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Role of *Saccharomyces* Probiotic in Addressing Heat Stress in Broiler Chickens

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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 *Saccharomyces cerevisiae* on growth performance parameters, some blood biochemical findings, antioxidant status in growing broilers exposed to heat stress. For this purpose, a total of 80 one-day-old chicks were randomly allotted into 4 dietary groups with 20 chicks each, which were fed for 35 days with a basal diet., Group1 served as the negative control group unexposed to heat stress (21-22 °C). While, Group 2 served as the positive control group exposed to heat stress (33-35 °C).While, Groups 3 un exposed to heat stress and supplemented with *Saccharomyces cerevisiae* (SC) and Groups 4 served as experimental groups exposed to heat stress (33-35 °C) and supplemented with *Saccharomyces cerevisiae* (SC). respectively The obtained results showed that SC dietary supplementation to basal diet in groups T3, T4 resulted in improved growth performance parameters (increased final B.W, BWG, F.C and F.C.R), improved liver and kidney function (reduced serum AST, ALT, creatinine and uric acid level), improved protein level (total protein& albumin & globulin), improved antioxidant status (reduced serum MDA levels, Cortisol and increased serum CAT, SOD, TAC and GPx activities), increase antibody titer against IB& ND, decrease level of glucose, PH, Haptoglobin, CRP. as compared to birds in group 2.

Keywords: Heat stress, Growth performance, Antioxidant capacity, *Saccharomyces cerevisiae*.

Introduction:

The poultry industry is growing across the world to fulfill the increasing demands of poultry meat and eggs. Poultry birds play a significant role in boosting the economy of many developing countries, providing nutrition and also contributing to the livelihood of many, particularly in rural areas (**Demeke 2004**).

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).**

Heat stress **(**HS**),** one of the most serious climate problems of tropical and subtropical regions of world, negatively affects the production performance of poultry and livestock. Concisely, HS is characterized by endocrine disorders, reduced metabolic rate, lipid peroxidation, decreased feed consumption, decreased BW gain, higher feed conversion ratio **(**FCR**),** immunosuppression, and intestinal microbial dysbiosis **(Sohail et al., 2011).**

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).**

Heat stress results from a negative balance between the net amounts of energy flowing from the animal's body to its surrounding environment And the amount of heat energy produced by the animal. This imbalance may be caused by variations of a combination of environmental factors

(e.g: sunlight, thermal irradiation, and air temperature, humidity and movement) and characteristics of the animal (e.g: species, metabolism rate and thermoregulatory mechanisms). Environmental stressors, such as heat stress, are particularly detrimental to animal agriculture (**Nienaber et al., 2007**–**Renaudeau et al., 2012**).

A recent study **(Felver et al., 2014)** showed that birds subjected to heat stress conditions spend less time feeding, more time drinking and panting, as well as more time with their wings elevated, less time moving or walking, and more time resting.

Animals utilize multiple ways for maintaining thermoregulation and homeostasis when subjected to high environmental temperatures, including increasing radiant, convective and evaporative heat loss by vasodilatation and perspiration **(Yousaf et al ,.2019).**

Probiotics are live microbes that are given as food additives and provide benefits to the host by improving the balance of intestinal microbial populations **(Abudabos et al. 2016)**.

Two basic Mechanisms by which probiotic act to maintain a beneficial microbial population include competitive exclusion and immune modulation. Competitive exclusion involves competition for substrates, production of antimicrobial metabolites that inhibit pathogen and competition for attachment sites **(Yang et al., 2009)**.by directly interacting with gut mucosal immune system, probiotics can modulate either innate or acquired immunity **(Dugas et al., 1999).** Further, specific immune modulatory effects of probiotics are dependent on the strain or species of bacteria included in the probiotics **(Edens, 2003 and Huang et al., 2004**).

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).**

Saccharomyces cerevisiae is arguably one of the most studied microorganisms. However, its mechanism of action as a probiotic has not been fully understood **(Ahmad., 2006).** It has been reported that it elaborates enzymes which aid in digestion and produces lactic acid which increases acidity and reduces pH of the digestive tract, thereby preventing the growth of pathogenic organisms and aiding in enzymatic activity. This increases digestibility and utility of minerals, proteins, and amino acids **(Gunal et al. 2006)**.

Saccharomyces cerevisiae has mannan oligosaccharide (MOS) in its cell wall which is a natural feed additive that is involved in helping the growth of beneficial microbes and inhibiting that of the pathogenic organisms **(Yang et al. 2008).**

2- Material & Method:

Dietary treatments

The broilers feeding programme consisted of starter (0–21 day) and finisher (21–35 day) basal diets that were processed to supply dietary nutrient requirements of the birds (NRC, 1994). Each basal diet was classified into 4 Groups, which were prepared as follows: G1: a negative control, basal diet with no supplementation without heat stress (21 -22 °C) exposure; G2: positive control, basal diet with no supplementation with heat stress (30 - 35 °C), G3: basal diet un exposed to heat stress and supplemented with *Saccharomyces cerevisiae* (SC), G4: basal diet under heat stress supplemented with *Saccharomyces cerevisiae* (SC)**.**

Management of birds and experimental design

The present experiment was conducted on 80,healthy one day old unsexed broiler (Arbor Ecars) chicks purchased from El-kahera Poultry Company .the chicks were randomly allocated into 4 groups. The broiler chicks were fed on well balanced ration. Starter diet was given till the 20 day of age after the chick were fed on finisher diet which was given from the 21 day till the end of the experiment .water & food were offered adlibitium. The chicks were housed in clean well ventilated separate experimental rooms, previously fumigated with formalin & potassium permanganate. The floor was bedded by fresh clean chopped wheat straw forming litter of 3.5cm depth. Each room was provided by suitable numbers of feeders & water utensils. The chicks were vaccinated against most common viral diseases which infect the broiler chicks.

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.

Probiotic:

Saccharomyces was obtained from biotechnology department at Animal Health Research Institute, Dokki, Giza, Egypt (ARC-AHRI).

Dose: 2.5 $\times 10^7$ CFU/g ration **(Aalaei et al., 2018**).

Route: on ration.

Blood sample(serum):

Serum was collected from 5 birds at the15th& 35stdays of ages in plain clean well dried centrifuge tube used for separation of serum to be used in estimation of biochemical parameters & serological studies.

Statistical Analysis:

Data statistical analyzed & each reading represents means $\pm$ standard deviation. The statistical evaluation of all data done using one-way analysis of variance (ANOVA) followed by dunnett's lest using a computer program (Statistical Package for Social science, version 16) P value $\leq$ 0.05 & P value $\leq$ 0.001 regarded as statistically significant SPSS program (2008).

Results:

1--Effect of *Saccharomyces* on growth performance

a) Body weight (B.W)

The effect of supplementation of *Saccharomyces's* on the B.W in broiler chicken is presented in Table (1)

Feeding of chicken on diet supplemented with *Saccharomyces's* (2.5 $*10^7$ CFU/g) induced a significant($P < 0.05$) increase in body weight compared with control group all over the experimental period.

Chicks experimentally exposed to heat stressed and fed on basal diet evoked a significant ($P < 0.05$) decrease in body weight all over the experimental period.

Feeding of heat stressed chicken on diet supplemented with *Saccharomyces's* (2.5 $*10^7$ CFU/g) displayed a significant($P < 0.05$) increase in Body weight compared with heat stressed group all over the experimental period.

b) B.W.G

The effect of supplementation of *Saccharomyces's s* on the B.W.G in broiler chicken is presented in Table (1)

Feeding of chicken on diet supplemented with *Saccharomyces's* (2.5 *10^7 CFU/g) induced a significant($P < 0.05$) increase in body weight gain compared with control group all over the experimental period.

Chicks experimentally exposed to heat stressed and fed on basal diet evoked a significant ($P < 0.05$) decrease in body weight gain all over the experimental period.

Feeding of heat stressed chicken on diet supplemented with *Saccharomyces's* (2.5 *10^7 CFU/g) displayed a significant ($P < 0.05$) increase in body weight gain compared with heat stressed group all over the experimental period.

c) Food consumption (F.C):

The effect of supplementation of *Saccharomyces's* on the F.C in broiler chicken is presented in Table (2)

Feeding of chicken on diet supplemented with *Saccharomyces's* (2.5 *10^7 CFU/g) induced a significant($P < 0.05$) increase in food consumption compared with control group on 15^{th} days of age and significant($P < 0.05$) decrease on 35^{st} days of age.

Chicks experimentally exposed to heat stressed and fed on basal diet evoked a significant ($P < 0.05$) decrease in food consumption all over the experimental period.

Feeding of heat stressed chicken on diet supplemented with *Saccharomyces's* (2.5 *10^7 CFU/g) displayed a significant($P < 0.05$) increase in food consumption compared with heat stressed group all over the experimental period.

D) Feed conversion ratio (F.C.R):

The effect of *Saccharomyces's* on F.C.R of heat stress exposed chicken was illustrated in table (2)

Feeding of chicken on diet supplemented with *Saccharomyces's* (2.5 *10^7 CFU/g) induced non-significant($P < 0.05$)change in food conversion ratio compared with control group on 15^{th} days of age and significant($P < 0.05$) decrease on 35^{st} days of age.

Chicks experimentally exposed to heat stressed and fed on basal diet evoked a significant ($P < 0.05$) increase in food conversion ratio on 15^{th} days of age and significant ($P < 0.05$) decrease on 35^{st} days of age.

Feeding of heat stressed chicken on diet supplemented with *Saccharomyces's* (2.5 *10^7 CFU/g) displayed a significant($P < 0.05$) decrease in food conversion ratio compared with heat stressed group all over the experimental period.

Table (1): **The effect of *Saccharomyces's* on body weight and body weight gain in broiler chicken exposed to heat stress on the15^{th}and35^{st} days of age.**

Days / Groups	15^{th}			35^{st}	
	Initial B.W	**B.W. (gm)**	**B.W.G (gm)**	**B.W. (gm)**	**B.W.G (gm)**
Group 1	70.86±1.79	386.00±4.30^{de}	315.4±4.65^{ed}	1823.6±66.97^{e}	1437.6±66.43^{d}
Group 2	72.88±1.62	244.4±4.69^{f}	171.52±3.53^{f}	1493.4±34.61^{f}	1249.0±30.88^{e}

Group 3	73.58±1.66	421.8±5.66^{ab}	348.22±7.06^{ab}	2023.4±8.63^{c}	1601.6±9.78^{c}
Group 4	73.64±1.25	394.4±4.30^{cd}	320.36±4.76^{cd}	1919.6±24.49^{d}	1525.6±23.68^{c}

Table 2: The effect of *Saccharomyces's* on food consumption and food conversion ratio in broiler chicken exposed to heat stress on the 15th and 35st days of age.

Days / **Groups**	**15th**		**35st**	
	FC	FCR	FC	FCR
Group 1	1237.8±19.22^{c}	3.53±0.42^{c}	1816±10.29^{a}	1.272±0.05^{a}
Group 2	1086.8±12.40^{d}	6.332±0.16^{a}	1438±20.34^{e}	1.1536±0.03^{ab}
Group 3	1306.4±6.23^{ab}	3.75±0.07^{c}	1763±8.60^{ab}	1.097±0.01^{abc}
Group 4	1304.4±3.41^{ab}	4.01±0.06^{bc}	1646±22.93^{d}	1.078±0.01^{abc}

Letter differ at p < 0.05.

2--Effect of *Saccharomyces's* on liver function test (ALT& AST)

The effect of *Saccharomyces's* on ALT& AST of heat stress exposed chicken was illustrated in table (3)

Feeding of chicken on diet supplemented with *Saccharomyces's* (2.5 *10^7 CFU/g) induced significant (P < 0.05) decrease in ALT & AST compared with control group all over the experimental period.

Chicks experimentally exposed to heat stressed and fed on basal diet evoked a significant (P < 0.05) increase in ALT & AST all over the experimental period.

Feeding of heat stressed chicken on diet supplemented with *Saccharomyces's* (2.5 *10^7 CFU/g) displayed a significant (P < 0.05) decrease in ALT & AST compared with heat stressed group all over the experimental period.

Table 3: The effect of *Saccharomyces's* on liver function test in broiler chicken exposed to heat stress on the15thand 35st days of age.

Days / **Groups**	**15th**		**35st**	
	ALT (µl/ml)	**AST (µl/ml)**	**ALT (µl/ml)**	**AST (µl/ml)**
Group 1	74.6±0.92c	99.8±2.15c	78.6±1.20b	109.4 ±0.87b
Group 2	93.6±2.01a	231±9.92a	95.2±1.85a	238.6 ±0.92a
Group 3	68.8±0.58d	96.8±0.66d	63±0.94e	89.4 ±1.46c
Group 4	80.2±1.06b	124±2.55b	74.2±1.49c	107.2 ±1.93b

Letter differ at p < 0.05.

3--Effect of *Saccharomyces's* on kidney function test (creatinine & uric acid)

The effect of *Saccharomyces's* on creatinine & uric acid of heat stress exposed chicken was illustrated in table (4)

Feeding of chicken on diet supplemented with *Saccharomyces's* (2.5 *10^7 CFU/g) induced significant ($P < 0.05$) decrease in creatinine & uric acid compared with control group all over the experimental period.

Chicks experimentally exposed to heat stressed and fed on basal diet evoked a significant ($P < 0.05$) increase in creatinine & uric acid all over the experimental period.

Feeding of heat stressed chicken on diet supplemented with *Saccharomyces's* (2.5 *10^7 CFU/g) displayed a significant ($P < 0.05$) decrease in creatinine & uric acid compared with heat stressed group all over the experimental period.

Table 4: The effect of *Saccharomyces's* on Kidney function test in broiler chicken exposed to heat stress on the15th and 35st days of age.

Days / Groups	15th		35st	
	Creatinine (µl/ml)	Uric acid (µl/ml)	Creatinine (µl/ml)	Uric acid (µl/ml)
Group 1	0.604±0.03bc	2.86±0.06bcd	0.772±0.02bc	3.52±0.15d
Group 2	1.08±0.04a	5.52±0.48a	1.244±0.03a	7.32±0.27a
Group 3	0.518±0.03cd	2.4±0.10def	0.68±0.02d	3.16±0.05de
Group 4	0.648±0.04b	3.14±0.12bc	0.794±0.01b	4.00±0.12bc

Letter differ atp < 0.05.

4--Effect of *Saccharomyces's* on protein level (total protein & albumin & globulin)

The effect of *Saccharomyces's* on total protein & albumin & globulin of heat stress exposed chicken was illustrated in table (5)

Feeding of chicken on diet supplemented with *Saccharomyces's* (2.5 *10^7 CFU/g) induced significant increase ($P < 0.05$) in total protein & albumin & globulin compared with control group all over the experimental period.

Chicks experimentally exposed to heat stressed and fed on basal diet evoked a significant ($P < 0.05$) decrease in total protein& albumin & globulin all over the experimental period.

Feeding of heat stressed chicken on diet supplemented with *Saccharomyces's* (2.5 *10^7 CFU/g) displayed a significant($P < 0.05$) increase in total protein& albumin & globulin compared with heat stressed group all over the experimental period.

Table 5: The effect of *Saccharomyces's* on protein level (total protein, albumin and globulin) in broiler chicken exposed to heat stress on the15thand35stdays of age.

Days	15th			35st		
	Total protein	Albumin	Globulin	Total protein	Albumin	Globulin

Groups						
Group 1	4.28±0.05b	2.32±0.03bc	1.96±0.04ab	4.58±0.07c	2.44±0.05bc	2.14±0.08cd
Group 2	3.17±0.05c	2.22±0.03cd	0.95±0.06d	3.38±0.03d	2.22±0.03d	1.16±0.04e
Group 3	4.68±0.27a	2.52±0.03a	2.1±0.26bc	4.82±0.03ab	2.48±0.03ab	2.34±0.06cb
Group 4	3.94±0.07b	2.38±0.03b	1.56±0.05c	4.4±0.07c	2.34±0.04bc	2.06±0.08d

Letter differ at p < 0.05.

5--Effect of *Saccharomyces's* on glucose & PH

The effect of *Saccharomyces's* on glucose& PH of heat stress exposed chicken was illustrated in table (6)

Feeding of chicken on diet supplemented with *Saccharomyces's* (2.5 $*10^7$ CFU/g) induced non-significant (P < 0.05) change on glucose & PH compared with control group all over the experimental period.

Chicks experimentally exposed to heat stressed and fed on basal diet evoked a significant (P < 0.05) increase in glucose & PH all over the experimental period.

Feeding of heat stressed chicken on diet supplemented with *Saccharomyces's* (2.5 $*10^7$ CFU/g) displayed a significant (P < 0.05) decrease in glucose & PH compared with heat stressed group all over the experimental period.

Table 6: The effect of *Saccharomyces's* on glucose& PH in broiler chicken exposed to heat stress onthe15thand35stdays of age.

Days / Groups	15th		35st	
	Glucose	**PH**	**Glucose**	**PH**
Group 1	141.4±2.15cd	7.18±0.05d	135.4±2.35d	7.36±0.05d
Group 2	198.6±5.22a	8.392±0.03a	208.8±3.13a	8.38±0.07a
Group 3	140.8±0.73cd	7.232±0.02d	135±3.27d	7.10±0.03e
Group 4	148.2±1.98bc	8.00±0.07c	159.8±2.15b	8.14±0.05b

Letter differ at p < 0.05.

6--Effect of *Saccharomyces's* on Haptoglobin & CRP

The effect of *Saccharomyces's* on Haptoglobin & CRP of heat stress exposed chicken was illustrated in table (7)

Feeding of chicken on diet supplemented with *Saccharomyces's* (2.5 $*10^7$ CFU/g) induced significant decrease (P < 0.05) on haptoglobin & CRP compared with control group all over the experimental period.

Chicks experimentally exposed to heat stressed and fed on basal diet evoked a significant (P < 0.05) increase in haptoglobin & CRP all over the experimental period.

Feeding of heat stressed chicken on diet supplemented with *Saccharomyces's* (2.5 *10^7 CFU/g) displayed a significant (P < 0.05) decrease in haptoglobin & CRP compared with heat stressed group all over the experimental period.

Table 7: The effect of *Saccharomyces's* on Haptoglobin & CRP in broiler chicken exposed to heat stress on the15thand35stdays of age.

Days / Groups	15th		35st	
	Heptoglobin	CRP	Heptoglobin	CRP
Group 1	40.4±0.50bc	4.86±0.47c	135.4±2.35bc	5.86±0.25c
Group 2	53.8±1.42a	13.38±0.66a	208.8±3.13a	17.66±1.29a
Group 3	40±0.74d	3.06±0.19d	135±3.27d	2.6±0.24d
Group 4	42.4±0.50b	6.58±0.18b	159.8±2.15b	8.64±0.40b

Letter differ at p < 0.05.

7--Effect of *Saccharomyces's* on oxidant & anti-oxidant stress (MDA & SOD & GPX & catalase & Total antioxidant capacity & Cortisol)

The effect of *Saccharomyces's* on oxidant & anti-oxidant **stress** of heat stress exposed chicken was illustrated in table (8,9,10)

1) MDA

Feeding of chicken on diet supplemented with *Saccharomyces's* (2.5 *10^7 CFU/g) induced non-significant (P < 0.05) change on MDA compared with control group on 15th days of age and significant (P < 0.05) decrease on 35st days of age.

Chicks experimentally exposed to heat stressed and fed on basal diet evoked a significant (P < 0.05) increase in MDA all over the experimental period.

Feeding of heat stressed chicken on diet supplemented with *Saccharomyces's* (2.5 *10^7 CFU/g) displayed a significant (P < 0.05) decrease in MDA compared with heat stressed group all over the experimental period.

2) SOD & GPX & catalase & Total antioxidant capacity (TAC)

Feeding of chicken on diet supplemented with *Saccharomyces's* (2.5 *10^7 CFU/g) induced significant (P < 0.05) increase on SOD & catalase & GPX &TAC compared with control group all over the experimental period.

Chicks experimentally exposed to heat stressed and fed on basal diet evoked a significant (P < 0.05) decrease in SOD & catalase & GPX &TAC all over the experimental period.

Feeding of heat stressed chicken on diet supplemented with *Saccharomyces's* (2.5 *10^7 CFU/g) displayed a significant (P < 0.05) increase in SOD & catalase & GPX &TAC compared with heat stressed all over the experimental period.

c) Cortisol

Feeding of chicken on diet supplemented with *Saccharomyces's* (2.5 *10^7 CFU/g) induced significant ($P < 0.05$) decrease on cortisol compared with control group all over the experimental period.

Chicks experimentally exposed to heat stressed and fed on basal diet evoked a significant ($P < 0.05$) increase in cortisol all over the experimental period.

Feeding of heat stressed chicken on diet supplemented with *Saccharomyces's* (2.5 *10^7 CFU/g) displayed a significant ($P < 0.05$) decrease in cortisol compared with heat stressed group all over the experimental period.

Table 8:The effect of *Saccharomyces's* on MDA& SOD in broiler chicken exposed to heat stress on the15thand35stdays of age.

Days / Groups	15th		35st	
	MDA	SOD	MDA	SOD
Group 1	14.2±1.24cd	2.94±0.18f	19.6±0.67bc	3.62±0.14e
Group 2	24.4±1.68a	2.5±0.20d	27.00±2.23a	1.16±0.05f
Group 3	12.2±0.58cd	5.78±0.17c	15.6±1.50d	6.38±0.05c
Group 4	19.2±0.58b	4.48±0.24e	20.2±0.37bc	3.24±0.14e

Letter differ at p < 0.05.

Table 9: The effect of *Saccharomyces's* on catalase& GPX in broiler chicken exposed to heat stress on the15thand35stdays of age.

Days / Groups	15th		35st	
	CAT	CPX	CAT	CPX
Group 1	3.56±0.16e	21.00±0.83cd	3.9±0.12d	20.80±0.73ef
Group 2	2.6±0.17f	6.40±0.67f	2.04±0.05f	4.60±0.60g
Group 3	6.02±0.32b	28.8±0.86b	6.66±0.22b	32.60±1.12c
Group 4	4.00±0.07de	14.40±1.02e	3.48±0.08e	19.40±0.50f

Letter differ at p < 0.05.

Table 10: The effect of *Saccharomyces's* on total antioxidant capacity& cortisol in broiler chicken exposed to heat stress on the15thand35stdays of age.

Days / Groups	15th		35st	
	TAC	Cortisol	TAC	Cortisol
Group 1	250.8±19.40cd	11.6±0.50cd	255.8±7.15d	12.00±0.70b
Group 2	191.4±5.30e	17.6±0.50a	168.4±3.90f	20.4±0.67a
Group 3	301.8±4.31b	9.2±0.66e	327.6±4.98c	8.00±0.44e
Group 4	224.8±3.05d	13.6±0.50b	230.4±3.12e	12.6±0.40b

Letter differ at p < 0.05.

8--Effect of *Saccharomyces's* on Antibody titer by ELISA against ND &IB

The effect of *Saccharomyces's* on Antibody titer against ND &IB of heat stress exposed chicken was illustrated in table (11)

Feeding of chicken on diet supplemented with *Saccharomyces's* (2.5 *10^7 CFU/g) induced significant (P < 0.05) increase on antibody titer of ND & IB compared with control group.

Chicks experimentally exposed to heat stressed and fed on basal diet evoked a significant (P < 0.05) decrease on antibody titer of ND & IB compared with control group.

Feeding of heat stressed chicken on diet supplemented with *Saccharomyces's* (2.5 *10^7 CFU/g) displayed a significant (P < 0.05) increase on antibody titer of ND & IB compared with heat stressed group.

Table 11: The effect of *Saccharomyces's* on antibody titer estimated by ELISA against ND& IB in broiler chicken exposed to heat stress.

Groups	ELISA ND	ELISA IB
Group 1	4.14±0.05^{d}	3.6±0.11^{d}
Group 2	2.2±0.07^{f}	2.44±0.05^{g}
Group 3	5.86±0.21^{b}	4.24±0.05^{c}
Group 6	3.34±0.05^{e}	2.94±0.05^{f}

Letter differ at p < 0.05.

9- Histopathological investigation:

The effect of *Saccharomyces's* on tissue histology (Liver- Heart- Lung- Intestine) of heat stress exposed chicken was illustrated in table (12)

table (12):

Groups	Liver	Heart	Lung	Intestine
Group (1)	normal tissue histology	normal tissue histology	normal tissue histology	normal tissue histology
Group (2)	1) severe degree of congestion of blood vessels 2) perivascular edema 3) degeneration of hepatic cells 4) multiple necrotic foci replaced with leukocytes' aggregation	1) severe degree of congestion 2) thickening of blood vessels wall 3) perivascular edema. 4) Degeneration of cardiac myofibril besides myocarditis and leukocytes' infiltration	1) severe degree of congestion of blood vessels with perivascular edema 2)diffuse interstitial hemorrhages 3)Pneumonia and bronchiolitis	1) severe degree of degeneration 2) desquamation of lining epithelium. 3) Congestion and thickening of blood vessels wall in lamina propria
Group (3)	normal tissue histology	normal tissue histology	normal tissue histology	normal tissue histology
Group (4)	revealed milder degree of the mentioned lesions in group 2.	moderate degree of congestion, edema	moderate degree of congestion, edema and inflammation.	slight elongation of length of villi.

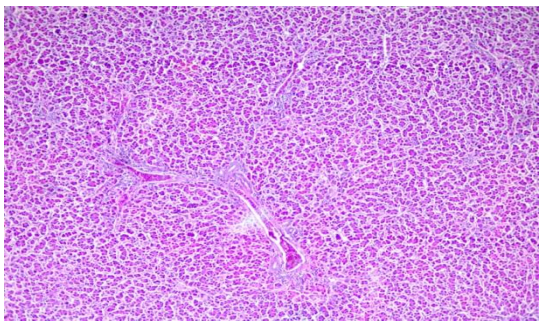

Figure (1): Liver of control negative group showing apparently normal hepatic cell HE, X100.

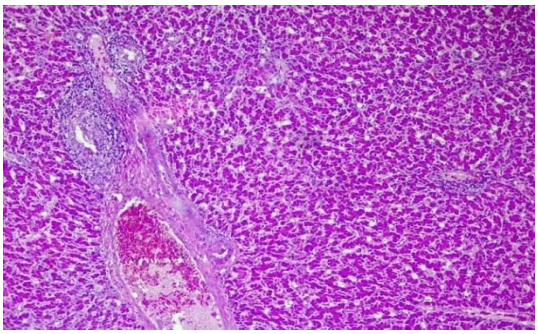

Figure (2): liver of heat stressed group showing congested blood vessels and sinusoids with few perivascular leukocytic infiltraion.HE,X100.

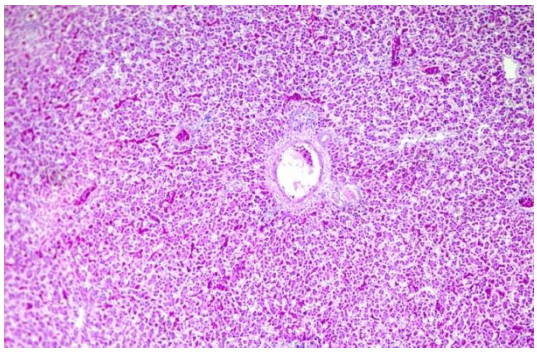

Figure (3): liver of supplemented heat stressed group by Saccharomycesshowing mild congested blood vessels and sinusoids .HE,X100.

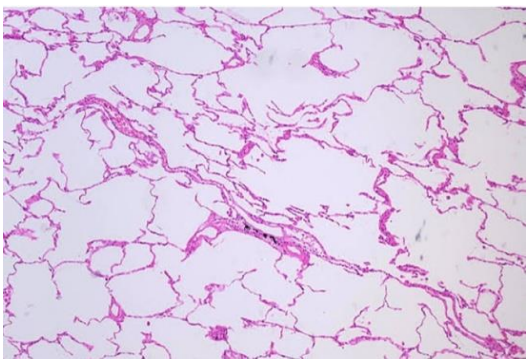

Figure (4): Lung of control negative group showing apparently normal alveoli HE, X100.

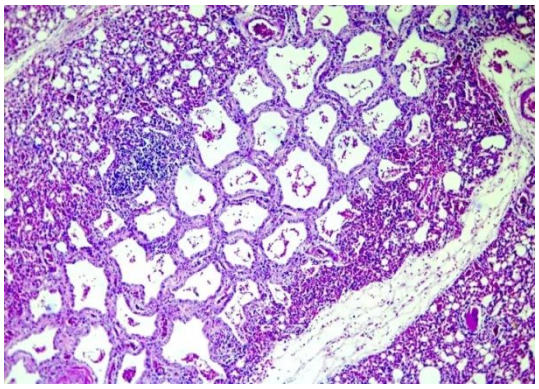

Figure (5): lung of heat stressed group, showing thickening of interalveolar septa , accumulation of esinophilic material inside the alveoli, hemorrhages and leukocytic agrrgation.HE,X100.

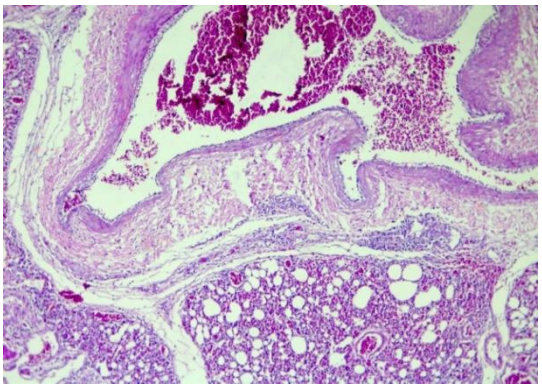

Figure (6):lung of supplemented heat stressed group by Saccharomyces, showing low degree of bronchiolitis and low leukocytes' aggregation HE, X100.

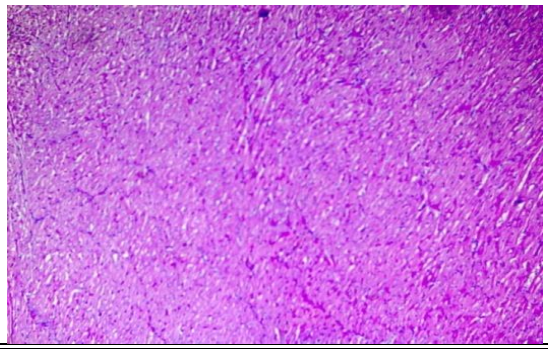

Figure (7): heart of control negative group showing apparently normal tissue HE, X100.

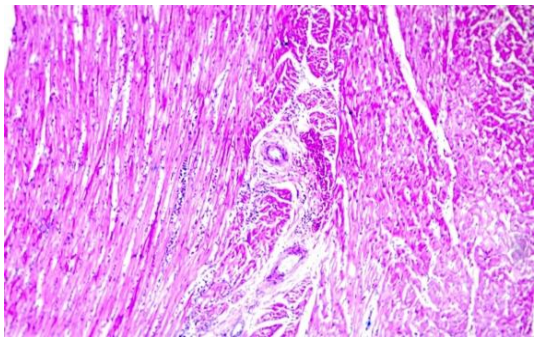

Figure (8): heart of heat stressed group showing hemorrhage and leukocytes' infiltration HE, X100.

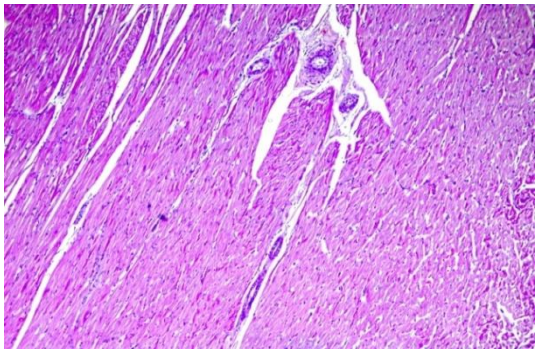

Figure (9):heart of supplemented heat stressed group by Saccharomyces showing mild perivascular edema and few leukocytes' infiltration HE, X100.

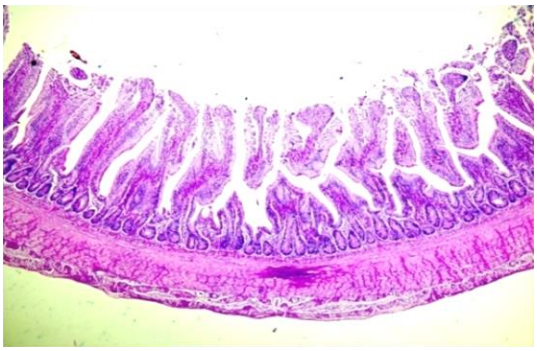

Figure (10): intestine of control negative group showing nearly normal histology HE, X50.

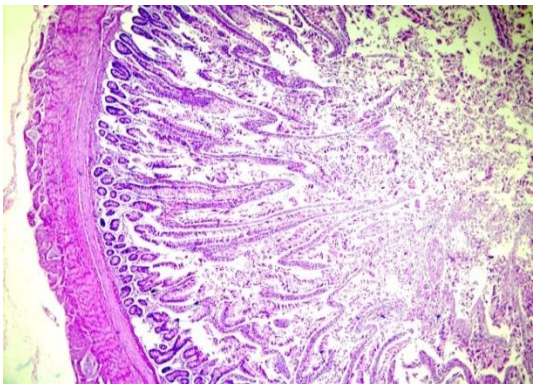

Figure (11):intestine of heat stressed group showing degenerated and sloughed epithelial lining HE, X50.

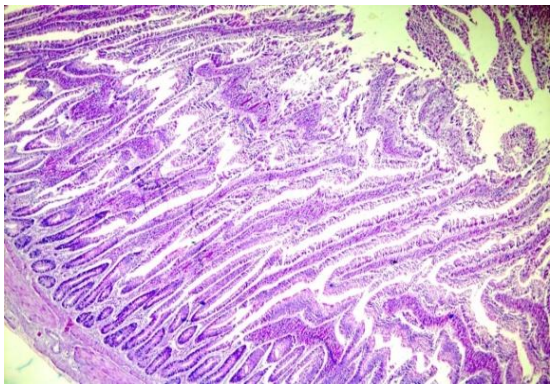

Figure (12):intestine of supplemented heat stressed group by Saccharomyces,showing longvilliwithfewdegenerated and sloughed epithelial lining.HE,X50.

Discussion

The effect on growth performance parameters

One of the most serious climate problems of tropical and subtropical regions of world negatively affects the production performance of poultry and livestock. Concisely, HS is characterized by endocrine disorders, reduced metabolic rate, lipid peroxidation, decreased feed consumption, higher feed conversion ratio **(FCR),** immunosuppression, and intestinal microbial dysbiosis **(Ashraf al., 2013; Sohail et al., 2012).**

Heat stress results from a negative balance between the net amounts of energy flowing from the animal's body to its surrounding environment and the amount of heat energy produced by the animal. This imbalance may be caused by variations of a combination of environmental factors (e.g: sunlight, thermal irradiation, and air temperature, humidity and movement) and characteristics of the animal (e.g: species, metabolism rate and thermoregulatory mechanisms). Environmental stressors, such as heat stress, are particularly detrimental to animal agriculture (**Nienaber et al., 2007**–**Renaudeau et al., 2012**).

In the present study, attempts were made to evaluate the use of *Saccharomyces's* to determine the effect of such feed supplement on growth performance, some biochemical parameters, oxidative stress, antioxidant status, and histopathological of *heat stressed* chickens.

In broiler nutrition, probiotic species have a beneficial effect on broiler performance **(Kalavathy et al., 2003 and Gil De Los Santos et al., 2005).**

In present study, a significant increase in body weight was recorded on 15^{th} and 35^{st} days of age in chicken supplemented with probiotics. This may be attributed to the optimum digestion of the feed due to the secretion of microbial digestible enzyme in these chicken and activation of normal flora by creation a favorable media for its growth and regeneration of intestinal villi enhancing nutrient absorption **(Kabir et al., 2004).**

Probiotic have been administered to counteract such stresses which subsequently improve body weight gain, food consumption in broiler chickens **(Hooge et al., 2004)**. Results of the present study showed that the inclusion of *S. cerevisiae* yeast in broilers ration improved body weight gain, feed intake and food consumption. The obtained results confirmed the previous findings of several researchers **(Peralta et al.,2018).** Also in agreement with our study, **Onifade et al. (1999)** reported that *SC* improved feed/gain ratio and BW gain.

Castro et al., 2012 reported that feed/gain ratio of broiler chicks from 0 to 9 wk of age improved significantly as the *SC* level in the diets increased.

Several studies gave evidence that the addition of probiotics to the diets of broilers leads to improved performance **(Khaksefidi and Ghoorchi, 2006**).

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).**

In the current study, we found that Heat Stress significantly decreased body weight, body weight gain, food consumption and significantly increased FCR and mortality in broiler chickens. It is reported that HS induces the secretion of stress hormones, which alter the chickens' neuroendocrine system by activating the hypothalamic-pituitaty-adrenal axis and thereby increasing the plasma corticosterone levels **(Quinteiro-Filho et al., 2012).** Corticosterone is associated with a higher degree of body protein breakdown **(Yunianto et al., 1997),** which affects the digestive system, nutrient utilization, and digestibility **(Olfati et al., 2018).** Furthermore, HS has been reported to disturb intestinal barrier function, lead to inflammatory responses, and compromise performance. A study by **Alhenaky et al. (2017)** showed that HS, whether chronic or acute, impaired intestinal integrity and increased intestinal permeability to endotoxins and *Salmonella* spp.

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).**

The positive impact of SC 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 **(Ghasemi *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.*, 2004)** and stimulate the immune organs **(Elfaky *et al.*, 2010).** 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 **Miazzo *et al.* (2005)** 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.* (2012)** who stated that Mannan oligosaccharide, 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.

The effect on Liver function (ALT &AST)

Measurement of serum transaminase ALT, AST activities are standard test for hepatocellular damage. It's well known that the enzyme are intracellular substances being located in the mitochondria and the cytoplasm, consequently, circulating levels increase following liver cell damage. **(Doxey, 1971).**

Regarding the biochemical findings, HS significantly ($P < 0.05$) elevated serum AST and ALT activities levels when compared to NHS birds group. 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.

In the current study the decrease in serum AST and ALT levels by SC supplemented groups was recorded it may collectively indicate the normal functions of liver and intestine of chickens fed feeds containing SC during HS conditions. This result is also confirmed by the obtained results of **Olugbemi *et al.* (2010)** who stated that SC 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 SC addition on hepatic tissues.

The effect on Kidney function (creatinine & uric acid)

Regarding the kidney function, several changes were recorded uric acid is the primary catabolic product of protein catabolism in birds **(Harrison and Harrison, 1986).** The avian kidney excretes uric acid primarily by tubular excretion, unlike the mammalian system that excretes urea entirely by filtration therefore the elevation of serum uric acid level indicate impaired renal function **(Osbaldiston,1968).**

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.

Huang *et al.* (2018) who stated that acute HS caused an increase in some hemato biochemical 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.

Huff *et al.* (2013) recorded that the dietary inclusion of *SC* in broilers ration, significantly ($P<0.05$) reduced uric acid and creatinine.

Effect on protein level

Serum protein is the total amount of protein in the blood in which albumin is the most abundant. Albumin helps keep the blood from leaking out of blood vessels as well as to carry some medicines and other substances through the blood that is important for tissue growth and healing. Low total protein levels can suggest a liver disorder, a kidney disorder, or a disorder in which protein is not digested or absorbed properly.

Heat stress significantly ($P < 0.05$) decreased serum total protein, albumin and globulin levels as matched to NHS group. In the current study, the obtained results seemed compatible to that recorded by **Sahin (2012)** also reported that heat stress inhibited the levels of protein and albumin and alleviated the effect of heat stress by supplement MOL in diet.

In the present study, SC supplementation adversely influenced serum protein and albumin concentrations, Several authors have identified that probiotic supplementation in diets increased serum protein and albumin concentrations, in broilers **(Agawane and Lonkar, et al., 2004, Capcarova *et al.*, 2011), piglets (Harding *et al.*, 2008) and fish (Zhou *et al.*, 2010)**reported that probiotics did not stimulate gastrointestinal protein synthesis or reduce the severity of intestinal colitis, but reported an unidentified signaling mechanism between the gut and liver which could be responsible for the probiotic-induced increase in liver protein and plasma protein synthesis. Furthermore, dietary probiotic supplementation may increase the serum protein and albumin concentrations by increasing iron absorption in the lower intestine **(Eizaguirre *et al.*, 2002).**

Effect on Glucose level

In the present study it has been shown that heat stressed chicks demonstrate a significant increase in the level of serum glucose. The increase in glucose concentration is directly responsive to an increase in glucocorticoids **(Borges et al., 2007),** which can result from various stressors including heat stress. Glucocorticoids have primary effects on metabolism, stimulating gluconeogenesis from muscle tissue proteins. **Habibian et al. (2014)** reported that high environmental temperature increased levels of plasma glucose and cholesterol and reduced protein level.

On the contrary, the dietary supplementation of SC in bird ration reared under HS significantly ($P<0.05$) lowered the serum glucose compared to HS non treated group. These results agree with findings of **Djouvinov et al. (2005)** who tested the probiotic strains in ducklings.

Effect on PH value

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).**

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.

Effect on Haptoglobin & CRP

Haptoglobin is an alpha-2 globulin that is known as PIT 54 in chickens. Haptoglobin's major role is to bind free hemoglobin produced from erythrocytes and decrease its oxidative activity **(Yang *et al.*, 2003).** It also binds hemoglobin to reduce iron losses via urine after hemolysis, hence sparing tissues from harm caused by free hemoglobin **(Nielsen *et al.*, 2006).** Haptoglobin has been identified as a potent angiogenic agent essential for endothelial cell proliferation and differentiation in the development of new blood vessels.

CRP was the first APP been described and is one of the major APP in humans and dogs **(Eckersall and Bell, 2010).** CRP has the ability to binds directly to degenerating cells, residues and polysaccharides onbacteria, fungi, and parasites, acting as an opsonin. It can activate the complement system when bound to one of its ligands and can bind to phagocytic cells, interacting together with humoral and cellular systems of inflammation **(Cray et al., 2009).**

In the current study the decrease in serum heptoglobin and c- reactive protein levels in SC supplemented groups was recorded it may collectively indicate the normal functions of liver of chickens fed feeds containing SC during HS conditions. This result is also confirmed by the obtained results of **Maoba *et al.* (2021),** who stated that SC possess a beneficial action to stimulate the immune responses and anti inflamatory in broilers.

Oxidant & Antioxidant stsatus

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), total antioxidant capacity (TAC) activities together with increased MDA levels and cortisol.

HS is considered one of the most important factors having the ability of enhancing ROS production **(Li *et al.*, 2020).** 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, catalase and SOD **(Emami *et al.*, 2021).**

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 Timo thee 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 GPX, 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).**

Effect on anti body titers against ND & IB

In the current study, mean antibody titer against ND and IB increased significantly in heat stressed group. It has been postulated that heat stress increases the carticosterone level, an immunosuppressive agent, while SC decrease the level of carticosterone **(Rama et al. 2004).**

Heat stress increases the production of free radicals and reduces the concentration of antioxidant vitamins like vitamin C, E, and A and minerals **(Khan et al., 2011)**. Findings of the present study are in agreement to that of **Khajavi et al. (2003),** who worked on chicken under heat-stressed condition and reported significant reduction in antibodies production. **Rama et al. (2004)** reported that heat stress reduces antibody production by increasing secretion of immunosuppressive agents (hydrocortisones), while SC reduces hydrocortisones production. Similarly, **Coelho and McNaughton (1995)** reported that higher vitamin supplementation enhanced broiler performance and immunity in stress condition, which are in agreement with the findings of the present study.

zhang et al. (2005) reported that antibody titers against NDV& IB was improved in broiler chickens fed SC.

Histopathological investigation

Prime examples of heat stress were increased heart rate and increased blood flow to muscle, brain, and heart **(Ewing *et al*., 1999).** This might cause congestion in kidney, liver and lung. Effects of heat stroke on the gross lesions were dominated by severe and generalized hyperemia, which was most severe in the respiratory tract, especially in the lung, tracheal and bronchial mucosae. Lungs may also be edematous and occasionally contain focal consolidations of bronchopneumonia. Other organs, such as heart, kidney, may also be severely congested. The microscopic lesions are compatible with those seen grossly. The vasculatures of the lung are severely engorged and edema are evident in alveoli. Centritubular necrosis, dissociation of hepatocytes and congestion are often found in the liver. Subendocardium and subepicardial hemorrhages are found in the heart. Besides, capillary congestion is evident in kidney and other structures **(Smith *et al*., 1979).**

Conclusion

It could be concluded that supplementation of chickens with probiotic (Saccharomyces) had a significant effect on growth performance & some biochemical parameters (ALT, AST, Creatinine, Uric acid, total protein, albumin, globulin, glucose, Ph, Haptoglobin, CRP) & oxidant and antioxidant parameter (GSH, SOD, Catalase, Total antioxidant capacity, cortisol) & histopathological condition. Based on the result of present study.

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