaling pathways and reducing oxidative stress, limit the stress-induced increase cortisol levels in birds (Olgun *et al.* 2021). L-Ascorbic acid does the activation of the HPA axis, inhibiting the release of corticotropin-releasing hormone (CRH) from the hypothalamus, thereby down-regulating cortisol production (Mortiz *et al.* 2020).

Results of the present study concluded a significant relationship between temperature and humidity of the environment and the levels of hormones cortisol, T3, and T4 of the experimental animals. The increased temperature during transportation reduces the level of thyroid hormone, as observed in birds of T₂ group. The reduced circulating level of cortisol in the CF-ZnONPs and the L-ascorbic

acid-supplemented group might be responsible for enhancing the concentration of T3 and T4 hormones in the supplemented groups because elevated concentrations of adrenal cortical hormones are considered to be responsible for hypothyroid activity. Antioxidant properties of CF-ZnONPs and L-ascorbic acid may safeguard thyroid tissue integrity, indirectly impacting TSH sensitivity.

Inflammatory markers: Present study identified a significantly (p<0.01) increased IL-6 value showing the modulation of immune responses in birds transported without any treatment (Table 4). Supplementation of CF-ZnONPs and L-ascorbic acid reduced the IL6 expression non-significantly and CRP levels remained relatively stable in the study. This protective effect may stem from the anti-inflammatory and antioxidant properties of zinc nanoparticles and ascorbic acid, respectively, mitigate the inflammatory cascade triggered by transportation stress (Al-Zghoul *et al.* 2019). Zinc nanoparticles possess immunomodulatory properties and have been shown to attenuate the inflammatory response and dampen the release of IL-6 in response to transportation stress. (Agarwal and Shanmugam 2020). Ascorbic acid serves as a potent antioxidant by neutralizing ROS and inhibiting NF-κB activation, ascorbic acid mitigates the inflammatory response and suppresses IL-6 production following transportation stress (Du *et al.* 2022).

Present study concluded that the transportation of birds significantly affects body weight, meat colour, pH, and drip loss percentage. Overall, CF-ZnONPs @100 μg/ml and ascorbic acid @82 μg/ml supplementation before pre-slaughter transport of birds showed promise in alleviating transport-induced stress and preserving meat quality in chickens, but also showed that L-ascorbic acid was more effective as compared to CF-ZnONPs and ensures the production of high-quality poultry meat for consumers.

REFERENCES

Agarwal H and Shanmugam V. 2020. A review on anti-inflammatory activity of green synthesized zinc oxide

- nanoparticle: Mechanism-based approach. *Bioorganic Chemistry* **94**: 103423.
- Al-Zghoul M B, Saleh K M and Ababneh M M. 2019. Effects of pre-hatch thermal manipulation and post-hatch acute heat stress on the mRNA expression of interleukin-6 and genes involved in its induction pathways in 2 broiler chicken breeds. *Poultry Science* **98**(4): 1805–19.
- Arikan M, Akin A, Akcay A, Aral Y, Sariozkan S and Cevrimli M. 2017. Effects of transportation distance, slaughter age, and seasonal factors on total losses in broiler chickens. *Brazilian Journal of Poultry Science* **19**: 421–28.
- Bala N, Saha S, Chakraborty M, Maiti M, Das S, Basu R and Nandy P. 2015. Green synthesis of zinc oxide nanoparticles using *Hibiscus sabdariffa* leaf extract: Effect of temperature on synthesis, anti-bacterial activity, and anti-diabetic activity. *RSC Advances* **5**: 4993–5003.
- Carrasco-García A A, Pardío-Sedas V T, León-Banda G G, Ahuja-Aguirre C, Paredes-Ramos P, Hernández-Cruz B C and Murillo V V. 2020. Effect of stress during slaughter on carcass characteristics and meat quality in tropical beef cattle. *Asian-Australasian Journal of Animal Science* **33**(10):1656–65.
- Du J, Shi Y, Zhou C, Guo L, Hu R, Huang C, Hu G, Gao X and Guo X. 2022. Antioxidative and anti-inflammatory effects of vitamin C on the liver of laying hens under chronic heat stress. *Frontiers of Veterinary Science* **9**:105–15.
- Fatima A, Zaheer T, Pal K, Abbas R Z, Akhtar T, Ali S and Mahmood M S. 2024. Zinc oxide nano-particles significant role in poultry and novel toxicological mechanisms. *Biological Trace Elemental Research* **202**(1): 268–90.
- Fehri N E, Kammoun M, Amraoui M, Ben Larbi M and Jemmali B. 2022. Poultry meat quality: Technological, nutritional, sensory and microbiological quality. *IOSR Journal of Biotechnology and Biochemistry* **8**(3): 9–14.
- Gegotek A and Skrzydlewska E. 2024. Lipid peroxidation products' role in autophagy regulation. *Free Radical Biology and Medicine* **212**: 375–83.
- Gou Z, Abouelezz K F, Fan Q, Li L, Lin X, Wang Y, Cui X, Ye J, Masoud M A, Jiang S and Ma X. 2021. Physiological effects of transport duration on stress biomarkers and meat quality of medium-growing yellow broiler chickens. *Animals* **15**(2): 100079.
- Gross W B and Siegel H S. 1983. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. *Avian Diseases* **27**(4): 972–79.
- Guo B and Dalrymple, B.P. 2017. Transcriptomics of meat quality. *New Aspects of Meat Quality*. Woodhead Publishing Series in Food Science, Technology and Nutrition.
- Hrynets Y, Omana D A, Xu Y and Betti M. 2011. Impact of citric acid and calcium ions on acid solubilization of mechanically separated turkey meat: Effect on lipid and pigment content. *Poultry Science* **90**(2): 458–66.
- Hussnain F, Mahmud A, Mehmood S and Jaspal M H. 2020. Influence of long-distance transportation under various crating densities on broiler meat quality during hot and humid weather. *Journal of Poultry Science* **57**(3): 246–52.
- Jiang Z Y, Jiang S Q, Lin Y C, Xi P B, Yu D Q and Wu T X. 2017. Effects of soybean isoflavone on growth performance, meat quality, and antioxidation in male broilers. *Poultry Science* **86**(7): 1356–62.
- Kleyn F J and Ciacciariello M. 2021. Future demands of the poultry industry: will we meet our commitments sustainably in developed and developing economies? *World's Poultry Science* **77**(2): 267–78.
- Ma M, Li L, Zuo G, Xiao, Chen J, He X and Song, Z. 2024. Effect of zinc amino acid complexes on growth performance, tissue zinc concentration, and muscle development of broilers. *Biological Trace Elemental Research* **202**(1): 291–306.
- Maness T J, Grace J K, Hirschak M R, Tompkins E M and Anderson D J. 2023. Circulating corticosterone predicts near-term, while H/L ratio predicts long-term, survival in a long-lived seabird. *Frontiers in Ecology and Evolution* **11**: 117–24.
- Moritz B, Schmitz A E, Rodrigues A L, Dafre A L and Cunha M P. 2020. The role of vitamin C in stress-related disorders. *Journal of Nutritional Biochemistry* **85**: 108–14.
- OECD. 2021. OECD-FAO Agricultural Outlook. *OECD Agriculture Statistics* 163–77.
- Olgun O, Abdulkader A F and Karabacak A. 2021. The importance of nutrition in preventing heat stress in poultry. *World's Poultry Science* **77**(3): 661–78.
- Ramakrishnan C, Karthiga S and Chilambarasan M. 2021. Physiological effects of transport duration on meat quality of Indian broiler chickens. *Pharma Innovation Journal* **10**(7): 403–05.
- Warner R D. 2023. The eating quality of meat: IV—Water holding capacity and juiciness. *Lawrie's Meat Science*. Woodhead Publishing Series in Food Science, Technology and Nutrition.
- Zhao W, He J, Yu P, Jiang X and Zhang L. 2023. Recent progress in the rubber antioxidants. *Polymers* **15**(16): 344–57.