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Impact of *Moringa olifera* leaves or saccharomyces supplementation on carcass quality, mRNA of heat shock proteins and antioxidants in broilers exposed to heat stress

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Abstract

Application of natural feed additives with a view to enhance production performance, carcass quality and state of health has constituted an important request in production of poultry especially under heat stress conditions. This study aimed to investigate the impacts of dietary supplementation of *Moringa oleifera* and *Saccharomyces cerevisiae* on growth performance parameters, some blood biochemical findings, antioxidant status, relative mRNA of heat shock proteins HSP70 and HSP90 expressions and quality and antioxidant properties of breast meat in growing broilers exposed to heat stress. For this purpose, a total of 120 one-day-old chicks were randomly allotted into 6 dietary groups (T) with 20 chicks each, which were fed for 35 days with a basal diet. At the 21st day, Group T1 served as the negative control group unexposed to heat stress (21-22 °C). While, Group T2 served as the positive control group exposed to heat stress (33-35 °C) and relative humidity (64± 2).While, Groups T3, T4 and T5 served as experimental groups exposed to heat stress (33-35 °C) and humidity (64± 2) and supplemented with 2%, 4% and 6% of *Moringa oleifera* leaves (MOL), respectively, and Group T6 served as experimental group exposed to heat stress (33-35 °C) and humidity (64± 2) supplemented with 5% of *Saccharomyces cerevisiae* (SC). The obtained results showed that MOL and SC dietary supplementation to basal diet in groups T3, T4, T5 and T6 resulted in improved growth performance parameters (increased final B.W, BWG and F.I), improved liver and kidney function (reduced serum AST, ALT, creatinine and uric acid level), improved antioxidant status (reduced serum MDA levels and increased serum CAT, SOD and GPx activities), down-regulated relative HSP70 and HSP 90 mRNA expressions and improved breast meat quality (increased pH value, decreased drip loss, lower L* and higher a* and b*, increased protein and decreased fat contents and reduced TBARS contents) as compared to birds in group T2. In conclusion, the *Moringa olifera* leaves and *Saccharomyces cerevisiae* dietary supplementation to basal diet of heat-stressed broilers mitigated heat stress negative impacts: enhanced growth performance, antioxidant capacity, controlled HSP70 and HSP 90 relative mRNA expressions and improved breast meat quality and its oxidative state.

Keywords: Heat stress, Growth performance, Antioxidant capacity, meat quality, *Moringa olifera*, *Saccharomyces cerevisiae*.

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Introduction

The poultry have a great contribution to worldwide nutrition and food safety. It aids in the production of reasonably priced protein, important micronutrients, and energy to human beings (Mottet and Tempio, 2017). In recent times, the environmental temperature increase globally becomes apparent severe challenge attacking the chicken farming in tropical and subtropical areas. In poultry, the heat injury happened if the ambient environmental temperature surpasses the thermo-neutral zone (El-Kholy *et al.*, 2018).

Broilers are incredibly producing residing organism reached high production ranges due to genetic improvement, alternatively, its metabolism turn out to be extra speedy, causing bad thermoregulation, and as therefore being not properly adapted to hot environments (Mariante *et al.*, 2009). Heat stress (HS) is a severe trouble in many countries and has been accompanied with oxidative stress. Moreover, it results in many physiological and serological changes in chickens (Deyhim and Teeter, 1991), which extremely harm organs or tissues. It causes feed intake and digestibility reduction, and alters different organs function in animals, resulting in growth rate suppression (Fulda *et al.*, 2010). Heat stress also increases peroxidation of lipid through increasing the free radical's generation. Peroxidation of lipid is also a great reason for deterioration of quality of meat, affecting texture, flavour, colour and nutritional benefits (Botsoglou *et al.*, 2010).

One of the most important acute HS indicators is heat shock proteins (HSPs). They are highly preserved proteins that invigorate cellular protection and initiate cellular heat tolerance in different organs and tissues during the heat stress times (Al-Aqil and Zulkifli, 2009). The HSPs also keep up the organs integrity by repairing harmed or misfolded proteins and increasing cell survival (Gabai *et al.*, 1997). Heat stress-produced transcription factors linked to the heat-shock-related genes promoters, improve the HSP genes expression, and subsequently the production of HSPs (Pirkkala *et al.*, 2001). The HSPs are grouped into many families of protein with variable molecular weights. HSP 70 and 90 are the most preserved and best-studied HSPs each possesses more inducible and constitutively expressed members showing variable functions.

In poultry farms in order to ameliorate the unfavorable effects of thermal injury; dietary modulation is considered an accepted way that followed; including incorporation of minerals, amino acids, vitamins, and the plant origin feed additives in the diets (Hosseini-Vashan *et al.*, 2015a, 2015b), the natural phytogenic feed additives which are considered type of commonly used feed additive received great attention owing to their safety, accessibility, little cost and their great antioxidant activities against HS (Farghly *et al.*, 2018). Hence, great attention is directed toward the dietary supplementation of different plant origin feed additives to overcome the adverse effects of increase temperature of the environment and increasing the avian performance and production (Wang *et al.*, 2008).

Meal manipulation is also important in this context for enhancing the quality and composition of poultry meat (Santhi *et al.*, 2020). Some plants are famous to have natural antioxidants (Hassan *et al.*, 2016

and Attia *et al.*, 2018). The inclusion of natural plant extracts in diets of animal enhanced the oxidative stability, shelf life and meat colour, due to the presence of antioxidant flavonoids and other phenolic compounds that exert anti-oxidative impacts on poultry products. So, discovery of new effective herbal feed additives as plant secondary metabolites to overcome environmentally-induced heat stress became direly required. Therefore, nutritional use of antioxidants might be beneficial.

One of the most famous medicinal herbs is *Moringa oleifera* (MO) which is a rapidly growing tree, 5- 10 m height, in tropical and subtropical arias. Nearly every plant part is of value in one way or another (Liu *et al.*, 2018). Its leaves are the most nutritious part, they are characterized by being a significant source of several vitamins and minerals such as vitamin C, vitamin K, manganese, vitamin B complex, pro-vitamin A as beta-carotene, and protein among other essential nutrients (Leone *et al.*, 2015). The leaves also have been reported to contain broad quantities of total phenolic compounds, proteins, potassium, magnesium, iron, calcium, and copper (Hekmat *et al*., 2015). MO can be incorporated in ration as a micronutrient source and as a supplement to diets in avian production (Makkar *et al.*, 2007). It has anti-oxidant activities that can prevent reactive oxygen species (ROS) and free radicals formation (Ogbunugafor *et al.*, 2011), therefore, protected the cellular biomolecules against the generated free radicals oxidative damage (Khalil *et al.*, 2020).

In addition, to counteract the adverse effects of heat stress, probiotic use is considered another natural feed additive of important concern (Zulkifli *et al.*, 2000). Probiotics are living nonpathogenic and nontoxic microorganisms, which when given via the digestive tract, are beneficial to the health of the host (Mohammed *et al.*, 2019). It was recorded that probiotics possess a positive effect on the performance of poultry, improve microbial balance and synthesize vitamins, release bacteriocins, improve feed intake in broilers and layers and altering bacterial metabolism (Cramer *et al.*, 2018). Probiotic is now widely used as a growth promoter in poultry industry due to its observed positive effects. In the last decade, the *Saccharomyces cerevisiae* (SC) yeast has received great attention. Supplementation of feeds with living yeast cells has been reported to improve digestibility of feed, enhance feed efficiency and performance of animal, decrease the pathogenic bacteria number, enhance the health of animal and decrease the pad environmental effects on livestock production (Elghandour *et al.*, 2019).

However, there are scarce and varying results regarding its effect on broilers bred under HS conditions. Also, up till now, the use of MO in animals of farm as a natural feed supplement to enhance the performance and health state has been limited. Even though it was known that MO is therapeutically important for the chickens' health, unfortunately the supplementation levels of MO in poultry feed and their mechanism of actions are still under consideration. Therefore, the present study aimed to investigate the impacts of dietary supplementation of *Moringa oleifera* and *Saccharomyces cerevisiae* on growth performance parameters, some blood biochemical findings, antioxidant status, relative

mRNA of heat shock proteins HSP70 and HSP90 expressions and quality and antioxidant properties of breast meat in growing broilers exposed to heat stress.

Material and Methods

Preparation of *M. oleifera* leaf meal (MOL)

Fresh, undamaged green mature *M. oleifera* leaves (MOL) were collected during June month from a number of trees from Ismailia, Ismailia governorate, Egypt. The MO leaves were dried in the air during the day without direct exposure to sunlight, with continuous turning over to avoid growth of fungi. Then after 5 days of drying, the leaves were powdered through grinding to a fine powder to pass via a 0.15-mm sieve. Finally, the powdered leaves meal was packaged in plastic bags made of polythene, tightly closed and stored at room temperature until used.

Dietary treatments

The broilers feeding programme consisted of starter (0–21 day), grower (22–28 day), and finisher (29–35 day) basal diets that were processed to supply dietary nutrient requirements of the birds (NRC, 1994). Each basal diet was classified into 6 treatment (T) groups, which were prepared as follows: T1: a negative control, basal diet with no supplementation without heat stress (21 -22 °C) exposure; T2: positive control, basal diet with no supplementation with heat stress (30 -35 °C and relative humidity 64± 2), T3: basal diet under heat stress supplemented with 2% MOL, T4: basal diet under heat stress supplemented with 4% MOL, T5: basal diet under heat stress supplemented with 6% MOL and T6: basal diet under heat stress supplemented with 5% of *Saccharomyces cerevisiae* (SC) (purchased from Starch and Yeast Company, Ismailia, Egypt).

Management of birds and experimental design

A total of 120-day-old Cobb-500 unsexed apparently healthy broiler chicks were sourced from Ismailia Misr for Poultry Company, Egypt (S.A.E). On day 1, birds were weighed individually, and allocated equally and randomly to one of 6 treatments, 20 bird/treatment in two rooms which were controlled environmentally. The two rooms are the same in terms of size, materials of construction, climatization equipment, drinkers and feeders, etc. The treatment (T) groups were as follow: T1- Control negative with no heat stress (NHS) exposure, T2- heat stressed with basal diet (HS), T3- heat stress with minced Moringa leaves 2% (HS+MO 2%), T4- heat stress with Moringa leaves 4% (HS+MO 4%), T5- heat stress with Moringa leaves 6% (HS+MO 6%) and T6- heat stress with *Saccharomyces cerevisiae* 5% (HS+SC 5%). For the first 21 day, all the chicks were reared according to the standard guidelines for broiler rearing on the floor pen system with free reach to *ad libitum* feed and water. After 21 days, birds in the HS, and HS+MO2%, HS+MO4%, HS+MO6% and HS+SC5% treatments were exposed to the heat of 33–35°C (from 8 am to 6 pm) and 21–22°C (from 6 pm to 8 am) with 50% relative humidity till 35 day (the end of the trial).

Ethical statement

All chickens included in this study were handled according to the regulations of the Animal Ethics of Institutional Animal Care Committee (ARC-IACUC) at Agriculture Research Center, Egypt (Approval Number: ARC-AH-22-14), with

good animal practice following the guidelines of the research code of ethics (ARC-IACUC) at Agriculture Research Center. All efforts were done to minimize painful sense and discomfort to the birds.

Growth performance parameters

The data of growth performance were estimated on day 35th. The initial body weights were subtracted from the final body weights to obtain body weight gain (BWG), the residual feed was subtracted from the offered feed to get the feed intake (FI). To calculate the feed– to-gain ratio (FGR), data for FI and BWG were used. Broiler mortalities were daily recorded to correct the FGR per pen and to calculate the birds' mortality rate (%) at 21 and 35 days of age.

Sampling and analysis

Blood sampling for biochemical analysis

On 35th day of age at the end of the experiment, 5 broiler birds from each group were randomly taken from each pen. Broilers were immediately euthanized by dislocation of the neck, and then 5-ml blood samples were collected from jugular vein into a plain (without anticoagulant) clean dry test tube. Then, the blood samples were stored by freezing at 4 ºC for 12 h and allowed to coagulate to produce sera and then the blood samples were centrifuged at 3,000 × g for 15 min at 4 ºC, and then sera were collected. Finally, the plasma sera were stored at –80 ºC until analyzed. Individual serum samples were analyzed for estimation of aspartate aminotransferase (AST, U/L) and alanine aminotransferase (ALT, U/L) activities (enzymatic colorimetrically) according to Reitman and Frankel (1957) and uric acid and kept for determination of quality and antioxidant properties of breast meat. (UA, µmol/L) according to Barham and Trinder (1972) and creatinine (µmol/L) levels (enzymatic colorimetrically) according to Schirmeister *et al.*, (1964). All used assay kits were purchased from Bio-diagnostic Co., Giza, Egypt. Also serum glutathione peroxidase (GPx) according to Paglia *et al.*, (1967), catalase (CAT) according to Aebi, (1984) and superoxide dismutase (SOD) activities were determined colorimetrically (enzymatically) according to Nishikimi *et al.*, (1972), using assay kits purchased from Bio-diagnostic Co., Giza, Egypt and serum malondialdehyde (MDA) level was determined colorimetrically (chemically) according to Sato *et al.*, (1978), using an Assay ELISA Kits (Cayman Chemical Co., Ann Arbor Ml,USA). All assays were performed according to the manufacturer's instructions without any modification.

Breast muscles meat sampling for determination of

Heat stress (HS)- induced Relative HSP 70 and HSP 90 genes expressions

Quality and antioxidant properties of breast meat

At the end of the experiment, on 35th day, five broiler birds were randomly chosen from each dietary treatment group and humanely slaughtered after overnight fasting. Afterwards, the slaughtered birds were scalded, de-feathered and carcasses were eviscerated. Breast muscle meat samples (nearly 5g weight) were collected directly into plastic bags and kept at –80ºC until analysis for determination of relative HSP 70 and HSP 90 genes expressions. The other part of breast muscles meat was collected directly into another plastic bag

Heat stress (HS) - induced Relative HSP 70 and HSP 90 genes expressions

Extraction, quantification and quality of total RNA

A. RNA extraction

RNA extraction from breast muscle tissue samples was applied using QIAamp RNeasy Mini kit (Qiagen, Germany, GmbH) as 30 mg from the tissue sample was added to 600 µl RLT buffer containing 10 µl β-mercaptoethanol per 1 ml. Tubes were placed into the adaptor sets, which are fixed into the clamps of the Qiagen tissue Lyser For homogenization of samples. In 2 minutes high-speed (30 Hz) shaking step disruption was done. O the cleared lysate, 70 % ethanol one volume was added, and the steps were done according to the total RNA purification from animal tissues protocol of the QIAamp RNeasy Mini kit (Qiagen, Germany, GmbH).

N.B. On column DNase digestion was done to remove residual DNA.

B. Oligonucleotide Primers

Primers used were supplied from Metabion (Germany) are listed in table (1).

Table (1): Primers sequences, target genes and cycling conditions for SYBR green rt-PCR.

Target gene	Primers sequences (5'-3')	Reverse transcription	Primary Denaturation	Amplification (40 cycles)			Dissociation curve (1 cycle)			Reference
				Secondary denaturation	**Annealing (Optics on)**	**Extension**	**Secondary denaturation**	**Annealing**	**Final denaturation**	
ß. Actin	CCACCGCAAATGCTTCTAAAC	50˚C 30 min.	94˚C 15 min.	94˚C 15 sec.	51˚C 30 sec.	72˚C 30 sec.	94˚C 1 min.	55˚C 1 min.	94˚C 1 min.	Yuan *et al.*, (2007)
	AAGACTGCTGCTGACACCTTC									
Hsp70	TGTGTCCATCCTTACCATTGAG				59˚C 30 sec.			59˚C 1 min.		Liu *et al.* (2014)
	GCTTGTGCTTACCCTTGAACTC									
Hsp90	TCAGACTTGATAACGGTGAACCT									
	TGTCTTCTCCTCCTTCTCCTCTT									

C. SYBR green rt-PCR

Primers were used in a 25- µl reaction containing 12.5 µl from the 2x QuantiTect SYBR Green PCR Master Mix (Qiagen, Germany, GmbH), 0.25 µl from RevertAid Reverse Transcriptase (200 U/µL)

(Thermo Fisher), 0.5 µl of each primer of 20 pmol concentration, 8.25 µl of water, and 3 µl of RNA template. The reaction was carried out in a Stratagene MX3005P real time PCR machine.

D. Analysis of rt-PCR results

By the stratagene MX3005P software, amplification curves and CT values were determined. The CT of each sample was compared with that of the positive control group to estimate the variation of gene expression on the RNA of the different samples, according to the "ΔΔCt" method stated by Yuan *et al*., (2006) using the following ratio: ($2^{-\Delta\Delta ct}$).

Whereas ΔCt reference – ΔCt target = ΔΔCt

Ct control – Ct treatment* = Δ*Ct target and ***Ct control- Ct treatment= Δ*Ct reference***

E: efficiency of amplification.

Quality and antioxidant properties of breast meat

Meat Quality Measurements

Ultimate pH of breast meat

The post-mortem ultimate pH (pH_u) was estimated on the breast meat of each bird using a portable pH meter equipped with a probe (CRISON pH25, CRISON Instruments SA, Spain). The carcasses were kept in refrigerator stored at 4 °C. At 24 hours post mortem, the chilled breast pH (pH_u) was determined. The pH value was expressed as the average of three measurements.

Drip loss percentage of breast meat

The difference between sample weights before and after chilling was used to calculate the drip loss (Honikel, (1987). Breast meat samples were weighed (W1) and kept individually in a cooler at 4°C. g of malonaldehyde per kg of breast meat according the procedure described by Then, each sample was removed from the cooler after 12 hours and then absorbent paper was used to wipe the sample and then the dry breast meat was weighed (W2). Finally, this equation [(W1 - W2) ÷ W1 × 100] was used to calculate the drip loss percentage (%).

Colour measurements

Color measurements (CIE Lab) for breast meat were directly performed using a colorimeter CR-10 (konica Minolta, Inc., Osaka, japan) 24 h after slaughter following the color system of the CIE-L*a*b* (Commission International De I' Eclairage (CIE) 1976). In this coordinate system, the L* refers to lightness, a* to redness and b* to yellowness. Three readings were taken on non-overlapping zones of the sample and the average values were calculated.

Proximate analysis of breast meat

The protein, fat and ash contents of samples were analyzed according to the standard methods of the Association of Official Analytical Chemists (AOAC, 2005). Protein content was determined by the Kjeldahl's method. Fat content was recorded by ether extraction by using Soxhlet extractor and ash content was determined by combustion of samples at 550˚C overnight.

Lipid Oxidation test (Measurements of Thiobarbituric Acid-Reactive Substances (TBARS)

The lipid oxidation degree was estimated by measuring the Thiobarbituric Acid-Reactive Substances (TBARS) for broiler breast meat at 1 and 7 days stored under refrigeration, and at 30 days stored under frozen conditions, and expressed as Strange *et al.*, (1977) and Abou Sekken *et al*. (2013a) as following:

A 20 g of meat sample mixed with 50 ml of cold 20% trichloroacetic acid (TCA) for 2 minutes. The blended contents were raised with 50 ml of water, mixed together, and filtered through Whatman No. 4 filter paper. This filtrate is termed TCA extract. A 5 ml of the TCA extract was mixed with 5 ml of 0.02 M of TBA. This solution kept for 14 h at room temperature. Colorimetric absorbance was measured using UV scanning spectrophotometer at 532 nm. Readings were converted to g of malonaldehyde per kg of meat and reported as TBARS values.

Statistical Analysis

Statistical analysis and graphs regarding the data of heat stress (HS)- induced Relative HSP 70 and HSP 90 mRNA expressions were done using R (A language and environment for statistical computing) (R Core Team, 2020). Results were described as mean ± SEM, comparison of treatments for each group was performed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for multiple comparisons, and calculation of p-value. The other data were analyzed using a one-way ANOVA procedure of SPSS 19.0 for windows (SPSS Inc., Chicago, IL, 2010); followed by the application of least significant difference (LSD) test for multiple comparisons, and calculation of p-value. Groups with a p-value less than 0.05 are significantly different.

Results

The effect of *Moringa oleifera* dried powder leaves (MOL) at (2, 4 and 6%) and *Saccharomyces cerevisiae* (SC) 5% on growth performance parameters

The results regarding the effect of MOL at (2, 4 and 6%) and SC 5% on growth performance parameters including final body weight (B.W), body weight gain (BWG) and feed intake (F.I) of broilers reared under normal or HS conditions were summarized in Table 2.

Table (2). The effect of *Moringa oleifera* leaves (MOL) at (2, 4 and 6%) and *Saccharomyces cerevisiae* 5% on growth parameter in broiler chickens under normal and HS conditions. (M±S.E, N=5).

	T1	T2	T3	T4	T5	T6
F. B.W.	2012.7 ±22.6^{a}	1632.48±26.2^{d}	1788.8 ±13.3^{c}	1861.5±16.9^{b}	1789.76± 18.7^{c}	1861.1± 29.20^{b}
B.W.G.(g)	1211.2±20.4^{a}	831.79±39.06^{c}	984.2±14.8^{b}	1058.1± 11.2^{b}	989.08 ±30.12^{b}	1052.24± 34.4^{b}
F. I	1753.9±37.8^{a}	1494±44.8^{c}	1660.8±48.9^{b}	1644.88±30.4^{b}	1621.9±39.5^{b}	1665.12±44.1^{b}
F.C.R.	1.57±0.04^{a}	1.77±0.09^{b}	1.61±0.03^{a}	1.61±0.03^{a}	1.60±0.34^{a}	1.59±0.06^{a}

Different letters show statistical differences ($p < 0.05$) among treatments according to LCD test ($p < 0.05$). The values are the means ± Standard Errors SE (n =5). T1: negative control fed basal diet without additives or heat stress, T2: positive control fed basal diet and heat stress, T3: fed basal diet with 2% *Moringa oleifera* leaves (MOL) with heat stress, T4: fed basal diet with 4% *Moringa oleifera* leaves (MOL) with heat stress, T5: fed basal diet with 6% *Moringa oleifera* leaves (MOL) with heat stress, T6: fed basal diet with 5% *Saccharomyces cerevisiae (SC)* with heat stress, F.B.W.: final body weight, B. W. G.: body weight gain, F.I.: body intake, F. C. R.: feed conversion ratio.

The obtained data revealed that birds fed on basal diet supplied with different MOL at (2, 4 and 6%) and SC 5% and kept in HS condition showed a

significantly (P<0.05) higher final B.W, BWG and FI as compared to birds fed on basal diet only and kept in HS condition. Additionally, birds fed on basal diet supplied with different MOL 4% and SC 5% and kept in HS condition showed a significantly (P<0.05) higher final BW as compared to birds fed on basal diet supplied with MOL 2 and 6 % and kept in HS condition. On the other hand, birds fed on basal diet supplied with different MOL (2, 4 and 6%) and SC 5% and kept in HS condition showed a non-significant (P>0.05) final BWG and FI as compared with each other

The effect of *Moringa oleifera* dried powder leaves (MOL) at (2, 4 and 6%) and *Saccharomyces cerevisiae* (SC) 5% on blood biochemistry

HS significantly increased serum AST and ALT activities, Creatinine and uric acid (P < 0.05) levels when compared to NHS group. While AST, ALT, Creatinine and uric acid level under all experimental treatments were significantly (P<0.05) reduced compared to HS group as showed in table 3.

Table (3). The effect of *Moringa oleifera* leaves (MOL) at (2, 4 and 6%) and *Saccharomyces cerevisiae* 5% on AST, ALT, uric acid and creatinine in broiler chickens under normal and HS conditions.(M±SE, N = 5).

	T1	T2	T3	T4	T5	T6
AST (IU)	68.05± 0.65^{c}	76.39±0.32^{a}	70.39±0.37^{b}	69.61±0.06^{bc}	70± 0.7^{b}	69.78±0.68^{b}
ALT (IU)	60.89± 0.31^{c}	65.32±0.27^{a}	63.23±0.73^{b}	62.38±0.50^{b}	62.45±0.34^{b}	62.07±0.4^{bc}
Creatinine (µmol/L)	0.62± 0.01^{d}	0.77± 0.01^{a}	0.68 ±0.01^{b}	0.63± 0.13^{dc}	0.69± 0.05^{b}	0.65±0.07^{c}
Uric acid (µmol/L)	4.67 ±0.24^{b}	5.48± 0.75^{a}	4.92± 0.5^{b}	4.7 ±0.89^{b}	4.85± 0.76^{b}	4.78±0.73^{b}

Different letters show statistical differences (p < 0.05) among treatments according to LCD test (p < 0.05). The values are the means ± Standard Errors SE (n =5). T1: negative control fed basal diet without additives or heat stress, T2: positive control fed basal diet and heat stress, T3: fed basal diet with 2% *Moringa oleifera* leaves (MOL) with heat stress, T4: fed basal diet with 4% *Moringa oleifera* leaves (MOL) with heat stress, T5: fed basal diet with 6% *Moringa oleifera* leaves (MOL) with heat stress, T6: fed basal diet with 5% *Saccharomyces cerevisiae (SC)* with heat stress, AST: aspartate aminotransferase, ALT : alanine aminotransferase

The effect of *Moringa oleifera* dried powder leaves (MOL) at (2, 4 and 6%) and *Saccharomyces cerevisiae* (SC) 5% on oxidant and Antioxidant status:

HS increased MDA concentration whereas decreased GSH-Px and serum catalase activities (P < 0.05). Moreover, serum SOD activity in the HS group was relatively decreased in comparison with the NHS group (P < 0.05). In contrast, dietary MOL at (2%, 4% and 6%) or SC 5% supplementation significantly (P < 0.05) reduced serum MDA concentration whereas significantly (P < 0.05) increased serum GSH-Px, catalase and SOD activities of broilers under HS as showed in table 4.

Table (4). The effect of *Moringa oleifera* dried powder leaves (MOL) at (2, 4 and 6%) and *Saccharomyces cerevisiae* (SC) 5% on serum oxidant and antioxidant parameters in broiler chickens under normal and HS conditions. (M±SE, N = 5).

	T1	T2	T3	T4	T5	T6
CAT	3.85±0.01^{a}	2.32±0.01^{c}	2.49±0.04^{c}	3.19±0.14^{b}	2.96±0.05^{b}	3.21±0.19^{b}
GSHpx	55.66±0.05^{a}	37.72±0.21^{e}	46.53±0.2^{c}	50.95±0.33^{b}	43.12±0.65^{d}	51.72±0.43^{b}
MDA	1.38±0.02^{d}	2.15±0.01^{a}	1.82±0.25^{b}	1.64±0.14^{c}	1.77±0.11^{b}	1.55±0.28^{c}
SOD	4.71±0.18^{a}	2.89±0.27^{d}	3.60±0.05^{d}	3.89±0.07^{bc}	3.56±0.16^{c}	4.09±0.11^{b}

Different letters show statistical differences ($p < 0.05$) among treatments according to LCD test ($p < 0.05$). The values are the means ± Standard Errors SE (n =5). T1: negative control fed basal diet without additives or heat stress, T2: positive control fed basal diet and heat stress, T3: fed basal diet with 2% *Moringa oleifera* leaves (MOL) with heat stress, T4: fed basal diet with 4% *Moringa oleifera* leaves (MOL) with heat stress, T5: fed basal diet with 6% *Moringa oleifera* leaves (MOL) with heat stress, T6: fed basal diet with 5% *Saccharomyces cerevisiae (SC)* with heat stress, CAT: catalyse, GSHpx: glutathione peroxide, MDA: malondahyde, SOD: superoxide.

The effect of *Moringa oleifera* dried powder leaves (MOL) at (2, 4 and 6%) and *Saccharomyces cerevisiae* 5% on heat stress (HS)- induced Relative *HSP 70* and *HSP 90* expressions

Referring to the control group, *HSP70* and HSP 90 mRNA expressions were up-regulated significantly ($p < 0.001$) in breast muscle of the heat stressed birds group (Figs.1 and 2). Meanwhile, treatment of heat-stressed broilers with MOL at (2, 4 and 6%) and SC 5% significantly ($p < 0.001$) down-regulated these expressions in dose dependent manner as compared to the HS group and restored them near to normal levels as matched with the control group. MOL 6% (T5) is the best.

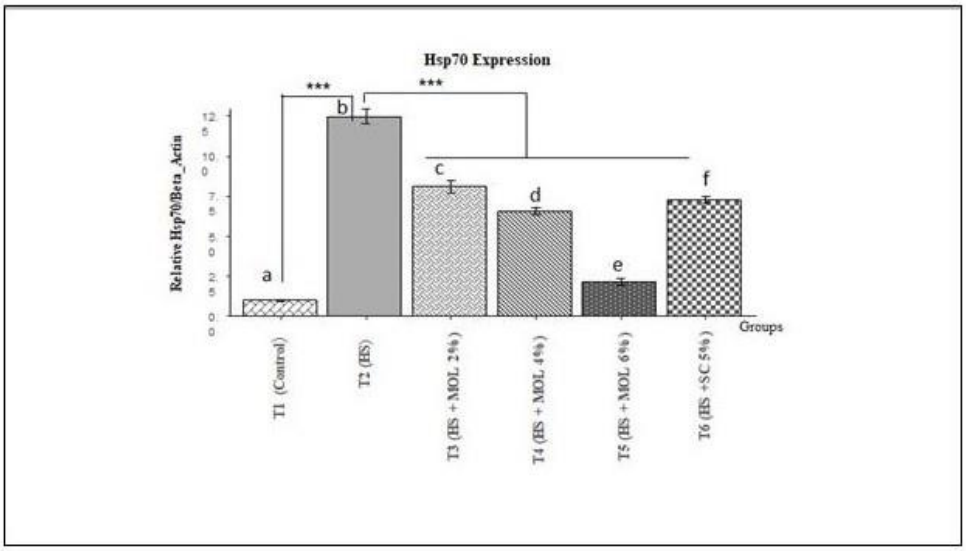

Fig.1: The impact of different treatments with *Moringa oleifera* dried powder leaves (MOL) at (2, 4 and 6%) and *Saccharomyces cerevisiae* (5%) on Relative *Hsp70* mRNA expression. *** = $p < 0.001$ in broiler chickens under normal and HS conditions. (M±SE, N = 5).

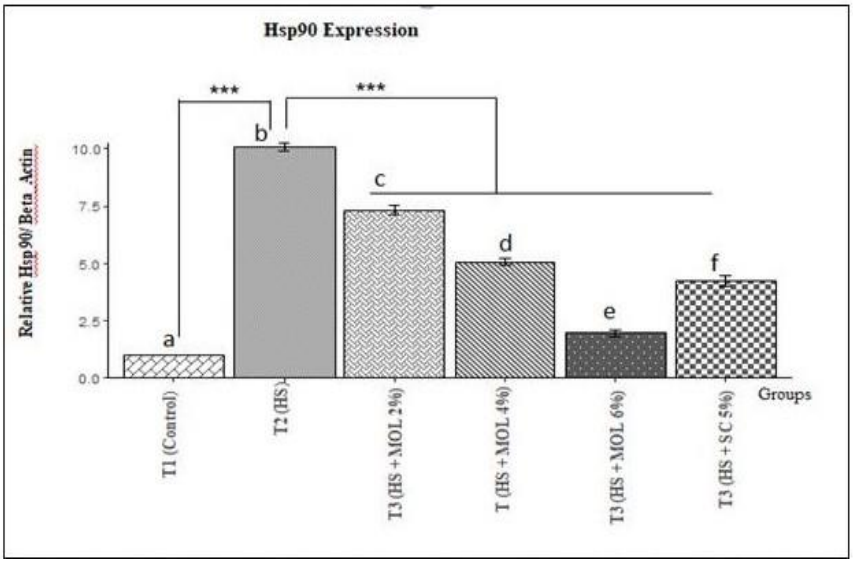

Fig.2: The impact of different treatments with *Moringa oleifera* dried powder leaves (MOL) at (2, 4 and 6%) and *Saccharomyces cerevisiae* (5%) on Relative *Hsp90* mRNA expression. *** = $p < 0.001$ in broiler chickens under normal and HS conditions. (M±SE, N = 5).

The effect of *Moringa oleifera* leaves (MOL) at (2, 4 and 6%) and *Saccharomyces cerevisiae* 5% on quality and antioxidant properties of breast meat

Ultimate pH (pH_u) of breast meat

The results recorded in table 5 revealed that breast meat of heat stressed birds fed only on basal ration had significant lower pH values (P<0.05) than that of the normal control group. Meanwhile, breast meat of heat stressed chickens fed on basal diet supplemented with MOL 2%, 4% and 6% and SC 5% showed significant (P<0.05) increased pH values as compared with heat stressed broilers group. No significant differences were recorded as matched with the control birds.

Drip loss percentage of breast meat

Drip loss percentages are showed in table 5. As a result of the HS exposure, drip loss percentages were higher in heat-stressed broilers than in normal control broilers (P<0.05). However, significant improving by decreasing drip loss was noticed in the heat stressed birds fed basal diet supplemented with SC 5% as no significant variance was recorded as matched with control birds (p<0.05). Also, significant improving by reducing drip loss was observed in the heat stressed birds fed basal diet supplemented with MOL 2, 4 and 6%.

Color of breast meat:

The findings recorded for meat color parameters, as an indication of meat quality were showed in table 5. The breast muscles meat of HS exposed chickens fed on basal diet only had significantly higher ($P < 0.05$) L* and lower ($P < 0.05$) a* and b* than those in normal control group. Meanwhile, the breast meat of heat stressed broilers fed 4% MOL and 5% SC

revealed significantly ($P < 0.05$) lower L* with heat stressed groups and divulged non-significant difference (P>0.05) as matched with control. However, there were and higher ($P < 0.05$) a* when compared no significant variability (P>0.05) of the b* parameter of meat across all the treatments subjected to heat stress.

Table (5). The effect of *Moringa oleifera* leaves (MOL) at (2, 4 and 6%) and *Saccharomyces cerevisiae* (SC) 5% on pH, drip loss % and colour parameters of broiler breast meat in broiler chickens under normal and HS conditions. (M±SE, N = 5).

Color measureme attributes	Different treatments (groups)						Sig
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	
pH_u	5.94±0.03^{ac}	5.52±0.06^{b}	5.93±0.01^{ac}	5.98±0.03^{a}	5.94±0.01^{a}	5.86±0.01^{c}	*
Drip loss%	2.86±0.09^{a}	3.89±0.09^{b}	3.60±0.05^{c}	3.14±0.05^{d}	3.61±0.05^{c}	3.04±0.03^{a} d	*
L*	51.51±0.73^{a}	63.76±0.37^{b}	61.92±0.32^{c}	52.56± 0.77^{a}	60.72±0.31^{c}	52.10±0.44 a	*
a*	1.60±0.12^{a}	0.44±0.08^{b}	0.76±0.05^{c}	1.34±0.04^{a}	1.04±0.09^{d}	1.51±0.11^{a}	*
b*	12.98±0.53^{a}	11.27±0.21^{b}	11.30±0.35^{b}	11.21±0.17^{b}	11.26±0.18^{b}	11.42±0.30 b	*

Different letters show statistical differences ($p < 0.05$) among treatments according to LCD test ($p < 0.05$). The values are the means ± Standard Errors SE (n =5). T1: negative control fed basal diet without additives or heat stress, T2: positive control fed basal diet and heat stress, T3: fed basal diet with 2% *Moringa oleifera* leaves (MOL) with heat stress, T4: fed basal diet with 4% *Moringa oleifera* leaves (MOL) with heat stress, T5: fed basal diet with 6% *Moringa oleifera* leaves (MOL) with heat stress, T6: fed basal diet with 5% *Saccharomyces cerevisiae (SC)* with heat stress, L*: lightness, a*: redness, b*: yellowness

Proximate analysis of breast meat:

The obtained results regarding the proximate analysis of breast meat of broilers were illustrated in **table 6**. The breast meat of broilers subjected to HS and fed basal diet only exhibited a significant (P<0.05) lower protein content and a higher fat content as matched with control. While those subjected to heat stress and fed MOL 4% and 6% had significantly (P<0.05) increased protein as compared with heat stressed birds. Moreover, heat stressed birds fed basal diet supplemented with MOL 4% and 6% and SC 5% had decreased fat contents as compared with heat stressed birds and non-significantly (P>0.05) different when compared with normal control. However, there is no significant variance (P>0.05) of ash content of breast meat from all treatments as matched with those in control.

Table (6). The effect of *Moringa oleifera* leaves (MOL) at (2, 4 and 6%) and *Saccharomyces cerevisiae* (SC) 5% on proximate analysis of broiler breast meat in broiler chickens under normal and HS conditions. (M±SE, N = 5).

Proximate analysis	Dietary treatments						Sig.
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	
Protein%	**26.44±0.58^{a}**	**20.82±0.23^{b}**	**22.99±0.24^{c}**	**25.83±0.32^{ad}**	**25.91±1.09^{ad}**	**24.60±0.36^{d}**	*****
Fat %	**0.87±0.05^{a}**	**2.12±0.08^{b}**	**1.67±0.18^{c}**	**0.89±0.12^{a}**	**0.99±0.16^{a}**	**0.86±0.18^{a}**	*****
Ash %	**1.45±0.15^{a}**	**1.39±0.07^{a}**	**1.41±0.07^{a}**	**1.43±0.07^{a}**	**1.44±0.08^{a}**	**1.41±0.07^{a}**	**NS**

Different letters show statistical differences ($p < 0.05$) among treatments according to LCD test ($p < 0.05$). The values are the means ± Standard Errors SE (n =5). (NS): Not significant. T1: negative control fed basal diet without additives or heat stress, T2: positive control fed basal diet and heat stress, T3: fed basal diet with 2% *Moringa oleifera* leaves (MOL) with heat stress, T4: fed basal diet with 4% *Moringa oleifera* leaves (MOL) with heat stress, T5: fed basal diet with 6% *Moringa oleifera* leaves (MOL) with heat stress, T6: fed basal diet with 5% *saccharomyces cerevisiae (SC)* with heat stress,

Lipid oxidation of breast meat

The results regarding the thiobarbituric acid-reacting substances (TBARS) as a measure of lipid oxidation in broilers breast meat were illustrated in **table 7**. The results revealed that at one day after storage in refrigerator, the degree of lipid oxidation was affected significantly by heat stress which increased ($P<0.05$) in all treatments, compared to control. Meanwhile, at 7 days after storage, TBARS content of breast meat from heat stressed birds of all treatments still significantly higher than those of control ($p<0.05$). However, MDA level was different after 30 days of freeze storage, there were no significant variations between breast meat TBARS contents of heat stressed birds that were fed basal diet supplemented with MLO, 4% and 6% and 5% SC and those in the control negative group.

Table (7). The effect of *Moringa oleifera* leaves (MOL) at (2, 4 and 6%) and *Saccharomyces cerevisiae* (SC) 5% on TBARS of broiler breast meat in broiler chickens under normal and HS conditions. (M±SE, N = 5).

TBARS		Dietary treatments						Sig.
		T1	T2	T3	T4	T5	T6	
Days of storage	1	0.277±0.01^{a}	0.593±0.03^{b}	0.423±0.00^{c}	0.334±0.01^{d}	0.327±0.00^{d}	0.325±0.00^{d}	*
	7	0.316±0.00^{a}	0.622±0.00^{b}	0.440±0.00^{c}	0.355±0.00^{d}	0.347±0.00^{e}	0.352±0.00^{d}	*
	30	0.412±0.00^{a}	0.702±0.02^{b}	0.504±0.00^{c}	0.422±0.00^{a}	0.418±0.00^{a}	0.430±0.00^{a}	*

Different letters show statistical differences ($p < 0.05$) among treatments according to LCD test ($p < 0.05$). The values are the means ± Standard Errors SE (n =5). T1: negative control basal diet without additives or heat stress, T2: positive control with basal diet and heat stress, T3: basal diet with 2% *Moringa oleifera* leaves (MOL) with heat stress, T4: basal diet with 4% *Moringa oleifera* leaves (MOL) with heat stress, T5: basal diet with 6% *Moringa oleifera* leaves (MOL) with heat stress, T6: basal diet with 5% *saccharomyces cerevisiae* (SC) with heat stress, TBARS: Thiobarbituric Acid Reactor Substances.

Discussion

The effect on growth performance parameters

In the present study, the obtained results revealed that the broilers chicken raised under HS conditions exhibited impaired growth performance parameters (decreased body weight, decreased body weight gain, reduced feed intake and increased feed conversion rate).

In the poultry industry, heat stress constitutes a significant problem leading to a serious economic loss because of its dangerous impacts on health and production (Wasti *et al.*, 2021). According to (Salles *et al.*, 2010), nutrient uptake of animal subjected to HS is diminished owing to a reduction in the capacity of intestine to absorb nutrients and reduce digestibility, likely because of the overproduction of ROS that oxidizes and destroys biological molecules in the cells, causing a variable degree of intestinal damages (Zhao and Shen, 2005). Furthermore, as a result of blood flow shift from intestine to periphery (Daghir, 2008) may lead to increased energy expenditure due to dissipation of heat which further decreased feed conversion rate (FCR) as recorded in broiler chickens (El-Deep *et al.*, 2019).

The dangerous impacts of HS on broilers growth performance have been recorded by (Akhavan-Salamat and Ghasemi, 2015). The decrease in performances of poultry exposed to HS have been accompanied with many reasons as poor appetite and lowered feed intake as a way to reduce heat increase (Sohail *et al.*, 2013), badly affected digestion as a result of intestinal morphology damage and decreased activity

of digestive enzyme (Chen *et al.,* 2014) and impaired metabolism owing to reduced activity of thyroid hormones (Sohail *et al.,* 2010), altered status of endocrine as increased level of corticosterone hormone (Sohail *et al.,* 2012).

On contrary, the broilers chicken grown under HS conditions and fed basal diet supplemented with MOL (2%, 4% and 6%) and SC5%, respond positively where growth performance parameters were advantageously regulated as they suppressed the stressful conditions. The MOL and SC treated birds showed increased body weight, body weight gain and feed intake while decreased feed conversion rate.

The positive impact of MOL could be attributed to high nutritional proprieties of MOL (Khalid *et al.*, 2020), as leaves have been recorded to contain many of essential amino acids and are considered a good source of alpha linoleic acid (Alabi *et al.*, 2017). Generally, the obtained results in this study emphasized on the previously obtained results indicated that MOL added to poultry diet possessed positive impacts on poultry growth and production which may be due to the nutrients and phytochemical contents of the plant (Kakengi *et al.*, 2007). The performance improvement in heat stressed MOL treated groups may be owing to the high vitamin C content of MO which has the ability to overcome the adverse impact of HS and enhance the productive responses. El-Moniary *et al.* (2010) recorded that vitamin C supplementation to chicken broiler feeds reared during summer HS conditions had improved the growth performance.

Addition of MOL mitigated the adverse impact of HS and therefore, aided the chicks to utilize more feed and consequently, gained more weight. The enhancement of BWG and FCR may be owing to crude protein digestibility improvement and the nutrients utilization as result of the flavonoids presence which act as antibacterial and antioxidant. Also, this improvement may be attributed to the beneficial effect of MOL on the microbes in the gut, which might improve nutrients digestion, absorption and utilization (Hassan *et al.*, 2016).

On the other side, the positive impact of SC 5% probiotic on broilers reared under normal and HS conditions may be due to offering competitive binding sites for harmful bacteria, as prebiotic microorganisms have been shown to maintain the expansion of undesirable intestinal pathogenic microorganisms in broiler birds (Tian e*t al.*, 2016). This activity has been shown to enhance GIT health state, nutrition absorption, and growth performance by reducing competition between pathogenic bacteria and the host for nutrients (Spring *et al.*, 2000). The yeast (SC) possesses a probiotic activity which decreased the infection chances in poultry (Gasemi *et al*., 2006). The probiotics impact of yeast has been found to enhance feed conversion efficiency (Ayanwale *et al.*, 2006), enhance BWG and decrease mortality (Jin *et al.*, 1997), decrease disease infection (Line *et al.*, 1997) and stimulate the immune organs (Havenaar and spon haak, 1994). The SC cell wall could improve the intestinal mucosa aspects and this is suggested that it could be the explanation for the improvement in broilers performance provided with SC Santin *et al.* (2001 and 2003). Concordantly, this was emphasized by Nilson *et al.* (2004) who stated that the broiler chicks fed on diet

supplemented with yeast to replace a part of the premix showed a better average WG and FC ratio. Yang *et al.* (2007) who stated that Mannanoligosaccharide, obtained from the outer cell wall of yeast (*Saccharomyces cerevisiae*), can be used as a growth promoter as well as a possible antibiotics alternative to in broiler rations. Sohail *et al.* (2012) who reported that supplementing *Saccharomyces cerevisiae* probiotic to broilers reared under HS conditions significantly improved growth performance which is consistent with the findings of this experiment. Recently (Ahiwe *et al.*, 2020) who stated that yeast as a hole and its cell walls dietary supplementation in broilers at 1.5–2 g/kg level could enhance growth performance and meat yield.

Biochemical findings

Regarding the biochemical findings, HS significantly ($P < 0.05$) elevated serum AST and ALT activities levels when compared to NHS birds group. On the contrary, the dietary supplementation of MO powdered leaves in bird ration reared under HS significantly ($P<0.05$) lowered the serum AST and ALT activities compared to HS non treated group. The hepatic tissue is recorded to contain enzymes like ALT and AST. These enzymes release into the blood when liver damaged (Sherwin, 2003). Increased serum AST and ALT levels can take place as a result of alteration of hepatocellular membrane either owing to blood hypoxia, toxins and toxemia exposure, metabolic disorders inflammation, or hepatic cells proliferation. Increased AST and ALT activities were also recorded with the damage of liver and intestine (Rani *et al.*, 2011).

These findings seemed compatible with (Lu-PingTang *et al.*, 2022) who reported that broiler chicks exposed to heat stress for 1 or 2 weeks displayed necrotic points on hepatic surface. Moreover, hepatic histological examination provoked inflammatory cells infiltration. These results indicated that heat stress induced hepatic tissue damage.

Antioxidant ingredients and sum of the phytochemicals found in plants may act together to decrease the levels of ROS more efficiently. Such effect could be attributed to better liver function and improved hepatic enzymes picture in birds fed diets supplemented with MOL (Hu *et al.*, 2012). This is confirmed by Divya *et al.* (2014) who stated that supplementation of broilers ration with MOL up to 1.5% significantly ($P<0.05$) reduced serum ALT and AST activities. These findings suggested that MOL could enhance the hepatic function.

Hence, in the current study the decrease in serum AST and ALT levels among MOL and SC treatment groups was recorded it may collectively indicate the normal functions of liver and intestine of chickens fed feeds containing MOL and SC during HS conditions. This result is also confirmed by the obtained results of Olugbemi *et al.* (2010) and Melesse *et al.* (2013), who stated that MOL possess a beneficial action to stimulate the immune responses and enhance intestinal health in broilers. Moreover, this could give a reflection about the protective effect of MOL addition on hepatic tissues. In the current study heat stress significantly ($P < 0.05$) increased serum creatinine and uric acid levels as matched to NHS group.

Serum uric acid and creatinine levels measurement is the most sensitive

indicators to evaluate renal functions and it is often considered an important tool in the evaluation of the Kidney state. In the current study, the obtained results seemed compatible to that recorded by Tang *et al.* (2018) who stated that birds subjected to HS displayed increased urea and uric acid levels in plasma, which indicated renal dysfunction. Moreover, histological renal tissue examination revealed tubular epithelial cells swelling, loss of brush border, necrosis, vacuolar degeneration and reduced blood flow to kidney; these findings are indicated ischemic damage of kidneys. Huang *et al.* (2018) who stated that acute HS caused an increase in some hematobiochemical parameters level such as creatinine, blood urea nitrogen, ALT and creatine kinase that may referee to the injury of some critical organs like kidney and liver.

On the other side, the dietary use of MOL in chickens ration raised under HS significantly ($P<0.05$) lowered the serum creatinine and uric acid levels compared to HS non treated group ($P<0.05$). This result is confirmed by Divya *et al.* (2014) who recorded that the dietary inclusion of MOL in broilers ration, significantly ($P<0.05$) reduced uric acid and creatinine.

Antioxidant status

Regarding to the antioxidant status, in the present study broilers fed basal diets and raised under HS conditions divulged significant reduction in catalase (CAT), super oxide dismutase (SOD), glutathione peroxidase (Gpx) activities together with increased MDA levels.

In the cells/tissues, oxidative stress occurs as a result of an imbalance between production of free radical and endogenous antioxidant defense and results in peroxidation of lipid, nitration of protein, damage of DNA, and apoptosis. Cells are subjected continuously to the free radicals generated during the physiological oxygen metabolism (Estevez, 2015). Over production of ROS and reactive nitrogen species (RNS) or their inefficient removal causes oxidative stress. ROS including superoxide ($O2^-$), hydrogen peroxide (H_2O_2), and the hydroxyl radical radicals (OH^-), are produced by oxygen metabolism and further balanced by the oxidant formation rate and the oxidant elimination rate. The intracellular decrease of ROS is physiologically removed by SOD, CAT and G_{PX} (Kurutas, 2016).

HS is considered one of the most important factors having the ability of enhancing ROS production (Li *et al.*, 2017). These phenomena resulted in oxidative stress via affecting function of mitochondria and changing ROS levels, therefore causing oxidative damage of lipids and proteins and change of oxidative stress markers levels, such as MDA, GPx, and SOD (Emami *et al.*, 2021). Indeed, the impairment of antioxidant enzyme activities caused an increase in free radicals levels leading to increased lipid peroxidation, compromised immune efficiency, pathophysiological conditions and DNA disturbance (Devi *et al.*, 2000).

On contrary, in present study, broilers chicken reared under heat stress conditions and fed basal diet supplemented with MOL (2%, 4% and 6%) and SC 5% improved the antioxidant capacity including significant reduction of the lipid peroxidation estimated parameter (MDA) and significant increase of SOD, CAT and G_{PX} enzymes activities. Indeed, his enhancement in the antioxidant efficiency is attributed to the antioxidant properties of MOL and SC supplementation. The

antioxidant efficacy of MOL is owing to its richness of numerous active ingredients such as (glycosides, tannins, polyphenols, anthocyanin, Beta-carotene, vitamins C and E, thiocarbamates, in addition to high metals contents of zinc, manganese, copper, and selenium) which are able to overcome free radicals, stimulate antioxidant enzymes and prevent oxidases (Khan *et al.*, 2017 and Rehman *et al.*, 2018).

Similarly, probiotic, as an antioxidant is able to restore the SOD activity and reduces serum MDA level in broilers raised under HS. This is emphasized by Bu *et al.* (2019) and Timothee Andriamialinirina *et al.* (2020) who noted that supplementation with products derived from Yeast involving culture of yeast were reported to improve both serum and liver tissue G_{PX}, SOD, and CAT enzyme activities and reduced MDA level in animals. Carbohydrates in yeast might share a role in enhancing antioxidant efficiency (Wang *et al.*, 2021).

The effect on relative *Hsp70* and *Hsp90* mRNA expression

Under environmental stress conditions including hyperthermia, all living organisms respond by creating a group of proteins known as heat shock proteins (HSPs) (Becker and Craig, 1994). The up regulation of HSP70 and HSP90 is a marked character of heat stress (Belhadj *et al.*, 2016). Indeed, this confirm our obtained results in the present study as HS significantly ($p < 0.001$) up-regulated HSP70 and HSP90 mRNA expressions in breast muscle of the heat stressed group as matched to the control birds group. These results also, seemed compatible with the finding of previously carried out studies of (Khan *et al.*, 2012; Song *et al.*, 2017 and Wan *et al.*, 2017a).

Meanwhile, treatment of heat-stressed broilers with MOL at (2, 4 and 6%) and SC 5% significantly down-regulated ($p < 0.001$) these up regulations in dose dependent manner in comparison to the heat stressed group and restored them near to normal levels in matching with the control. MOL 6% is the best. Previously, it is reported that HS induces oxidative damage in the different tissues in broilers (Rehman *et al.*, 2017 and Wan *et al.*, 2017b). The ROS production is considered one of the factors that cause the HSPs response and consequently the creation of the different HSPs type. Hence, oxidative stress has been suggested as a main mechanism that causes HSPs induction and biosynthesis (Mahmoud *et al.*, 2004). Antioxidant systems including both enzymatic and non-enzymatic division play an important role in maintaining redox status balance by eliminating excessive generated free radicals (Cheng *et al.*, 2020). Therefore, nutritional interventions with antioxidants which have the ability of scavenging of ROS and free radicals might be beneficial. The antioxidants decreased the need for HSPs gene expression stimulation (Sahin *et al.*, 2012). Since MOL have anti-oxidant activities because of its total phenol, vitamin c, vitamin A, vitamin B complex and Vitamin K constituents (Hekmat *et al*., 2015) can prevent ROS and free radicals formation (Ogbunugafor *et al.*, 2011) and successfuly safguarded the cellular biological molecules against the generated free radicals oxidative damage (Khalil *et al.*, 2019); Mohamed *et al.*, 2019 and Khalil *et al.*, 2020) with no need for HSPs gene expression stimulation. Therefore, in

the same context, the MOL supplementation to boilers attenuated HSP70 and HSP90 mRNA expression in breast muscles of heat stressed chickens. This suggestion is confirmed by previous findings of (Tiloke *et al.*, 2016) who stated that MOL gold nanoparticles decreased HSP70 mRNA and protein level expression in pulmonary tumor cells. Heat stressed broiler chicks fed basal diet provided with Vitamin C decreased the expression of HSP70 in the broilers liver (Jang *et al.*, 2014). Similarly, science the dietary application of natural antioxidants is considered an effective approach that is appropriate to reduce the deleterious effects of HS in birds (Seven, 2008) and feeding probiotics to heat-stressed chickens enhances antioxidant efficiency in organs as well as in muscular tissue (Jahromi *et al.*, 2016). Therefore, the SC supplementation to boilers weakened HSP70 and HSP90 mRNA expression in breast muscles of broiler chickens exposed to HS.

Quality and antioxidant properties of breast meat

Ultimate pH of breast meat

The breast meat of broiler chickens exposed to HS fed only on basal diet had significant lower pH_u values ($P<0.05$) than that of the control birds. Meanwhile breast meat of heat stressed broilers fed on basal diet supplemented with MOL 2%, 4% and 6% and SC 5% showed significant ($P<0.05$) increased pH_u values as matched with broiler chickens exposed to HS, and no significant differences were recorded as matched with the normal control group.

In the muscle, HS significantly increased the production of lactic acid thereby fasting the pH decline rate and consequently decreasing the breast muscle quality, which lastly leads to pale, soft, and exudative meat (PSE-like meat) in poultry (Wang *et al.*, 2017). HS could induce a sharp decrease in the metabolism of birds, which in turn will produce dangerous complications, such as a color change, decrease in muscle pH and water-holding capacity (WHC) of chicken meat (Gonzalez-Rivas *et al.*, 2020). MOL is a good source of vitamin E and selenium (Moyo *et al.*, 2011). As a result of existence of these contents, it can be hypothesized that the MOL dietary addition might have stabilized the muscle membrane by stimulating antioxidants and inhibiting free radicals production (Alabi *et al.*, 2017). Moreover, increasing muscle pH values in MOL supplemented groups might have contributed towards the stabilization of the volume of myofibrils decreasing the protein denaturation, thus leading to conservation of water within the muscle cells (Honikel, 1998). In addition, Cramer *et al.* (2018) reported that providing with probiotic could mitigate the pH decrease of breast muscles from heated stressed broiler by likely affecting the rate of postmortem glycolysis metabolism.

These findings are consistent with many previously carried out studies. Zhang *et al.* (2012), Tang *et al.* (2013) and Wang *et al.* (2017) recorded that the final pH of muscles was greatly reduced in chickens exposed to HS before slaughter. Wapi *et al.* (2014) and Rehman *et al.* (2018) noted that breast muscles of the birds feed on basal diet supplemented with MOL had higher ($p<0.05$) pH values when matched to the non-supplemented group. Moreover, Cramer *et al.* (2018) stated that probiotic supplementation significantly ($P < 0.05$) increased ultimate pH of broiler chickens breasts within birds group exposed to heat

stress. Aksu, *et al.* (2005) and Benamirouche *et al.* (2020) recorded an increase in breast muscle pH in broilers fed on a diet provided with SC compared to those fed unsupplemented diet.

On the other side, these findings are not in agreement with Petracci *et al.* (2001) and Schneider *et al.* (2012) who revealed that high thermal degrees did not affect the values of pH_u of broiler chicken breast muscle. Moreover, Łukasiewicz *et al.* (2014) noted that there was no significant difference caused by dietary supplementation with plant additives on the final pH of broiler meat. In addition, Nkukwana *et al.* (2015) and Cui *et al.* (2018) recorded that pH was not affected by MOL addition to the diet ($P > 0.05$). In addition, Aristides *et al.* (2018) reported a significant reduction ($P < 0.05$) of the pH of breast meat at the inclusion of different levels of SC. Stęczny and Kokoszynski (2019) mentioned that probiotic did not possess effect on pH of the broiler meat. Recently, Nduku *et al.* (2020) reported that there was not any variation in the pH values of meat across the treatments of moringa or probiotics.

Drip loss percentage of breast meat

Drip loss increases obviously with decreasing the ultimate pH_u via stronger shrinkage of myofibrils caused by decreased electrostatic inconsistency between the filaments. In this study, as a result of the HS exposure, drip loss percentages were higher in heat-stressed birds than in normal control broilers ($P<0.05$). This increased drip loss % in chickens subjected to HS could be attributed to the recorded reduced ultimate pH of heat stressed birds. Moreover, this result is confirmed by Strasburg and Chiang (2009) who reported that heat stress causes anaerobic glycolysis inside the muscles during and after slaughtering of the animal, thus, more H^+ and lactic acid accumulate in the muscles owing to ATP hydrolysis. This leads to a fast decrease in the pH of muscles leading to low water holding capacity. Łukasiewicz *et al.* (2014) recorded that a drop in pH value is accompanied by increased drip loss. Zaboli *et al.* (2019) reported that HS increases drip loss of the meat.

However, significant improving by decreasing drip loss was noticed in the heat stressed birds fed basal ration supplied with SC 5%. Moreover, birds fed on basal diet provided with SC 5% showed no significant ($p<0.05$) difference as matched with control. These results seemed compatible with Nduku *et al.* (2020) who recorded a decreased ($P<0.05$) drip loss percentage in probiotic fed chickens.

On contrary, the results are not in consistence with Cramer *et al.* (2018); Stęczny and Kokoszynski (2019) who found that the feeding of probiotics possessed no significant effect on drip loss of the breast muscles.

Also, significant improving by reducing drip loss was observed in the heat stressed birds fed basal diet supplemented with MOL 2, 4 and 6%.

These findings were confirmed by Rehman *et al.* (2018) who recorded that the supplementation of MOL in feed decreased drip loss of breast meat.

In contrast, Cui *et al.* (2018) reported that drip loss was not affected by MOL supplementation to the diet.

Color of breast meat

When matched with normal control birds group, breast meat of heat stressed broilers with basal feed had significantly ($P < 0.05$) higher L* and lower ($P < 0.05$)

a* and b*. Pre-slaughter HS could hastens the development of rigor mortis , which induced higher L* value in chicken meat (McKee and Sams, 1997). Pale meat color could be attributed to increase light scattering associated with denaturation of protein and changes in sarcomere length (Swatland *et al.,* 2004). While, the decreased a* value results indicated that there was more oxidized myoglobin in the muscle of heat-exposed birds, so improved oxidative stability should be the main cause for improved meat color (Mancini and Hunt, 2005). These results go hand in hand with Tang *et al.* (2013) and Wang *et al.* (2017) who noticed that the value of meat color lightness significantly increased with exposure to heat stress. However, the redness value decreased significantly.

Meanwhile, the breast muscle meat of broilers exposed to HS fed 4% MOL and 5% SC revealed significantly ($P < 0.05$) lower L* and higher ($P < 0.05$) a* parameters when matched with heat stressed groups but divulged non-significant difference (P>0.05) in comparison with control group. However, there were no significant variability (P>0.05) of the b* parameter of meat across all the treatments subjected to heat stress.

These results confirmed by Cui *et al.* (2018) who recorded that MOL addition to the diet increased a∗ values. Moreover, Nduku *et al.* (2020) noted that birds fed on MOL treatment showed a higher a* value as compared with control. The higher levels of redness in meat obtained from chickens that fed on diet supplemented with MOL could be attributed to the presence of high levels of iron in moringa leaves. The iron content is indicated by concentration of iron of muscular pigment and it founds in the oxymyoglobin, which caused the meat red color (Kadim *et al.,* 2003). Moringa leaves powder reported to contain 28 mg of iron, meanwhile, beef meat has only 2 mg (Gopalakrishnan *et al.*, 2016).

On the contrast, the results are not in accordance with Aristides *et al.* (2018) and Stęczny and Kokoszynski (2019) who recorded no variations in color standards of broiler breast meat samples when provided diet with components of SC as a prebiotic. Additionally, Cui *et al.* (2018) stated that addition of MOL to the diet decreased b∗ values while L∗ values were not affected.

Proximate analysis of breast meat

The broilers breast meat subjected to HS and given basal diet exhibited a significant (P<0.05) lower protein content and a higher content of fat as matched with normal controls. These findings are in accordance with Geraert *et al.* (1996) who recorded more fat and low protein contents in broiler-chickens subjected to HS. Lu *et al.* (2007) and Zhang *et al.* (2012) reported also increased fat deposition.

Meanwhile, broiler chickens breast meat exposed to HS and given MOL 4% and 6% had significantly (P<0.05) increased protein and decreased fat contents, and those exposed to HS and fed 5% SC had significantly decreased fat contents (P<0.05) as matched with heat stressed chicken birds given basal feed, and non-significantly (P>0.05) different when matched with control. However, there is no significant variation (P>0.05) in ash content of breast meat from all treatments as matched with those in control group.

These findings were emphasized by Mickdam *et al.* (2021) who found that

MOL addition significantly increased (P<0.01) protein content and decreased fat content. In addition, Benamirouche *et al.* (2020) recorded those dietary probiotics supplementation had non-significant impact on ash contents of breast meats but significantly ($p < 0.05$) decreased total lipid contents. Moreover, Zhou *et al.* (2010) and Inatomi (2015) reported no effect of probiotic on the protein and ash contents of broilers breast muscles. On the other side, Macelline *et al.* (2017) and Stęczny and Kokoszynski (2019) reported no effect of probiotics and SC yeast on the content of fat of chickens breast muscles meat.

It has been markedly obvious that HS decreases synthesis of protein by altering transcription of ribosomal gene. Thus, the increases of ambient temperature can decrease the capacity of ribosomes resulting in reduction of protein synthesis rate, and consequently the decrease of the protein deposition. While, it increases deposition of fat which could be correlates to decrease in basal metabolism and physical activity (Zhang *et al.*, 2012). High environmental temperature deactivates lipolysis via down regulation of the enzymes included in lipid decomposition leading to more deposition of fat and decreased protein content in muscles (Lu *et al.*, 2019). MO leaves have the capability to enhance absorption and retention of protein (Lu *et al.* 2016). They are characterized by the presence of nutritious elements, highlighting the content of crude protein of the dried leaves, meaning they are a good probable source of protein supplement (Moyo *et al.*, 2011). It should be mentioned that leaves of MO are considered a very wealthy source of essential amino acids, which are sometimes lacking in many vegetables (Mahmood *et al.*, 2010). The decrease in percentage of fat could be attributed to fat metabolism variation caused by MOL supplementation (Mickdam *et al.*, 2021). Several previous studies displayed that MOL supplementation decreased synthesis (Sangkitikomol *et al.*, 2014) and prevent fat accumulation by inhibiting the adipogenesis and enhancing the lipolysis (Xie *et al.*, 2018).

Oxidation of lipid of broiler breast meat

Regarding the thiobarbituric acid-reacting substances (TBARS) as a an indicator of oxidation of lipid in broilers breast muscle meat, the results revealed that at one day after storage in refrigerator, the degree of lipid oxidation was affected significantly via heat stress which increased significantly (P<0.05) in all treatments as compared to control group. Meanwhile, at 7 days after storage, TBARS content of breast muscles meat samples from birds of all treatments exposed to HS still significantly ($p<0.05$) higher than those birds of normal control. However, the concentration of malonaldehyde (MDA) was different after 30 days of storage by freezing, there were no significant variations between breast meat sample TBARS contents of birds exposed to HS that were given basal feed provided with MLO 4% and 6% and SC 5% and those birds in the control group.

These findings are in the same line with AbouSekken (2015), Gbore *et al.* (2021) and Mickdam *et al.* (2021) who also highlighted significant decrease in oxidation of meat lipid of broiler given different doses of MOL levels. Zhang *et al.* (2005) reported no variations in values of TBARS of breast meat samples ($P > 0.05$) among the SC treated groups until 6 days

of incubation, but at 10 days, the values of TBARS of SC treated bird groups were significantly ($P \leq 0.05$) lower as compared to the control. Additionally, Aristides *et al.* (2018) reported that the supplementation of SC significantly lowered ($P < 0.05$) oxidation of lipid, which is considered an important factor regarding the decision of any consumer in purchasing the product as it indicates quality as a change in odor or shortened shelf life. Moreover, Chao *et al.* (2019) stated that feeding of probiotic to broilers subjected to HS may minimized breast muscle degradation possibly via muscle's increased antioxidant capacity and reduced phospholipid content.

Antioxidants had an important role in decreasing oxidative stress by scavenging free radicals during the oxidative process, thereby acting as scavengers of ROS and releasing catalysts (Goyal and Brahma, 2014). The antioxidant enzymes save cells against the deleterious impacts of ROS, thereby having a protective function towards oxidative damage to prevent degradation of meat tissue and consequently improve meats shelf life (Varasteh *et al.*, 2015). One of the plants that can be utilized as an antioxidant is *Moringa oleifera*. It has nearly 46 antioxidants, that, it is considered one of the most potent sources of naturally occurred antioxidants that aid cells to overcome the free radicals impact (Umar *et al.*, 2018). MOLs are privileged in flavonoids and essential micronutrients such as zinc or selenium that have antioxidant action or are directly linked to this process, (Moyo *et al.*, 2011). Additionally, both vitamin C and E found in *M. oleifera* also act as antioxidants (Trigo, *et al.*, 2021). These findings could be attributed to existence of phenolic antioxidants in both powdered moringa and its ethanolic extract which enhanced the oxidative steadiness of abdominal fat in poultry (AbouSekken *et al.*, 2013b). In addition, Zhang *et al*. (2005) reported that in SC supplements there are some antioxidant components that may alter the profile of oxidative fat or fatty acids in meat. Glucose tolerance factor fractions is present and act as an antioxidant (Ampel *et al.*, 2000) and also copper-zinc superoxide dismutase (acts as oxidation-retarding factor) (Meyer *et al.*, 1994).

Conclusion

It could be concluded that the *Moringa olifera* leaves 4% and *Saccharomyces cerevisiae* 5% dietary supplementation to basal diet of heat stressed broilers mitigate heat stress negative impacts manifested by enhanced growth performance, antioxidant capacity, controlled relative HSP70 and HSP 90 mRNA expressions and give the best improvement of quality of pectoral meat and its oxidative state.

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