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Effects of probiotic (*Saccharomyces cerevisiae*) and ascorbic acid on oxidative gene damage biomarker, heat shock protein 70 and interleukin 10 in broiler chickens exposed to heat stress

Victory Osirimade Sumanu [a,*], Charles Byaruhanga [b], Anna-Mari Bosman [b], Sunday Ochonu Ochai [b], Vinny Naidoo [c], Marinda Catharina Oosthuizen [b], Joseph Panashe Chamunorwa [a]

[a] Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa
[b] Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa
[c] Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa

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ABSTRACT

Heat stress is a prominent factor responsible for losses economically in poultry meat industry due to adverse effects on the general performance of broiler chickens. In this study, we evaluated the effects of probiotic (*Saccharomyces cerevisiae*) and ascorbic acid on oxidative gene damage biomarker, heat shock protein 70 (*HSP70*) and interleukin 10 (*IL-10*) in broiler chickens exposed to heat stress under natural conditions. Fifty-six broiler chickens served as the subjects, they were divided into 4 groups of 14 as follows: group I (control), group II (probiotic *S. cerevisiae* at 1 g/kg of feed), group III (ascorbic acid at 200 mg/kg of feed) and group IV (probiotic + ascorbic acid at 1 g/kg and 200 mg/kg of feed, respectively). The treatments were administered via feed for 35 days (D1 to D35). Enzyme-linked immunosorbent assay (ELISA) and one step real time reverse transcription polymerase chain reaction (RT-PCR) was utilised to study the effects of heat stress on the expression levels of 8-hydroxy-2′-deoxyguanosine (*8-OHdG*), *HSP70* and *IL-10* respectively, in broiler chickens raised during the hot summer season. The level of *8-OHdG* gene was significantly lower in the probiotic administered group. The expression level of *HSP70* was lowest in the ascorbic acid group while, *IL-10* level of expression was highest in the probiotic + ascorbic acid group. The administered antioxidants were efficient in exhibiting anti-stress effects at the level of gene expression. We conclude that probiotic, ascorbic acid and probiotic + ascorbic acid reduced oxidative gene damage, affected the expression of *HSP70* and increased the level of *IL-10* gene respectively, in broiler chickens exposed to heat stress.

1. Introduction

The world has been faced with the detrimental effects of heat stress arising from global warming (Moyo and Nsahlai, 2021). Global warming, especially in the sub-tropical and tropical regions of the world, has negatively affected the surface of the earth due to the emission of trapped solar radiation by several greenhouse gases. With the impact of global warming being widespread, negative impacts are now extending to agricultural systems (Oke et al., 2021). Of the various species farmed, intensively farmed poultry are highly susceptible species, due to the absence of sweat glands in the breed, making them highly dependent on other mechanisms for heat dissipation (Ouchi et al., 2022). In this species, increased thermal strength leads to heat stress and subsequent oxidative stress in broiler chickens and other animals (Chen et al., 2018; Domenici and Seebacher, 2020; Shakeri and Le, 2022). Oxidative stress occurs when the production of reactive oxygen species (ROS) such as

Abbreviations: ROS, Reactive oxygen species; DNA, Deoxyribonucleic acid; HSP, Heat shock proteins; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; IL-10, Interleukin-10; OVARU, Onderstepoort Veterinary Animal Research Unit; EDTA, Ethylenediamine tetraacetic acid; RNA, Ribonucleic acid; EL, Erythrocyte lysis; RLT, Lysis buffer; RW, Washing buffer; RPE, Mild washing buffer; RT-PCR, Reverse transcription polymerase chain reaction; ELISA, Enzyme-linked immunosorbent assay; HRP, Horseradish peroxidase; OD, Optical density; ROM, Reactive oxygen metabolites.

* Corresponding author.
E-mail address: devicpet@gmail.com (V.O. Sumanu).

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superoxide anion radical, hydroxyl radical, nitric oxide radical, hydrogen peroxide, peroxynitrite radical and oxygen singlet exceeds the body's capability to reduce them with resultant cellular damage (Amevor et al., 2022; Lin et al., 2022).

More specifically increased levels of ROS have a negative impact on the nervous and endocrine systems which subsequently affect both cell-mediated and antibody immune responses (Alaqil et al., 2022; Rattanasrisomporn et al., 2022). Alterations in the hypothalamic-pituitary-adrenal axis leads to an increase level of corticosterone as a response to the stressor (Ogbuagu et al., 2018). During chronic inflammation, the response of the body to inflammatory processes could eventually damage cells, tissues and organs that are apparently healthy (Fu et al., 2022). This can result to deoxyribonucleic acid (DNA) damage, other subcellular structures (plasma membrane, nucleus, mitochondria) damage, death of tissue and scarring over time which are sequel to several diseases such as cancer (Hu et al., 2019). ROS effects on cells could also trigger the induction of autoimmune response.

Heat shock proteins (*HSP*) are molecular chaperones termed as stress protein; they are made when the cell is briefly exposed to temperatures above normal. They are also produced in response to oxidative stress (Roushdy et al., 2018). Their up-regulation is as a result of an individual's response to stressful conditions (Siddiqui et al., 2020). *HSP* are responsible for the folding of proteins that are newly synthesized and the refolding of misfolded or damaged proteins (Siddiqui et al., 2020). In addition to upregulating ROS production, hydroxyl radicals can react with mitochondrial or nuclear DNA resulting to the formation of 8-hydroxy-2′-deoxyguanosine (*8-OHdG*), which is widely considered a biomarker of oxidative gene damage (Attia et al., 2019; Dai et al., 2019). ROS induce the modification of DNA bases by attacking guanine and leaving it unpaired (Attia et al., 2019; Zhu et al., 2019; Hu et al., 2019; Qing et al., 2019). Following the release of inflammatory cytokines, their release triggers the release of anti-inflammatory cytokines (interleukin-10 (*IL-10*)) that inhibits further secretions of the previous cytokines and repair the damages caused by inflammation (Saleh and Al-Zghoul, 2019; Jiang et al., 2022; Sedghi et al., 2022). The balance between cell-based and humoral immune responses is effectively modulated by the cytokines (Saleh and Al-Zghoul, 2019). ROS exposure is known to interfere with this process with resultant increases in pro-inflammatory cytokine gene which could inhibit the release of anti-inflammatory cytokine like *IL-10* gene if not mitigated (Jiang et al., 2022).

The net impact of oxidative stress in poultry production system are decreases in productivity and potentially death of birds (Aluwong et al., 2017). To understand the latter impact, one needs to just consider the importance of poultry meat as a food source in the world. Poultry production, a form of animal husbandry encompasses the raising of domesticated birds like chickens, turkeys, etc., to produce meat or egg (Deng et al., 2022). Broiler chickens are bred for rapid growth to attain maximum meat production. Broiler meat is universally accepted because it is less expensive, has a quick turn-over and is thought to be more healthy than red meat, hence its value in countries with poorer economy (Sumanu et al., 2022).

To allow for optimal production, birds need to be either managed under controlled conditions which are expensive or through therapeutic means. For the latter the antioxidants, like ascorbic acid (Van Hieu et al., 2022), are attractive therapeutic agents as they are known to inhibit the process of oxidation (Fu et al., 2022). Yeast probiotic, a living organism, may offer an alternate treatment as they possess both antioxidant and anti-stress potencies (Aluwong et al., 2017; Kim et al., 2022). The current study was therefore carried out to evaluate the effect of probiotic (*Saccharomyces cerevisiae*) and ascorbic acid in mitigating oxidative gene damage biomarker, *HSP70* and *IL-10* genes in broiler chickens exposed to heat stress under natural conditions.

2. Materials and methods

2.1. Experimental sites and thermal environmental conditions

The study was undertaken at the Onderstepoort Veterinary Animal Research Unit (OVARU), Faculty of Veterinary Science, University of Pretoria, located in latitude 25° 39′ 5"S and longitude 28° 10' 41.8"E, South Africa. The broiler chickens were raised in a natural environment of fluctuating ambient temperature during the hot period of the South African summer. The study was approved by the Animal Ethics Committee of the University of Pretoria (REC050–20).

2.2. Experimental animals and management

Fifty-six (one-day-old) certified healthy male and female broiler chicks (Ross), sourced from Alpha chicks, Gauteng, were used as the subjects. They were housed in an intensive management system (same building with four separate pens); brooding was done for 14 days with the aid of infra-red bulbs with an upper end of 32 °C. Feed (Epol feeds, South Africa; constituted as shown in Table 1) and water were made available to the chicks ad libitum, while wood shavings were used as litter. The poultry pen was constructed with concrete floor, cement blocks and aluminium roof. Efficient biosecurity measures were ensured during the study.

2.3. Experimental design

The individually marked chickens ($n = 56$) were allotted into four groups of fourteen each. Group I (control); Group II [probiotic (*Saccharomyces cerevisiae*); Sigma-Aldrich (Pty) Ltd. 1 Friesland Drive, Modderfontein, Johannesburg 1645, South Africa], Group III (ascorbic acid; Medico Herbs, 80 Lourensford Road, Somerset West 7130, South Africa), Group IV (probiotic and ascorbic acid). Probiotic and ascorbic acid were administered at a dose of 1 g/kg of feed (Parlat et al., 2001) and 200 mg/kg of feed (Egbuniwe et al., 2021), respectively for 35 days.

2.4. Determination of 8-OHdG, HSP70 and IL-10

Four (4) mL of blood sample was collected from the wing vein of 28 broiler chickens (7 per group) into 2 mL plain tubes and tubes containing ethylenediamine tetraacetic acid (EDTA) at D35 of the study before euthanising the entire (56) broiler chickens. The samples were transferred to the Department of Veterinary Tropical Diseases Laboratory for analysis. Plasma samples were utilised for the *HSP70* and *IL-10* analyses while serum samples were used for *8-OHdG*.

2.4.1. 8-hydroxy-2′-deoxyguanosine analysis

The *8-OHdG* gene was measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit specific for the gene (BIOCOM Africa, Clubview, Gauteng, 0014, South Africa). The manufacturer's instructions were adhered to accordingly. Briefly, 50 μL of the sample was added to each well and 40 ng·μL^{-1} of biotinylated detection antibodies was added immediately to the wells. It was incubated at 37 °C for 45 min

Table 1
Feed composition utilised for the study.

Feed composition	Starter	Grower	Finisher
Ingredients (g/kg)			
Protein	180	170	160
Total lysine	11	10	9
Total methionine	4.2	3.8	3.4
Moisture	120	120	120
Fat	25	25	25
Fibre	50	60	70
Calcium	10	10	10
Phosphorus	5	4.5	4

and washed thrice. Forty nanograms per microlitre (40 ng·μL^{-1}) of HRP conjugate was added to the wells and incubated for 30 min at 37 °C and washed, hence, 90 μLof substrate reagent was added and incubated at 37 °C for 15 min. Finally, 50 μL of stop solution was added and the optical density (OD) was determined immediately at 450 nm.

2.4.2. Procedure for ribonucleic acid (RNA) extraction from whole blood

Two microgram per millilitre (2 μg/mL) of plasma was mixed with 800 μL of buffer EL. The mixture was placed on ice for 15 min. The mixture was centrifuged at 400 ×*g* for 10 min at 4 °C. The supernatant was completely removed and discarded. Fifty millimolar (50 mM) of buffer EL was added to the cell pellet and it was vortexed briefly. It was then centrifuged at 400 ×*g* for 10 min at 4 °C. The supernatant was completely removed and discarded. Three hundred and fifty (350 μL) of buffer RLT was added to the pellet. Vortexing or pipetting was done to mix in order to remove any clumps. Lysate was pipetted directly into a QIAshredder spin column in a 2 mL collection tube. It was centrifuged for 2 min at maximum speed to homogenise. The spin column was then discarded, and the homogenised lysate was saved. Three hundred and fifty (350 μL) of 70% ethanol was added to the homogenised lysate and mixed by pipetting. We carefully pipetted the sample (lysate +70% ethanol), including the precipitate that was formed into a new spin column. We centrifuged for 15 s at 10000 rpm. The spin column was transferred into a new collection tube. We added 700 μL of buffer RW1 to the collection tube. Centrifuged again for 15 s at 10000 rpm. The spin column was placed into another new collection tube, 500 μL of buffer RPE was added into the collection tube and centrifuged for the third time for 15 s at 10000 rpm. The supernatant was discarded. The collection tube (spin column) was carefully opened and 500 μL of buffer RPE was added. It was closed and centrifuged for 3 min at maximum speed. The spin column was placed in a new collection tube. The old collection tube was then discarded with the lysate and buffer. It was centrifuged for 1 min at full speed (without buffer). Finally, the spin column was transferred into a new microcentrifuge tube (final collection tube). We pipetted 50 μL of RNase-free water into the membrane (tube), we then centrifuged for 1 min at 10000 rpm to elute. Afterward, we stored the RNA in −80 °C.

2.4.3. Target genes

Three target genes *IL-10, HSP70* and *8-OHdG* that are involved in the regulation of heat stress in broiler chickens were evaluated in three treatment groups and one control group. The expression of target genes for the *IL-10* and *HSP70* were measured using reverse transcription polymerase chain reaction (RT-PCR), while the concentration of *8-OHdG* was determined using enzyme-linked immunosorbent assay. The description of the target genes and corresponding primers are shown on Table 2.

2.4.4. Standard curves and RT-PCR optimisation

Quantitative reverse transcription polymerase chain reaction was used to determine the differential gene expression *of IL-10* and *HSP70*. The RT-PCR for each gene was optimised first to determine the most appropriate annealing temperature for efficiency and specificity of amplification. Stock samples with relatively high RNA concentration (PP4, 55.50 ng/μl and CP2, 103.45 ng/μl) was used for the *HSP70* and *IL-10* gene, respectively. The number of copies of single stranded RNA was calculated using the formula:

Table 2
Primers (F, forward; R, reverse) for quantitative real-time PCR.

Gene	Symbol	Nucleotide sequence (5′-3′)
Heat shock protein 70	*HSP70*	F: 5′-CCAAGAACCAAGTGGCAATGAA-3′ R: 5′-CATACTTGCGGCCGATGAGA-3′
Interleukin 10	*IL-10*	F: 5′-AAGGCAGTGGAGCAGGTGAA-3′ R: 5′-CCAGCAGACTCAATACACAC-3′

$$\text{Number of copies} = [6.022\text{E}+23\ (\text{copies/mol})\ \text{x Concentration}\ (\text{g}/\mu\text{l})\ ] / [\text{DNA/RNA length (bp)} \times 340\ (\text{g/mol})\ ]$$

where, 6.022 × 10^{23} molecules/mol is the Avogadro's constant number, concentration as 5.5 × 10^{-8} g/μl for *HSP70* and 1.0345 × 10^{-7} g/μl for *IL-10*, and 340 Da is the average weight of a base in RNA. The length of RNA for the *HSP70* and *IL-10* are taken to be 2692 bases and 2300 bases, respectively.

Standard curved were generated using four 10-fold serial dilutions, with the aim of optimising and determining the efficiency of the RT-PCR assays. The diluent was nuclease free water. Each dilution was analysed in triplicate in each RT-PCR run. We used annealing temperatures 53, 57, 60, 63 °C and primer concentrations 0.2 and 0.4 μM. The mean of the C_q values from the dilutions in each RT-PCR test run were plotted against the logarithm of copy number of each gene to generate a standard curve. The PCR efficiency [E] (Livak and Schmittgen, 2001; Pfaffl, 2001; Vandesompele et al., 2002), expressed as a percentage, was determined using the following formula:

$$\%\text{E} = \left(10^{(-1/\text{Slope})} - 1\right) \text{x } 100$$

where slope = slope of the derivative (tangent line) of the calibration curve. Dissociation curves were also evaluated for specificity of the PCR assays. After optimisation, the most appropriate annealing temperature and primer concentrations were used in the PCR reactions.

2.4.5. RT-PCR for experimental samples

All the 28 experimental chicken (7 per group) were analysed for the two genes in separate arrays. Each RT-PCR reaction comprised x1 (final concentration) Luna® Universal One-Step RT-qPCR Mix, x1 (final concentration) Luna WarmStart RT Enzyme Mix (New England BioLabs Inc., Ipswich, Massachusetts, USA), 0.4 μM of each primer and 1 μL of template RNA in a total reaction volume of 10 μL. The RT-qPCR conditions were set with an initial reverse transcription stage at 55 °C for 10 min, followed by initial denaturation at 95 °C for 1 min, 45 cycles of denaturation at 95 °C for 10 s, annealing at optimised temperature for each primer for 10 s (60 °C for *HSP70* and 57 °C for *IL-10* and an extension step at 60 °C for 60 s). We included an additional stage of 1 cycle for the melt curve analysis, with temperatures of 95 °C for 15 s, 60 °C for 1 min and 95 °C for 15 s. A no-template control (without RNA template) was included in each PCR run. The RT-PCR was performed using a StepOnePlus™ Real-Time PCR System and the RT-PCR run data were collected using the StepOne™ Software 2.0 (Applied Biosystems, Life Technologies, Johannesburg, South Africa).

Efficiency testing of the *HSP70* and *IL-10* primers was done prior to performing gene expression analysis. The optimal annealing temperatures determined for the *HSP70* and *IL-10* primers were 60 °C and 57 °C, respectively, and the primer concentrations were 0.4 μM for each gene. The amplification efficiencies were 106.9% and 104.4%, corresponding to slopes −3.1676 and − 3.2201 respectively. The coefficients of determination (R2) were higher than 0.99, i.e., 0.9957 and 0.9934, respectively. The specificity of the primers was assessed using melting curves, which showed one peak indicating good performance.

2.4.6. Gene expression analysis

The RT-PCR quantification cycle (Cq) values of all experimental samples as well as 10-fold RNA dilutions were exported to Microsoft Excel to compute the Cq means and standard errors (SE) for each treatment group in each gene. Cq values of wells with undetectable values were excluded from further analyses. The RT-PCR data were quality controlled with the criteria that Cq values above 35 were excluded from the analyses. The standard curves generated were used to estimate *HSP70* and *IL-10* copy number (copies per ml of blood) in the experimental chicken samples, using the corresponding linear regressions.

2.4.7. Malondialdehyde concentration

The homogenates of breast muscle tissue were utilised to determine the Malondialdehyde (MDA) concentration using an ELISA kit (BIOCOM Africa, Clubview, Gauteng, 0014, South Africa), the manufacturer's instructions were adhered to accordingly.

2.5. Data analyses

Data (*HSP70* and *IL-10* genes) were log transformed to obtain a normal distribution. Comparison of gene expression levels across treatment groups in each target gene were performed using Generalised Linear Models with Gaussian distribution for *HSP70* and *IL-10* genes. While one-way analysis of variance (ANOVA) was used to compare the means between the groups for *8-OHdG* gene. This was followed by the Tukey's Honest Significant Difference (Tukey's HSD) tests to compare differences between treatment pairs. Statistical analyses were performed using R software version 4.1.2 (R Core Team, 2021) at a significance level of 0.05.

3. Results

3.1. 8-hydroxy-2′-deoxyguanosine gene

Results are presented as mean ± standard error of mean (SEM) for all the treatment groups. There was a highly significant difference between *8-OHdG* gene obtained in the control and probiotic groups ($P < 0.0001$), but no significant difference ($P > 0.05$) between the control and ascorbic acid groups. Also, the difference between probiotic and ascorbic acid groups was significant ($P < 0.05$), however, there was no significant difference ($P > 0.05$) between probiotic and probiotic + ascorbic acid groups, and between ascorbic acid and probiotic + ascorbic acid groups, respectively (Fig. 1).

3.2. Heat shock protein 70 and interleukin 10 gene

Analysis of Cq values showed that *IL-10* (mean 27.14 and median 27.19) had stronger mRNA expression than *HSP70* (mean 20.35 and median 19.88). The RT-PCR mean copy number expressions of the two different gene markers (*HSP70 and IL-10*) in the probiotic, ascorbic acid and co-administered groups compared to the control are shown in Table 3. The copy number is reported as mean ± standard error of mean for all experimental groups.

The Generalised Linear model showed that the expression of *HSP70* was 1.66 and 1.60 in the probiotic and probiotic + ascorbic groups, respectively compared with the control group. In contrast, *HSP70* had a

Table 3
Gene expression of heat shock protein and interleukin 10 in four groups of chicken. This was in an experiment to determine the effect of ascorbic acid and probiotic treatments on diverse effects of heat stress.

Parameter	Groups	Mean copy numbers ± SE ($x10^{12}$)	Coefficient	*P*-value
Heat shock protein 70	Control	1.633 ± 0.479		
	Probiotic	3.140 ± 0.684	1.662	0.149
	Ascorbic acid	1.155 ± 0.310	0.849	0.635
	Probiotic + ascorbic acid	3.110 ± 0.837	1.603	0.178
		$x10^{8}$		
Interleukin 10	Control	12.488 ± 4.725		
	Probiotic	12.623 ± 3.144	1.050	0.896
	Ascorbic acid	5.044 ± 0.282	0.951	0.897
	Probiotic + ascorbic acid	16.666 ± 5.689	1.156	0.699

Statistical analysis conducted using logarithm-transformed copy number data. SE, standard error of mean. $n = 7$.

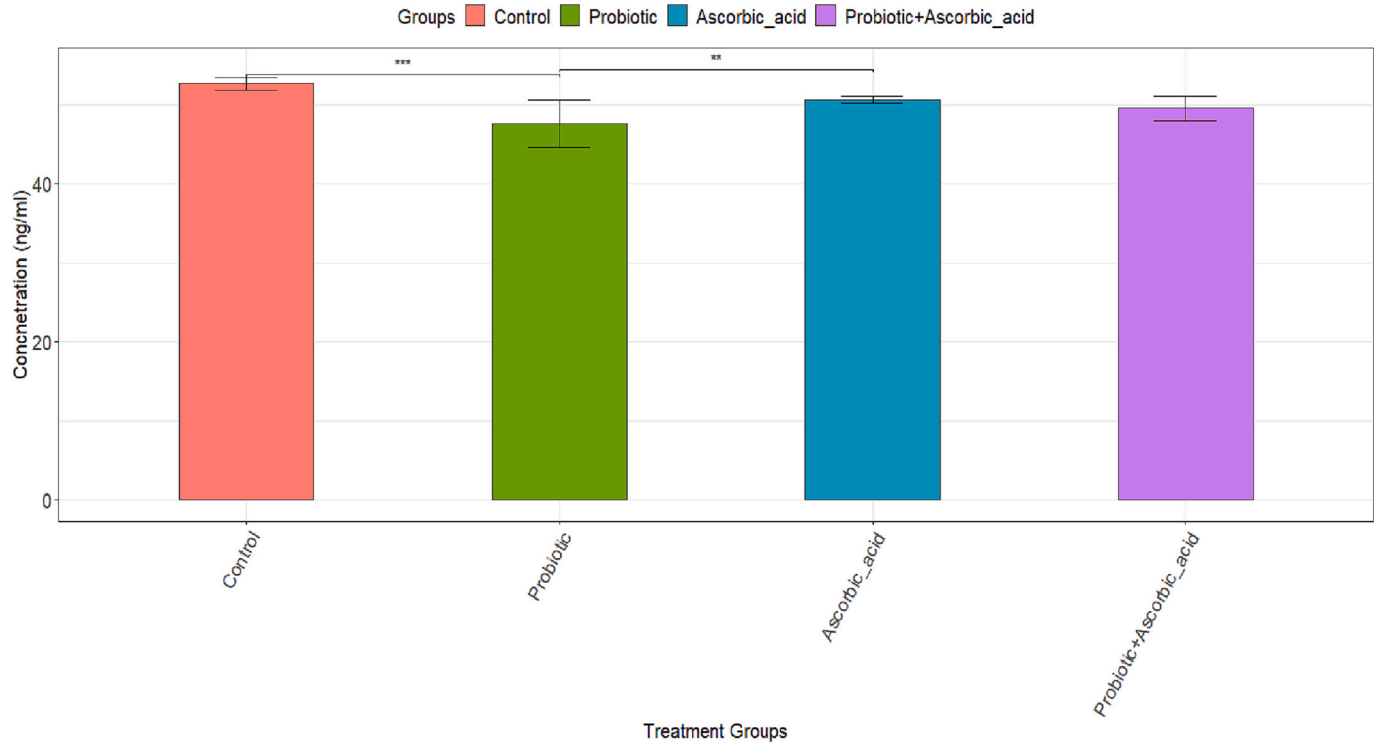

Fig. 1. 8-hydroxy-2′-deoxyguanosine (*8-OHdG*) gene in blood from broiler chickens with respect to the adverse effects of heat stress. Four treatment groups: control, probiotic, ascorbic acid and probiotic + ascorbic acid. Data were expressed as mean fold change in gene expression; the vertical bars represent standard error of mean. ($n = 7$).

lower expression (0.85 times) in the ascorbic group than the control group (Table 3). The *IL-10* gene had higher expression in probiotic + ascorbic group (1.16 times) compared with the control group, but similar expression in probiotic group and lower expression in ascorbic group (Table 3). The differences in both genes were not statistically significant ($P > 0.05$). Pairwise analysis using the Tukey's HSD showed no significant differences ($P > 0.05$) in *HSP70* and *IL-10* gene expression between groups.

3.3. MDA concentration

The concentration of MDA was significantly lower ($P < 0.02$) in the probiotic, ascorbic acid and probiotic + ascorbic acid groups of broiler chickens when compared to the control group during the study period (Fig. 2).

4. Discussion

Heat stress induces oxidative stress which promotes damages to the cell membranes; this process further stimulates various cellular activities as a response to the initial damages (Yan et al., 2022). The experimental period was thermally stressful (20–30 °C) to the broiler chickens and this fact was supported by the environmental temperature which outranged the thermoneutral zone (18–24 °C) for broiler chickens. To our knowledge, the use of yeast probiotic and ascorbic acid in alleviating oxidative stress using *HSP70, IL-10* gene expression and especially *8-OHdG* as biomarkers, have not been evaluated in broiler chickens. During this study, performance indices were negatively affected by heat stress in the group of broiler chickens void of antioxidants administration (control), while the treatment groups had better performance evident by an increase in body weight gain.

The serum concentration of *8-OHdG* was higher in the control group of broiler chickens, which could indicate that the broiler chickens were subject to heat stress during this study. This finding conforms with that of Soria-Meneses et al. (2022) who reported an increase in sperm concentration of *8-OHdG* in ram exposed to oxidative stress. The group of broiler chickens treated with probiotic had the least concentration of serum *8-OHdG*. With *8-OHdG* reported to be a genotoxicological effect via the cellular uptake of nanoparticles that are responsible for oxidative stress (Dai et al., 2019), it could be speculated that *Saccharomyces cerevisiae* probiotic was efficacious in modulating the production of ROS which triggers oxidative gene damage. *Saccharomyces cerevisiae* probiotic functions as both anti-stress and antioxidant agent hence, its efficacy in inhibiting oxidative processes may be attributed to the decrease concentration of serum *8-OHdG* observed in this group of broiler chickens. This singular process may further confer immunity and a sense of wellness to the broiler chickens during the thermally stressful season. Ascorbic acid alone was not effective in down regulating the gene in broiler chickens.

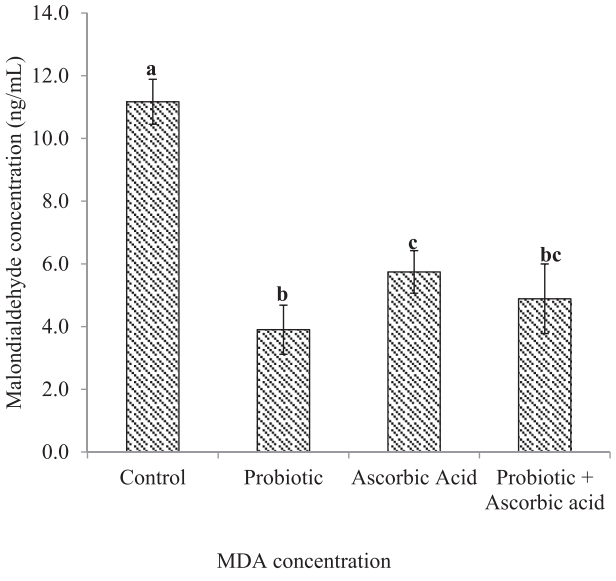

Fig. 2. Malondialdehyde concentration in broiler chickens administered probiotic and ascorbic acid and exposed to heat stress. Data are expressed as Mean ± SEM. Vertical bars represent standard error of mean.

There was no significant difference in the expression of *HSP70* during the study although, the ascorbic acid group showed the down regulation of *HSP70* gene in comparison to the control group. Heat stress is responsible for the induction of reactive oxygen metabolites (ROM) and *HSP70* (Gorman et al., 1999). Fatty acid oxidation increases to meet the requirement for energy in animals during heat stress, hence it is suggestive that the administration of ascorbic acid was effective in decreasing the accumulation as expected due to the potent antioxidant effect of the compound. The availability of ascorbic acid via diet could heighten the quantities of ascorbic acid oxidised to dehydroascorbic acid which would enhance the reduction of α-tocopheroxyl to α-tocopherol and functions to scavenge ROM produced during heat stress. Antioxidants generally slow down or decrease the process of oxidation, hence minimising the expression level of *HSP70* in birds (Sur et al., 2023). Hence, it could be speculated that animals treated with antioxidant and/or antistress agents during heat stress may inhibit the level of expression of *HSP70* in cells. That is, absence of oxidative stress could decrease the level of *HSP70* expression and vice versa. Gu et al. (2012) reported that the treatment of H_2O_2-stressed fish with melatonin decreased the level of *HSP70* due to its protective effects against oxidative stress. Mahmoud et al. (2004) has previously reported a decrease in expression of *HSP70* in broiler chickens administered ascorbic acid and exposed to chronic stress. This was attributed to the ability of the supplement to act on the cellular antioxidant cascade to reduce the accumulation of reactive oxygen species. Our findings were the same as in the literature. The administered probiotic was not effective on the expression level of *HSP70* during the study. It could be speculated from our study that yeast probiotic had an inverse proportion between the expression levels of *HSP70* and *8-OHdG* genes in broiler chickens exposed to heat stress, which requires further investigation to ascertain the cause.

The combined administration of the probiotic + ascorbic acid was effective in increasing the expression level of *IL-10* gene which indicated that systemic inflammation induced by heat stress in broiler chickens was being mitigated. Stress induces inflammatory responses, hence the up regulation of *IL-10* an anti-inflammatory cytokine serves as a protective measure against oxidative stress effect in the cells. This also revealed that ascorbic acid functioned best when combined with probiotic in enhancing the production of *IL-10* gene. Broiler chickens treated with the probiotic or ascorbic acid singly showed a decrease level of expression of *IL-10* gene in comparison with the co-administered group. This result shows that the effect of probiotic and especially ascorbic acid administration on a host could be affected by several factors like the duration and type of stressors, animal species and environment in enhancing immune response (El-Senousey et al., 2017; Wang et al., 2018). Hence, the broiler chickens could have also been susceptible to the ascorbic acid administered in stimulating anti-inflammatory cytokine release and this could be responsible for the lowest value obtained in this group. The control group had a decrease expression of *IL-10* in comparison with the co-administered group. This may be attributed to the intensity of heat stress the broiler chickens were exposed to during the study. Arendt et al. (2019) reported a decrease in expression of *IL-10* in the jejunum and cecum of broiler chickens infected with *Eimeria* which is a form of stressors to the chickens.

Antioxidants are responsible for converting free radicals to more stable products. Therefore, they play a vital role in enhancing the stability of lipids in the cell by preventing the oxidation of lipid during oxidative stress in broiler chickens (Ogbuagu et al., 2018). This could be

attributed to the decrease in concentration of MDA in the treatment groups in comparison with those of the control. This finding supports that of Deng et al. (2022) who reported the antioxidant effect of yeast probiotic in broiler chickens.

The mechanisms responsible for the modulation of *8-OHdG* and *HSP70* gene expression inversely in broiler chickens administered probiotic and exposed to heat stress are unknown and should be threads for future research. Though the sample size may be seen as a limitation of this study, the biostatistician's calculation found 56 birds to be enough for the objectives of the study and the research ethics and animal ethics committees are strict on using the minimum number of animals when approving ethics application with respect to the animal welfare policy of the University of Pretoria.

5. Conclusion

Based on the above findings, we concluded that probiotic (*Saccharomyces cerevisiae*) reduced oxidative gene damage by mitigating the excessive production of ROS which could be responsible for genetic mutation in broiler chickens. Ascorbic acid was effective in reducing the production of *HSP70*, while probiotic + ascorbic acid increased the expression level of *IL-10* genes in broiler chickens exposed to heat stress. Hence, it could be deduced that the administration of probiotic and ascorbic acid both singly and in combination were effective in alleviating the adverse effect of heat stress in broiler chickens.

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Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

No data was used for the research described in the article.

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None.

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