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Comparative study between zinc and selenium supplementation in diets of heat stressed broiler.

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Abstract

One hundred and twinty- five one day- old hubbard chicks were used to investigate the effect of dietary Zinc (organic and inorganic) and Selenium (organic and inorganic) on growth performance, biochemical parameters, immuneresponse and histopathological, immunohistochemistry (IHC) and intestinal morphology changes of heat stressed broiler chickens from July till half of August. The birds were randomly separated into 5 groups fed on basal diet under good ventilation and environmental condition (ambient tempreture and relative humidity). After 2 weeks till 6 weeks of age ,broilers were reared under natural ambient tempreture (36°c- 38°c) and relative humidity(80%) of summer condition, groups1 (control) fed on basal diet without minerals supplementation, group 2, 3, 4 and , 5 fed on basal diet supplemented with (40 ppm Zinc methionine, 40ppm Zinc sulfate, 0.15ppm Selenium proteinate, 0.15ppm Sodium selenite) respectively. Body weight, weight gain and feed conversion ratio were calculated weekly. At the 20th, 30th and 40th days of age the sera were analyzed for glucose, total protein, albumin, triglycerides, cholesterol, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Glutathione, Malondialdehyde (MDA) and HI titer against NDV and total antibodies. At the end of the experiment specimens from intestine, liver, kidney, spleen and heart were examined for histopathological changes, morphological analysis of intestine (villi length, villi width, crypt depth and villi length /crypt depth ratio) and Immunohistochemistry (IHC) staining for detection of localization and density of heat shock protein 70(HSP70) in myocardial cells.The results revealed that supplementing with zinc methionine and selenium proteinate (organic sources) at the 4th, 5th and 6th weeks of the experiment significantly ($p<0.05$) improved the body weight, body weight gain and feed conversion ratios, the blood glucose regulating effect was clearer in serum, decreased the serum triglyceride, decreased the cholesterol, serum total protein, albumin and globulin increased and improve the immune response. Significantly decreased AST,ALT and malondialdhyde and enhanced the glutathione activity. Decrease the severity of the pathological changes which may be occure due to exposure to severe heat stress, improve intestinal villi length, width and decreased the crypt depth. In addition, decreased IHC staining density of HSP70 in myocardial cells especially in organic selenium (Se proteinate). Therefore, from the present results it could be concluded that, Zn and Se ,especially the organic sources are a suitable dietary supplements for broilers to ameliorate the negative effects of summer heat stress condition.

Keywords: *Broiler, zinc, selenium, heat stress, biochemical analysis, Malondialdhyde, immune response, histopathology, immunohistochemistry.*

Introduction

Heat stress is one of the major problems facing the poultry industry in tropics reflecting directly in the economic returns, as the birds resort to many strategies and one of them is the reduction of the feed intake to reduce the production of heat from the body **(Gregory, 2010)**. The decreased feed intake results in reduce growth rate, poor meat quality, decreased egg production, poor egg quality and reduction in the efficiency of feed utilization. (**Habibian *et al.*, 2016)**. Supplementation of minerals like, zinc, copper,, magnesium, selenium,, manganeseandiodine help to sustain the production in animals, improve nutrient utilization and at the same time effectively neutralize the oxidant stress and enhance the compromised immune system of heat stressed birds (**Mir *et al.,* 2018)**. The heat stress induced biochemical as well as physiological changes potentially enhances the formation of reactive oxygen species (ROS) (**Mujahid *et al*., 2007; and El-Kholy *et al.,* 2017, 2018)** which in turn disturb the balance of oxidation as well as antioxidant defense systems to induce lipid peroxidation which increase malondialdhyde more than two fold and cause oxidative damages to biological molecules including proteins and DNA leading to accumulation of heat shock protein70 which work as abiomarker for tissue damage by astrong stress **(Lin *et al*. 2006 and kumbhar *et al.,* 2018).**

Dietary zinc supplementation Improveing weight gain and feed efficiency in heat stressed broilers, and tends to increase the antibody titre (IgM and IgG) **(Yang *et al.*, 2010).** Selenium used in the antioxidative defense system in the heat-stressed poultry. Organic selenium in the form of selenomethionine and Se–yeast is more bioavailable than theinorganic forms in chicken **(Habibian *et al*., 2016)**. Selenium enhancing the absorption from the gut and protecting the cell membrane fats from oxidative damage **(Liang *et al*., 2014)** and can alleviate the deleterious effect of heat stress on immune systems by increasing the antibody titre (IgG and IgM) and phagocytic functions **(Liang 2010).**

The pathogenesis of chronic heat stress in broilers may explain the phenomenon of body changes and becomes a potent cause of death **(Aengwanich and Simaraks)** Fatty degeneration is the accumulation of neutral lipids in the cytoplasm. This is a diagnostic clue for liver injury. Excess lipids in hepatocytes indicate that sublethal injury has occurred. However, the swollen, yellow, greasy appearance of fatty degeneration is characteristic of liver and less common in kidney and heart **(Sahine *et al.,* 2009)**. Besides, necrosis due to oxygen deficiency. In kidney, There were generalized edema and hemorrhage in the kidney, especially in renal papillae, renal tubular and subrenal capsule. Heterophils accumulation **(Salim *et al.*, 2011).** The present study was conducted to investigate the effects of supplemented diet with organic and inorganic Zn and Se on growth performance, biochemical changes, immune response, histopathology, intestinal morphology, immunohistochemistry and phagocytic activity in broiler reared under high ambient temperature.

Materials and Methods

Experimental design:

One hundred and twinty five one day old hubbard chicks were divided into 5 groups each group contain 25 chicks at the beginning of 3rd week of age. The chick groups were accomodated in separate penson deep litter and maintained under good ventilation and suitable (31-35C) temperature for the first 2 weeks of age,, after which they were kept under natural environmental conditions (ambient temperature and relative humidity) and free access to feed, water and continuous lightening program. The experiment lasted for 6 weeks during July and Augustmonths, 2019 at summer season where's the ambient temperature in the broiler pens were 36°c- 38°c. The maximum daily ambient temperature, and relative humidity were recorded and the mean was tabulated weekly as compared to the recommended ideal temperature and humidity (Table1). All experimental birds were subjected to vaccination program against Newcastle disease by Hitchner B1 at 7 days of age and Lasota at 18 and 28days of age and Gumboro diseases at 13 days of age. The chicks were fed according to the two phases feeding program from 1 to 21 days and from 22 to 42 days on starter and finisher diets, respectively. The diets were formulated to meet the recommended requirements nutrient for broilers **(NRC, 1994).** The control was fed the

basal control starter diet during the first three weeks of age then finisher one till the end of the experiment at 6 weeks without adding excess supplemental minerals. At the beginning of the 3rd week of the experiment, the chicks in other experimental groups were fed the normal control diet (starter and finisher) but supplemented with40 mg Zn/Kg from 2 sources, organic as zinc meth. Chelated or inorganic as zinc sulfate or 0.15 ppm selenium/Kg from 2 sources, organic as selenium enriched yeastor inorganic as sodium selenite above the recommended levels of these minerals.

Growth performance:

Average body weight growth, body gain, fed intake and feed conversion ratio for each group were calculated every week throughout the experimental period.

Table (1). The means of weekly recorded maximum ambient temperature and relative humidity as compared to the recommended ideal ones:

Experimental. period/ week	Ambient temperature°c		Relative humidity%	
	Recorded	Ideal	Recorded	Ideal
1	36	33.3-35	86.55	60-70
2	36	31.1-32.2	85.42	60-70
3	38	28.3-29.4	88.30	60-70
4	37	25.5-26.6	88.33	60-70
5	38	22.7-23.8	85.00	60-70
6	36	21.1	86.71	60-70

Table (2). Ingredient composition and chemical composition of the experimental diets.

Ingredient	Experimental diets	
	Starter	Finisher
Yellow corn	50.2	66.4
Soybean meal (44% CP)	31.8	22.3
Fish meal (72%)	4.2	2.0
Corn gluten	2.82	1.8
Cotton seed oil	7.7	4.8
Dicalcium phosphate	1.2	0.7
Limestone	1.4	1.4
Common salt	0.3	0.3
Trace minerals*	0.05	0.05
Vit-premix**	0.25	0.25
Dl-methionine	0.08	___
Calculated chemical composition***		
M. E. (kcal/kg)	3199	3203
Crude protein (%)	23.03	18
Lysine%	1.21	0.86
Methionine %	0.5	0.32
Zinc mg/kg	45	41
Manganese mg/kg	63	61
Selenium mg/kg	0.2	0.15
Analyzed chemical composition %****		
CP	22.84	17.88
EE	8.21	6.32
CF	3.67	3.54
Ash	4.09	3.81

* Each 1kg of trace mineral premix supply Mn 100gm; Zn 35gm; Fe 40gm; Cu 10gm; I1gm; Se 0.12gm and cobalt 0.15gm.
**each 2kg of vitamin premix supply A 12×10^6 IU; D_3 3×10^6 IU; E 1.5×10^4mg; $k_3 0.3\times10^4$mg; B_1 0.2×10^4 mg;B_2 0.6×10^4mg;$B_6 0.3\times10^4$ mg; B_{12} 15mg; Nicotin0.4×10^5mg; folic1.5×10^3mg; Biotin 75mg and Ca pant othianate 10×10^4 mg
*** According to feed composition tables of NRC (1994).
**** Chemical analysis according to AOAC (1984).

Blood sampling and serum biochemical analysis:
Blood samples were collected from 20 chicks of the control group and each subgroup at the 20th, 30th and 40th days of age via the wing vein puncture. Sera were obtained and used for determation of serum glucose **(Trinder and Ann, 1969),** total protein **(Henry, 1964),** albumin **(Doumas, 1971),** triglycerides **(Koditscheck and Umbreit, 1969),** cholesterol **(Roeschlau *et al.*, 1974),** alanine aminotransferase (ALT), aspartate aminotransferase (AST), glutathione and malondialdhyde (MDA), **(Mujahid *et al.*, 2009)** using already prepared analyzing chemical kits, while serum globulin was estimated as the difference between total protein and serum albumin. The serum samples were used for determining of haemagglutination inhibition test for antibody titer against Newcastle disease virus **(Anon, 1980)** and total antibodies **(Erhard *et al.*, 1992).**

Pathological examination:
1-Histopathology:
At the end of the trial, five birds from each group were killed by cervical dislocation. Clinical signs and gross lesions of visceral organs of broilers were examined. Intestine, liver, kidney,spleen and heart were taken from necropsied birds, fixed in 10% buffered formalinsolution, dehydrated in gradual ethanol (70-100%),cleared in xylene, and embedded in paraffin. Five-micron thick paraffin sections were prepared and then routinely stained with hematoxylin and eosin (H&E) dyes and then examined microscopically. **(Bancroft and Marilyn, 2013).**

2-Intestinal morphology:
About 2.5cm in length were excised from the middle of the jejunum (from the distal portion of the duodenal loop to Meckel's diverticulum). Fixed intestinal samples were prepared by using conventional paraffin embedding techniques. Samples were sectioned at a 5-µm thickness and routinely stained with hematoxylin and eosin. Villus height, villus width and crypt depth were measured according to **(Law *et al.*, 2007).** Under a light microscope, the histomorphometric analysis was performed using Image J analysis software. A total of 10 intact, well-oriented, crypt-villus units were selected in triplicate for each intestinal cross-section. Villus height was measured from the tip of the villus to the villus-crypt junction, villus width at the mid of the villus, crypt depth was defined as the depth of the invagination between adjacent villi (from the crypt-villus junction to the base of the crypt, and then villus height: crypt depth (V/C ratio) was calculated.

3-Immunohistochemical Staining (IHC) for Detection the localization and Density of heat stress protein (HSP70) in Chicken Heart: Was carried out according to the manufacturer's instructions using DAKO, an Agilent Technologies Company ,USA as following:
Heart samples were taken from each group. Formalin-fixed and paraffin-embedded tissue sections(4-mm thickness) using a standard microtome. In a 55°C water bath, floating paraffin tissue sections are gently adhered to positively charged slides. Slides are then dried at 37°C overnight. Dewaxed heart tissue sections were fixed with HCl solution for antigen retrieval (2N HCL) in distilled water, pH (0.6–0.9) for 20 min at room temperature (RT). After washing with PBS three times, endogenous peroxidase activity was inactivated by incubation in 3% H2O2 for 10 min at RT. Subsequently, the sections were blocked with 5% bovine serum albumin (BSA) for 30 min at 37° C and then incubated with HSP70 primary antibodies at 1 : 100 dilution for 2 h at 37°C. After washing with PBS three times, sections were incubated with a horseradish peroxidase goat anti-mouse secondary antibody at 1 : 500 dilution for 1 h at 37°C. The sections were washed with PBS three times and then treated with two drops of ready-made 3,3′-diaminobenzidine(DAB) substrate chromogen solution for 15 min until the desired brown color ppeared. The sections were counterstained with hematoxylin and observed under a light microscope .

Statistical analysis:
Data obtained in this work, was statistically analyzed using the general linear models **(GLM) of SAS, (2004)** using one-way analysis of variance to test the effect of different dietary

treatment according to the control group. Duncan's Multiple Range Test **(Duncan, 1955)** was used to separate means when separation was relevant. Statistical significance was accepted at a probability level of 0.05.

Results and Discussion

Effects of dietary zinc sources and supplementation on growth performance

Supplementing the diets with zinc in the form zinc sulfate and zinc methionine significantly improved the body weight, absolute weight gain and feed conversion ratio of the heat stressed broiler chicks at the 3rd, 4th, 5th and 6th weeks of the age (Tables 3).**(Kucuk *et al.,* 2003)** reported that supplemental zinc (30 mg Zn/Kg diet) significantly increased live weight gain and improved feed efficiency ($p<0.05$) of heat-stressed broilers. With the same concept, **(Hess *et al.,* 2001 and Ao *et al.,* 2006)** showed high significant improvements in body weight, feed conversion ratio in broiler chickens fed diets supplemented with zinc methionine. Other studies have shown no effect of zinc source on growth performance **(Anil *et al.,* 2012, Salim *et al*., 2012 and Zakaria *et al.,* 2017).**

Effects of dietary selenium sources and supplementation on growth performance

Feeding the heat stressed broilers on the diets supplemented with selenium selenite or selenium proteinate increased the average body weight and body weight gain and reduced the feed conversion ratio (Tables 3). The improvement in growth performance parameters in the Se-supplemented diets could be attributed to the effect of Se on thyroxine hormone. **Jianhua *et al*., (2000).** Selenium positively affects feed utilization through participation in the metabolism of carbohydrates, lipids, and proteins, **Stapleton (2000)**.

supplementation of the diet with selenium enriched yeast (organic selenium) improved body weight, feed conversion ratio, and decreased mortality in both heat stressed and enteropathogenic Escherichia coli-challenged chicks **Mahmoud and Edens (2005)**. **Doaa *et al.,* (2019)** reported that groups supplemented with organic selenium showed a significant increase in body weight, body weight gain and improved FCR when compared with groups supplemented with in organic selenium while there is no difference in the feed intake in organic and non organic supplemented groups. **Da Silva *et al.,* (2010)** not detected any significant effect of selenium levels and sources on performance. However, **Rahimi *et al*., (2011)** investigated the effect of organic and inorganic selenium (0.3 mg/kg) supplementation on performance responses in broilers reared under heat stress or thermo neutral condition and reported that live weight gain, feed conversion ratio, and dressing percentages were significant with both selenium supplementations in both conditions.

Mortality rate was increased (12%) in heat stressed group (control) in comparison to zinc supplemented groups where's the mortality was (4%) for organic and non-organic zinc respectively and was(4%)for organic and non-organic selenium respectively. **Al-Fataftah *et al.,* (2007)** recorded mortality rate of 11.79 - 5.73- 5.16 and 2.53% at ambient temperature 35^{o}C, natural (24-28^{o}C), 30^{o}C and 25^{o}C. **Zakaria *et al*., (2017)** recorded a significantly lower mortality rate in birds fed organic zinc (1.25%) compared with those fed in organic zinc (4.80%). This reflect the role of zinc in stimulating the development of the immune system providing a sufficient bioavailable zinc source results in healthier birds and decrease mortalities, **Salim *et al.,* (2011). Peric *et al*., (2009) and Da- Silva *et al.,* (2010)** reported non-significant difference in mortality among organic and non-organic selenium treated groups .

Table (3). Effect of zinc and selenium source and supplementation level on feed intake, body weight and feed conversion of broilers (2-6wk) exposed to heat stress (over 30c) with high relative humidity.

Age (wk)		Miniral supplement (mg/kg)				
		control	Zinc (40)		Selenium (0.15)	
			Meth	Sulfate	Proteinate	Selenite
3rd	F1 BW FC	518.2 599.1±9.58^{b} 1.78	544.7 645.3±7.69^{a} 1.61	540.2 624.9±10.15^{ab} 1.7	520.8 526 .5±10.48^{ab} 1.63	530,8 617.8±10.46^{ab} 1.73
4th	FI	670.7	741.7	752.5	767.6	753.8
	BW	971.7±17.40^{b}	1076.5±13.09^{a}	1054.3±16.96^{ab}	1079.7±16.90^{a}	1041.3±29.60^{ab}
	FC	1.80	1.72	1.90	1.71	1.78
5th	FI	1006.1	1015.8	1017.1	966.7	1001.7
	BW	1463±31.80^{b}	1644±28.83^{a}	1580.6±32.82^{a}	1645±32.80^{a}	1591.7±29.42^{a}
	FC	1.93	1.79	1.90	1.71	1.282
6th	FI BW FC	1286.4 1962.5±31.80^{c} 2.10	1312.4 2209.7±36.70^{a} 2.6	1324.9 2102.2±31.80^{ab} 2.75	1293.8 2193.2±28.40^{a} 2.36	1102 2031±32.45^{bc} 2.51
3-6 WKs	FI FC	3481.4 2.10	3614.6 1.90	3634.7 2.03	3548.9 1.88	3388.9 1.97

Values are means ± standard error. Means within the same row of different litters are significantly different at ($P < 0.05$)

Serum protein:

Exposure of the broiler chickens to long period of heat stress significantly decreased the levels of serum total proteins and albumin (Table 4). The significant decline in serum protein with rising temperature seems to be due to depression of anabolic hormonal secretion such as growth hormone **(Sahine *et al.*, 2009)**, thyroxine **(El-Masry, 1987)** and insulin **(Habeeb, 1987)**. Also, **(Kamal, 1965)** reported that the decrease in serum protein may also be due to the decrease in feed nitrogen and mineral intake which occur under heat stress condition and / or the increase in the catabolic hormones such as glucocorticoid and catecholamines. Similarly heat stress decrease total protein levels in broiler chicks **(Yousef, 2005)**. However, supplementing the diets of the heat stressed broilers with zinc and selenium raised the serum total protein to near the normal level (before exposure to heat stress). Zinc has numerous biological roles, including protein metabolism **(Forbes, 1984)** DNA synthesis **(Lieberman *et al.*, 1963)** and cell division and multiplication **(Rubin, 1972 and Rubin and Koide, 1973)**. Also, the increase in serum total protein, albumin and globulin due to dietary zinc supplementation could be attributed partially to increased feed consumption (Table 4) and so protein intake and stimulation of anabolic hormones.

Effects of minerals supplementation on immunocompetance of heat stressed broiler chicks:

The effect of minerals (Zn and Se) supplementation in broilers exposed to long period of heat stress (3-6 wks) on the humoral immune response for vaccines (NDV) at 20, 30 and 40 days of age are presented in table (4). No statistical differences in the mean immune titers between the broiler groups in spite of Zn (40ppm) and Se (0.15ppm) supplementation during heat stress at 20 days. At 30 and 40 days of the broilers age the antibody titers against NDV were significantly increased in the broiler groups supplemented with either of Zn (40ppm from methionine or sulfate source) or, Se (0.15ppm from organic selenite) when compared to heat stressed group. Heat stress is known to cause a reduction in antibody production in broilers **(Habibian *et al.*, 2014)**. Zinc Methionine supplemented group had significantly higher antibody titer against Newcastle disease vaccine when compared to zinc sulfate supplemented group. **(Ahmed *et al.*, 2017)** showed that different levels of dietary zinc methionine had a positive effect on anti-

body titer against Newcastle disease vaccine. Organic selenium supplemented group had significantly higher antibody titer against Newcastle disease vaccine when compared to in organic selenium supplemented group at 30 days of age but not at 40 days of age. Antibody titer against Newcastle disease vaccine did not affected with different concentrations of organic selenium, **(Savaram *et al.,* 2013).**

Table (4). Effect of dietary mineral (zinc and selenium) supplementation on serum levels of total protein, albumin, globulin, haemagglutination inhibition (HI) and total antibodies titers of broiler chicks exposed to heat stress

Items	Control	Mineral supplement			
		Zinc (40 ppm)		Selenium (0.15 ppm)	
		Methionine	Sulfate	Proteinate	Selenite
Total protein (g/dl) at:					
20 day	4.22±0.25^{d}	4.91±0.03^{ab}	4.74±0.41^{b}	5.10±0.13^{a}	4.52±0.27^{c}
30 day	4.11±0.19^{c}	5.25±0.10^{a}	4.84±0.12^{b}	4.80±0.05^{b}	4.31±0.13^{c}
40 day	4.09±0.10^{e}	5.19±0.12^{a}	4.65±0.06^{bc}	4.88±0.04^{b}	4.60±0.23^{cd}
Albumin (g/dl) at:					
20 day	2.15±0.03^{bc}	2.57±0.16^{a}	2.49±0.25^{a}	2.23±0.05^{bc}	2.05±0.08^{c}
30 day	2.21±0.09^{bc}	2.35±0.06^{a}	2.33±0.04^{ab}	2.25±0.04^{ab}	2.08±0.07^{c}
40 day	2.15±0.08^{bc}	2.41±0.09^{a}	2.35±0.03^{a}	2.29±0.07^{ab}	2.28±0.13^{ab}
Globulin (g/dl) at:					
20 day	2.07±0.21^{d}	2.34±0.14^{bc}	2.25±0.16^{cd}	2.87±0.19^{a}	2.47±0.12^{b}
30 day	1.90±0.14^{d}	2.90±0.04^{a}	2.51±0.10^{b}	2.55±0.18^{b}	2.23±0.08^{c}
40 day	1.94±0.01^{c}	2.78±0.03^{a}	2.30±0.06^{b}	2.59±0.04^{a}	2.32±0.13^{b}
HI titer at:					
20 day	2.13±0.15	2.21±0.13	2.14±0.41	2.10±0.13	2.22±0.27
30 day	2.21±0.09^{c}	2.75±0.10^{a}	2.44±0.12^{bc}	2.80±0.05^{a}	2.51±0.13^{b}
40 day	2.09±0.10^{d}	3.19±0.12^{a}	2.65±0.06^{c}	2.98±0.04^{ab}	2.80±0.23^{bc}
Total antibodies at:					
20 day	3.11±0.13^{c}	3.37±0.16^{ab}	3.29±0.15^{b}	3.43±0.15^{ab}	3.25±0.06^{bc}
30 day	3.09±0.10^{d}	3.67±0.11^{b}	3.69±0.12^{b}	3.93±0.09^{a}	3.65±0.16^{b}
40 day	3.25±0.09^{c}	3.91±0.07^{a}	3.75±0.11^{b}	3.99±0.07^{a}	3.68±0.13^{b}

Values are means ± standard error. Means within the same row of different litters are significantly different at ($P < 0.05$).

Serum glucose:
Zinc and selenium supplementation of the broiler diets especially from zinc methionine and selenium proteinate, during exposure to heat stress resulted in decreasing the level of serum glucose compared to the un-supplemented (control) group (Table5). The lowered glucose level due to zinc supplementation may either partly resulted from depressed pancreatic enzyme activities by excess zinc **(Uyanik *et al.,* 2001)** or increased zinc uptake of pancreas with dietary zinc concentration **(Habibian *et al*, 2010)** because of the putative effect of zinc on insulin metabolism **(Mir *et al,* 2018)** indicating the increased glucose utilization.

Serum cholesterol and triglycerides:
Concerning serum triglycerides and cholesterol levels, supplementing the diets of heat stressed broiler with zinc and selenium lowered the serum triglycerides and cholesterol levels which increased due to exposure to heat stress (Table 5). The lowering effects were more pronounced in the broilers fed the diets supplemented with zinc methionine, selenium proteinate (the level of 90 ppm). The relationship between dietary zinc and plasma cholesterol homeostasis is not well characterized. Zinc supplementation did not influence serum cholesterol in chicks **(Lü and Combs, 1988)**. In contrast, reduced serum total cholesterol concentrations were reported in other studies **(Boukaiba *et al.,* 1993 and Uyanik *et al.,* 2001).**

AST, ALT, Glutathione and Malondialdehyde:
The comparison of the blood metabolites and oxidative stress parameters in control group and supplemented groups (Table 5) demonstrated that the control group significantly ($P < 0.05$) increas AST, ALT, Glutathione and Malondialdehyde. Moreover, the supplemented treatments improved the blood profile parameters and the oxidative status of birds. Measurment of Malondialdehyde as an oxidative stress indicator in the serum revealed a significant increase in its concentration in the control group compared to that of the supplemented other groups. Heat stress increased lipid peroxidation as a consequence of increased free radical generation. The rise of lipid peroxidation resulted in increased Malondialdehyde level in blood and tissues **(Okutan *et al*., 2005 and Ates *et al*., 2006)**. Zn supplementation decreased serum Malondialdehyde level ,this might attributed to that Zn induces production of metallothionein, which is an effective scavenger for hydroxyl radical **(Sahin *et al.,* 2009)**.

Glutathion peroxidase, present in cytosol and mitochondrial matrix, catalyzes the degradation of varios peroxides by oxidizing glutathione. Se is an essential component of Se-dependent glutathione peroxidase enzyme, which reduces peroxide and protects cells against the damaging effects of oxidation **(Reddy *et al.,* 2009)**. **(Jianhua *et al.,* 2000)** and **(Payne and Southern, 2005)** recorded that thatdietary Se supplementation increased the plasma glutathion activity in the broiler chichens**. (Khajali *et al.,* 2010)** found that the organic Se significantly elevated plasma glutathion activity.

Table (5). Effect of dietary mineral (zinc and selenium) supplementation on some Biochemical parameters of broiler chicks exposed to heat stress.

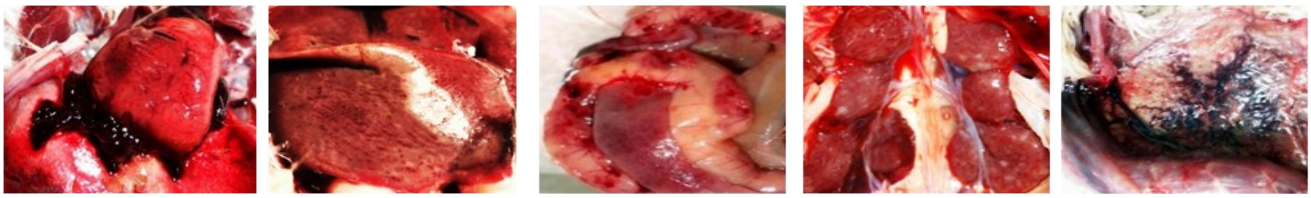
Items	Control	Mineral supplement			
		Zinc (40 ppm)		Selenium (0.15 ppm)	
		Methionine	Sulfate	Proteinate	Selenite
AST (U/L)at:					
20 day	129.71±0.528 a	101±1.154 d	111.377±1.89 c	100.42±1.421 d	118.12±0.594 b
30 day	134.87±1.970 a	109±3.214 bc	114.3±3.592b	101.38±2.739 c	117.126±1.514 b
40 day	135.41±1.873 a	102.33±1.763 c	119.11±1.494b	99.33±1.201 c	120.45±2.221 b
ALT (U/L) at:					
20 day	35.46±2.369 a	29.76±0.788 b	31±1.04ab	27.33±1.20b	28.41±1.185 b
30 day	36.4±1.32 a	27.7±1.222 bc	30.30±1.113b	26.166±1.092 c	27.60±1.193 bc
40 day	37.47±1.227 a	28.67±1.092 b	32.16±0.978 b	28.33±1.166b	29.56±1.202b
Glutathione (U/L) at:					
20 day	51.33±0.881 c	57.43±1.245 ab	55.30±0.901 b	59.63±1.489a	55.63±1.146b
30 day	53.30±1.123 b	59.30±0.984a	58.70±0.611 a	61.30±1.171 a	59.10±0.757 a
40 day	55.50±0.680b	62.80±1.096a	60.13±0.548 a	62.83±1.223a	62.63±0.696 a
Malondialdehyde (Nm/ml) at:					
20 day	6.20±0.700a	4.90±0.611a	5.93±0.425a	5.1±0.416a	5.83±0.523a
30 day	6.83±0.606a	4.86±0.578b	5.56±0.405 ab	5.06±0.523b	5.36±0.371 ab
40 day	7.23±0.240a	5.26±0.440b	5.76±0.592b	4.9±0.416b	5.13±0.548b
Glucose (mg/dl) at:					
20 day	247.1 ± 6.82^{a}	198.3 ± 2.69^{c}	237.7 ± 7.98^{a}	201.0 ± 7.61^{c}	234.1 ± 5.61^{a}
30 day	267.3 ± 7.88^{a}	211.0 ± 3.21^{c}	229.0 ± 5.35^{bc}	146.2 ± 6.76^{d}	218.0±7.6
40 day	269.5 ± 5.23^{a}	190.7 ± 6.23^{c}	252.0 ± 3.32^{a}	167.6 ± 4.13^{c}	249.7 ± 8.97^{ab}
Triglycerides (mg/dl) at:					
20 day	356.0 ± 9.0^{a}	210.2 ± 6.51^{e}	309.1 ± 12.39^{b}	229.7 ± 9.80^{de}	253.7 ± 7.59^{cd}
30 day	313.7 ± 8.89^{a}	250.7 ± 8.52^{c}	308.7 ± 8.99^{ab}	231.3 ± 13.87^{c}	285.7 ± 13.20^{b}
40 day	289.2 ± 6.91^{a}	251.0 ± 12.06^{b}	287.7 ± 11.86^{a}	251.7 ± 14.62^{b}	271.3 ± 4.72^{a}
Cholesterol (mg/dl) at:					
20 day	153.3 ± 5.17^{a}	115.7 ± 2.40^{d}	123.2 ± 2.42^{cd}	96.0 ± 0.58^{e}	141.1 ± 4.04^{ab}
30 day	131.4 ± 4.58^{a}	104.7 ± 2.03^{c}	119.4 ± 2.33^{ab}	107.0 ± 2.52^{bc}	96.3 ± 1.67^{c}
40 day	135.7 ± 5.78^{a}	112.7 ± 2.03^{d}	131.2 ± 8.10^{ab}	116.3 ± 0.38^{cd}	122.1 ± 2.01^{bc}

Values are means ± standard error. Means within the same row of different litters are significantly different at ($P < 0.05$).

Pathological findings:

Clinical signs: Control group (HS) ,exhibited many clinical signs of decreased activity and feed intake with opened wings, Gasping, panting, increased drinking water, diarrhoea and terminal convulsions, cannibalism, increased output of urine , loss of body weight and increased mortality .These signs mostly improved in groups supplemented with organic minirals (Zn and Se) comparing to inorganic forms, that in agreement with **(Aengwanich and Simaraks, 2004)** and **(Kumar *et al.*, 2013, Pearce *et al.*, 2013, Huang *et al.*, 2018)** who reported that at 35 oC, the broilers panted with high respiratory frequency, the ratio of water to feed intake increased up to 9: 1 and the excreta had the consistency of soup, and the dry matter content dropped as low as 12%. Besides, the increased water content of the excreta during heat stress was due to an increased flow of urine.

Gross lesions: Control group (HS), showing right atrium hypertrophy, heart enlargement with blood accumulation **(Fig.1, A)**, enlarged yellow pale liver **(Fig.1, B)**, congestion and hemorrhage of intestine**(Fig.1,C**),generalized edema and hemorrhage in kidney **(Fig.1, D)**, subcutaneous hemorrhage **(Fig.1, E)**. Muscles are dry and sticky to touch. Blood is thicker and darker than normal. Crop and gizzard are empty and dry. Gizzard lining peels off easily, that agree with **(Aengwanich and Simaraks, 2004).** These lesions significantly decreased in Zn and Se supplemented groups especially with organic forms, which were improved by **(Kumar *et al.*, 2013 and Pearce *et al.*, 2013).**



A **B** **C** **D** **E**

Fig. (1): Heat stressed broiler chicken showed right atrium hypertrophy of the heart muscle with significant blood accumulation **(Fig. 1, A)**. swollen, yellow, greasy liver **(Fig. 1, B)**. Sever congestion and haemorrhage of intestine **(Fig. 1, C).** Swelling a congestion of kidey **(Fig. 1, D)**. Sub cutaneous haemorrhage **(Fig. 1, E).**

Histopathological lesions:

Intestine: HS broilers (control gp. Fed diets with normal reqirments of Zn, Se, Mn) shows severe villus congetion (mid to basal), hemorrhage in villus tip, desquamation of epithelial lining villi and leucocytic infiltration in lamina propria (Fig.2). Broilers supplemented with Zn methionine (organic Zn,40ppm) shows normal intestinal villi with intact lining epithelium and mild leukocytic infiltration in lamina propria (Fig.3). Broilers supplemented with Zn sulfate (inorganic Zn, 40ppm) shows moderate hemorrhage in villus base, mild desquamation of epithelial lining villi with leukocytic infiltration in the lamina propria (Fig.4). Broilers supplemented with Se proteinate (organic Se, 0.15ppm) shows prominent crypts with hyperplasia of lining epithelium, thin mucosa and neumerous leucocytic infiltration in the lamina propria near the base of the crypt (Fig.5) Broilers supplemented with Na selenite (inorganic Se, 0.15ppm) shows short destructed intestinal villi with aggregation of eosinophilic cellular debris, hypertrophy of crypts and thick mucosa with leucocytic infiltration (Fig.6). Our findings are in agreement with **(Lambert *et al.*, 2002, Prosser *et al.*, 2004, Singleton & Wischmeyer, 2006, Lambert, 2009 and Quinteiro-Filho *et al.* 2010)** who reported that HS affects the integrity of the intestinal barrier, which is composed of enterocytes, tight junctions, secreted mucus and immunecells, such as macrophages .The loss of intestinal barrierintegrity leads to increased intestinal permeability, inducinglocal intestinal inflammation **(Chappell *et al.*, 2009)**. **(Hao *et***

***al.* 2012 and Quinteiro-Filho *et al.* 2012)** reported that hyperplasia of the crypt epithelium was induced to increase the absorptive area.

Liver: HS broilers (control, gp) shows vasculitis with coagulation necrosis, vacuolation of liver cell cytoplasm due to the presence of fat, besides focal replacement of hepatic paryncyma with mononuclear cells (Fig.7).Broilers supplemented with Zn methionine shows mild congestion of the portal blood vessels and hepatic sinusoids with normal hepatic parenchyma (Fig.8) .Broilers supplemented with Zn sulphate shows vasculitis with fibrinous exudate and lymphocytes and heterophils infiltrations in the hepatic parenchyma (Fig.9). Broilers supplemented with Se proteinate shows subcapsular hemorrhage and autolysed tissue mild focal coagulative necrosis of hepatocytes and hypertrophy of Kupffer cells (Fig.10). Broilers supplemented with Na selenite shows focal replacement of the hepatic paryncyma with mononuclear cells and hepatocytic vacuolation due to presence of fat (Fig.11). These finding agree with **(Cheville, 2000 and (Aengwanich and simaraks, 2004)** who reported that fatty degeneration is adiagnostic clue for liver sublethal injury due to accumulation of excess neutral lipids in the cytoplasm, especially in centrolobular zones of liver and hepatocyte mostly susceptible to oxygen deficiency and this might be caused after exposal to high environmental temperature. However, minerals (Se and Zn) are negatively correlated with high damage caused by heat stress in poultry **(Sahin *et al.* 2002).**

Kidney: HS broilers (control) shows coagulative necrosis of some renal tubules and dissociation the epithelial lining, focal replacement of the renal paryncyma with mononuclear infiltration, besides interstitial hemorrhage and edema (Fig.12). Broilers supplemented with Zn methionine shows congestion of the renal tubeules and blood vessels, flattening of tubular epithelium with normal renal parenchyma and interstitial leukocytic infiltration (Fig.13). Broilers supplemented with Zn sulphate shows clusters of tubular necrosis, focal leukocytic aggregation replaced some renal tubules, besides interstitial nephritis (Fig.14). Broilers supplemented with Se protein ate shows congestion, moderate leukocytic infiltration among some degenerated renal tubules with normal renal parenchyma (Fig.15). Broilers supplemented with Na selenite shows focal leukocytic infiltration replaced the degenerated renal tubules and hemorrhage (Fig.16).These finding are agree with **(Cheville, 2000 and Aengwanich *et al.*, 2005)** who reported that after heat stroke ocures, renal failure caused bydegenerative and necrotizing changes of renal tubules besides leukocytic aggregation and destruction of glomeruli.

Spleen: HS broilers (control gp) shows hemorrhage and hemosiderosis in the red pulp and depletion of the lymphoid cells in the white pulp (Fig.17). Broilers supplemented with Zn methionine shows subcapsular hemorrhage and autolysed tissue mild focal coagulative necrosis of hepatocytes and hypertrophy of Kupffer cells (Fig.18). Broilers supplemented with Zn sulphate shows severe depletion of the lymphoid cells in the white pulp and necrosis (Fig.19). Broilers supplemented with Se proteinate shows mild depletion of the lymphoid cells from the white pulp (Fig.20). Broilers supplemented with Na selenite shows severe necrosis and depletion of lymphoid cells from the white pulp (Fig.21). These finding are agree with those obtained with **(Soni *et al.*, 2013 and El-Katcha *et al,* 2017)** who concluded that immune system (cellular and humoral immun) is dependant on cellular metabolism which improved with organic memirals (Zn and Se). On the other hand low level of these menirals showed relative reduction in size of lymphoid organs (spleen, thymus and bursa) and depletion of lymphocytes.

Heart: HS group (control gp) shows myocardial fiber fractures, karyopyknosis and acute degeneration were observed (fig22.A); whereas in Zn methionine group, lesions were relatively less severe with some fiber fracture and hemorrhage (fig 22 .B). In Zn sulfate group, moderate degree of myocardial fiber fractures, hemorrhage and degeneration (fig 22. C). In Se proteinate group, normal basic structure with mild pyknosis and hemorrhageof cardiac muscle fibers (fig 22. D); however, Na selenite group, showed karyopyknosis and degeneration (fig 22. E). Increased heart rate and blood flow is the main response against heat stress to maintain a steady state, this might cause congestion, hemorrhage, myocardial fiber fractures and degeneration**, (Tang *et al,* 2018).**

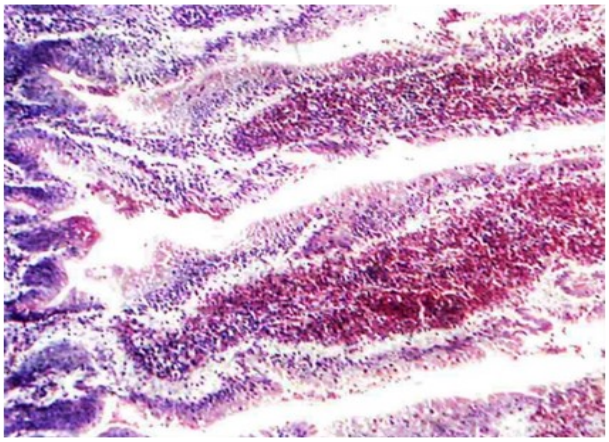


Fig. (2): Small intestine of HS broilers (control gp. Fed diets with normal reqirments of Zn, Se, Mn)shows severe villus congetion (mid to basal), hemorrhage in villus tip, desquamation of epithelial lining villi and leucocytic infiltration in lamina propria. H&E.X400

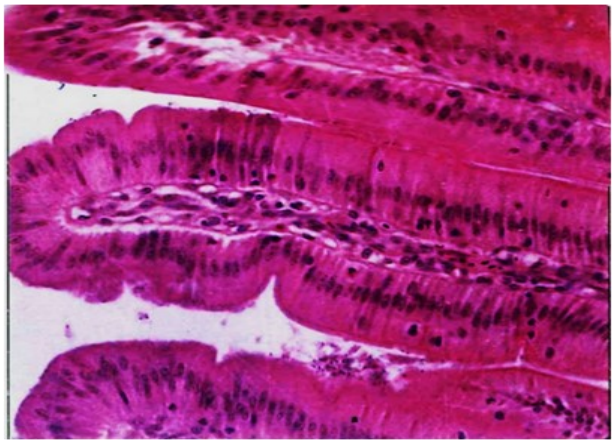


Fig. (3): Small intestine of broilers supplemented with Zn methionine (organic Zn,40ppm) shows normal intestinal villi with intact lining epithelium and mild leukocytic infiltration in lamina propria. .H&E.X400

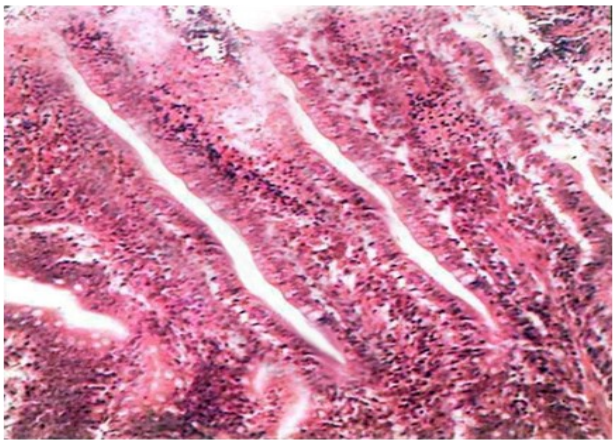


Fig. (4): Small intestine of broilers supplemented with Zn sulfate (inorganic Zn,40ppm) shows moderate hemorrhage in villus base, mild desquamation of epithelial lining villi with leukocytic infiltration in the lamina propria.H&E.X400

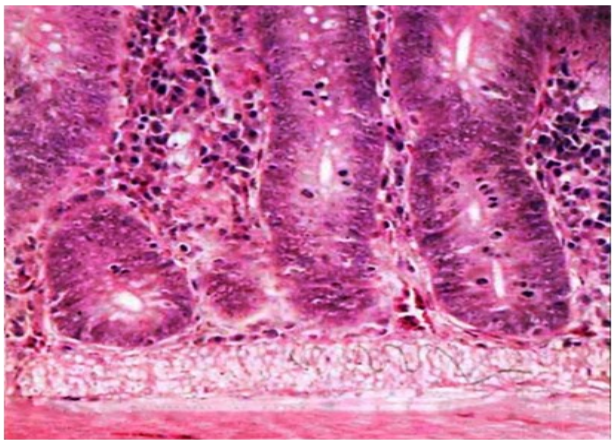


Fig. (5): Small intestine of broilers supplemented with Se proteinate (organic Se,0.15ppm) shows prominent crypts with hyperplasia of lining epithelium, thin mucosa and neumerous leucocytic infiltration in the lamina propria near the base of the crypt.H&E.X400.

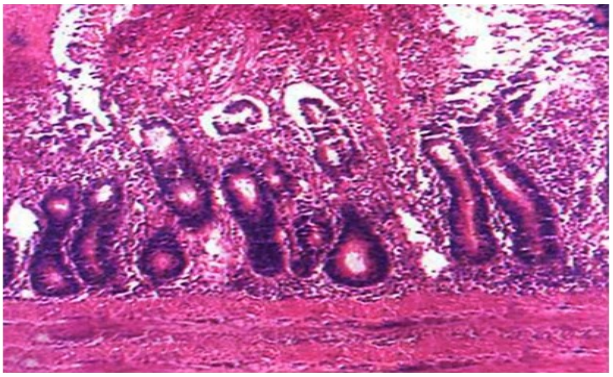


Fig. (6): Small intestine of broilers supplemented with Na selenite (inorganic Se,0.15ppm) shows short destructed intestinal villi with aggregation of eosinophilic cellular debris, hypertrophy of crypts and thick mucosa with leucocytic infiltration. H&E.X400.

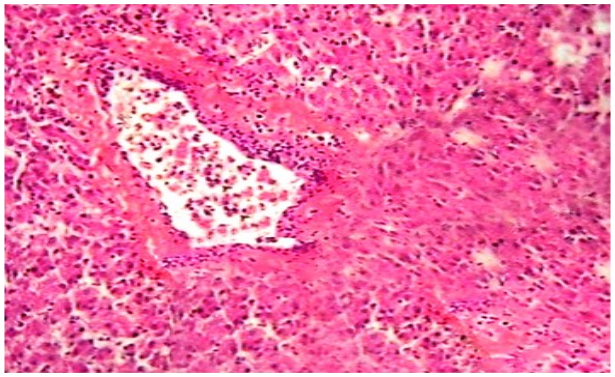


Fig. (7): Liver of HS broilers (control, gp) Fed diets with normal reqirments of Zn, Se) shows vasculitis with coagulation necrosis, vacuolation of liver cell cytoplasm due to the presence of fat, besides focal replacement of hepatic paryncyma with mononuclear cells. H&E.X400.

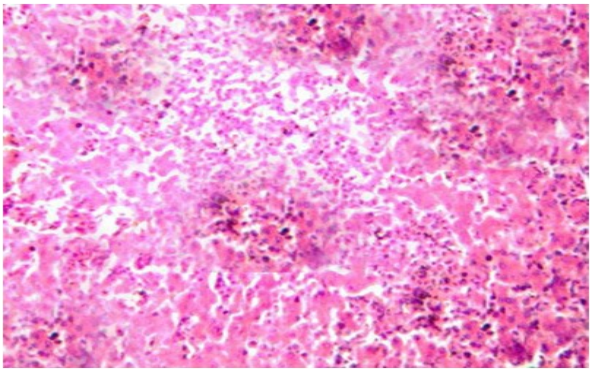


Fig. (8): Liver of broilers supplemented with Zn methionine (organic Zn,40ppm) shows mild congestion of the portal blood vessels and hepatic sinusoids with normal hepatic parenchyma. H&E.X400.

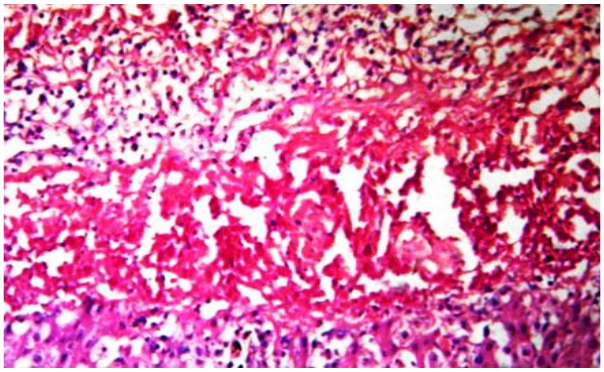


Fig. (9): Liver of broilers supplemented with Zn sulphate (inorganic Zn,40ppm) shows vasculitis with fibrinous exudate and lymphocytes and heterophils infiltrations in the hepatic parenchyma.H&E.X400

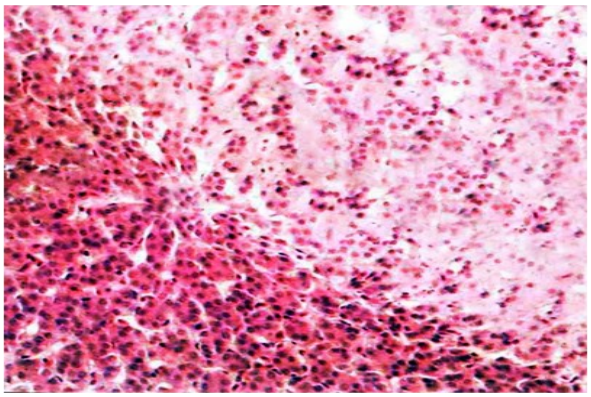


Fig. (10): Liver of broilers supplemented with Se proteinate (organic Se,0.15ppm) shows subcapsular hemorrhage and autolysed tissue mild focal coagulative necrosis of hepatocytes and hypertrophy of Kupffer cells.H&E.X200

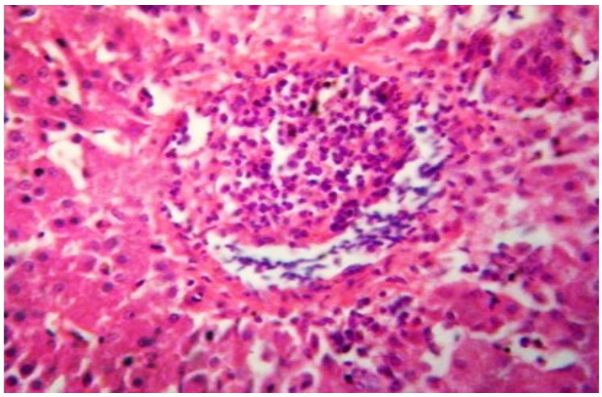


Fig. (11): Liver of broilers supplemented with Na selenite (inorganic Se,0.15ppm) shows focal replacement of the hepatic parynchyma with mononuclear cells and hepatocytic vacuolation due to presence of fat.. H&E.X400

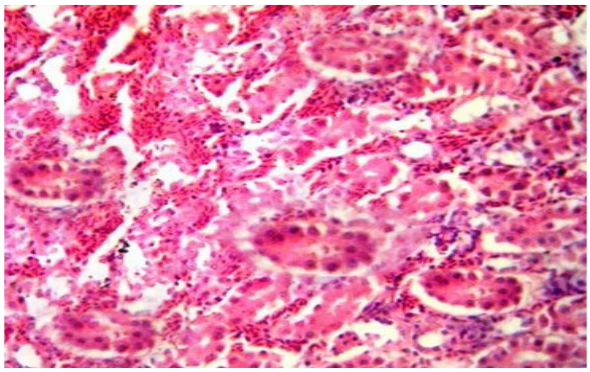


Fig. (12): Kidney of HS broilers (control,gp. Fed diets with normal reqirments of Zn, Se) shows coagulative necrosis of some renal tubules and dissociation the epithelial lining, focal replacement of the renal parynchyma with mononuclear infiltration ,besides interstitial hemorrhage and edema. H&E. X200.

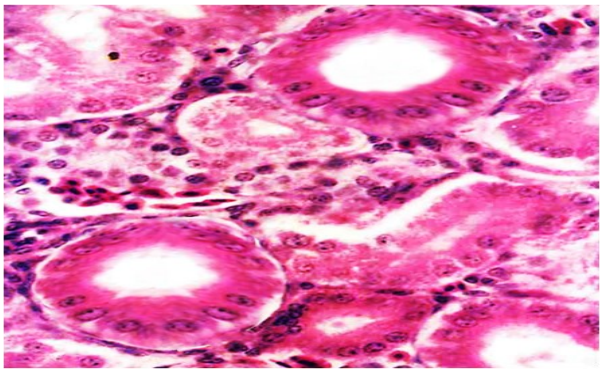


Fig. (13): Kidney of broilers supplemented with Zn methionine (organic ZC,40ppm) shows congestion of the renal tubeules and blood vessels, flattening of tubular epithelium with normal renal parenchyma and interstitial leukocytic infiltration..H&E.X400.

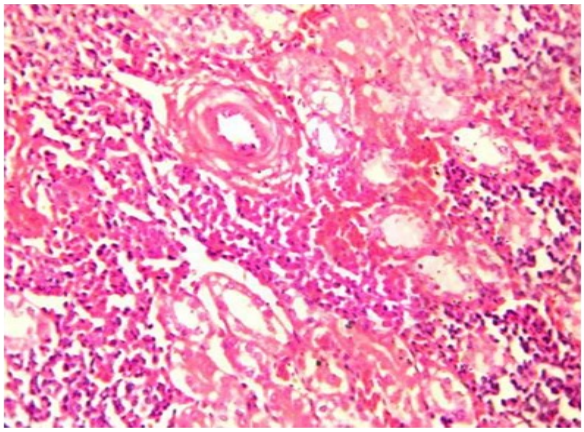


Fig (14). Kidneyof broilers supplemented with Zn sulphate (inorganic Zn,40ppm) shows clusters of tubular necrosis, focal leukocytic aggregation replaced some renal tubules, besides interstitial nephritis. H&E.X400

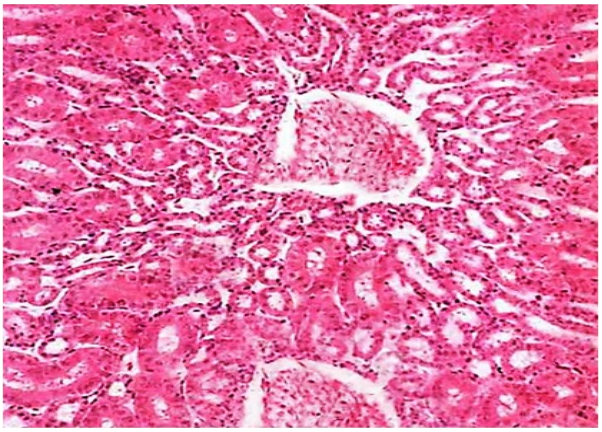


Fig (15). Kidney of broilers supplemented with Se proteinate (organic Se,0.15ppm) shows congestion, moderate leukocytic infiltration among some degenerated renal tubules with normal renal parenchyma. H&E.X200.

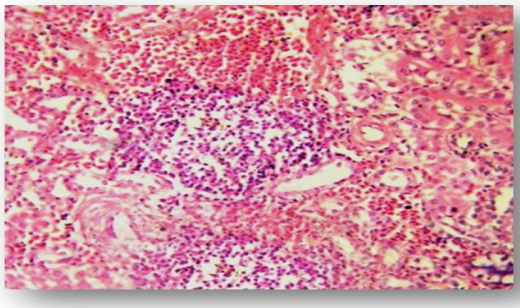


Fig (16). Kidney of broilers supplemented with Na selenite (inorganic Se, 0.15ppm) shows focal leukocytic infiltration replaced the degenerated renal tubules and hemorrage..H&E.X400

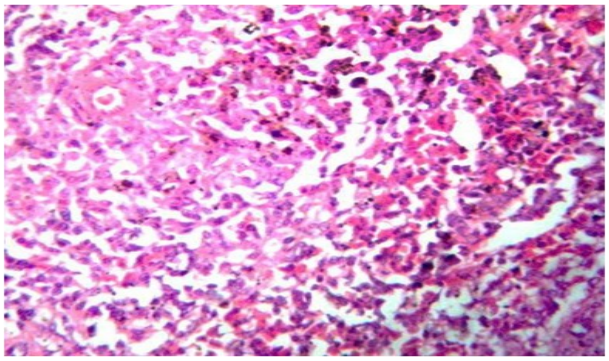


Fig (18). Spleen of broilers supplemented with Zn methionine (organic Zn,40ppm) shows congestion of splenic sinusoids and depletion of some lymphoid cells. H&E.X400

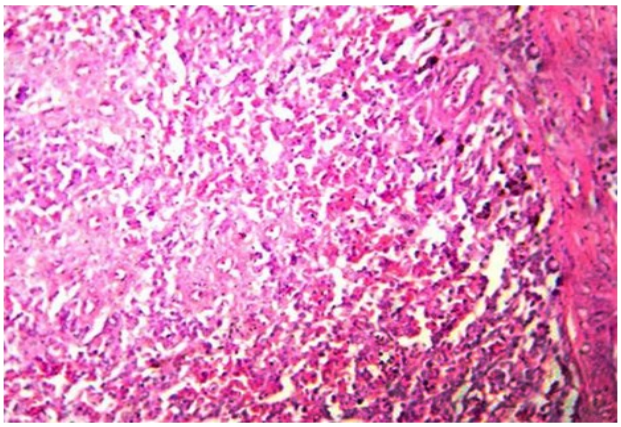


Fig (19). Spleen of broilers supplemented with Zn sulphate (inorganic Zn,40ppm) shows severe depletion of the lymphoid cells in the white pulpand necrosis. H&E.X200

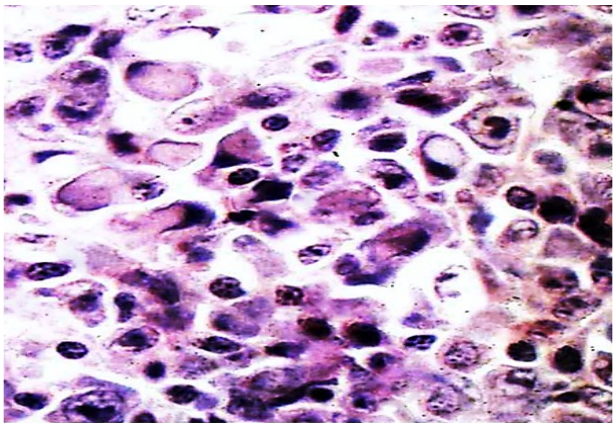


Fig (20). Spleen of broilers supplemented with Se organic (organic Se,0.15ppm) shows mild depletion of the lymphoid cells from the white pulp. H & E.X400.

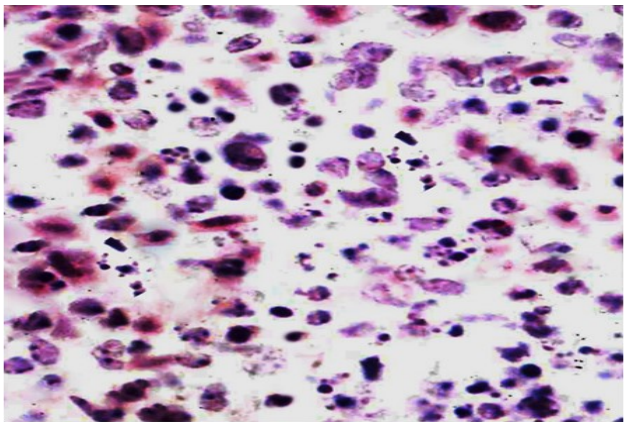
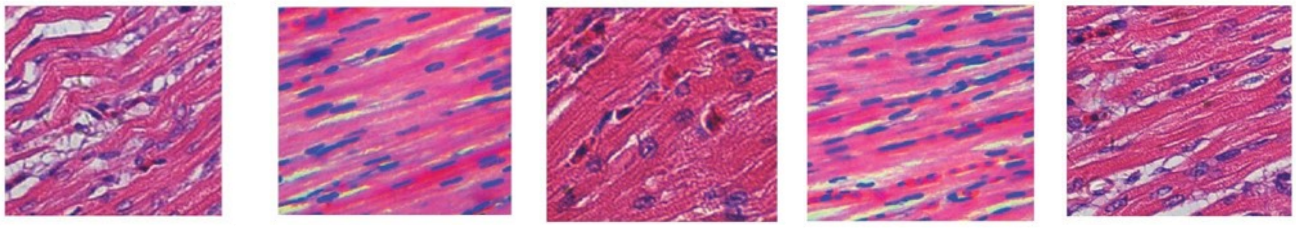


Fig (21). Spleen of broilers supplemented with Na selenite (inorganic Se,0.15ppm) shows severe necrosis and depletion of lymphoid cells from the white pulp. H&E.X400.

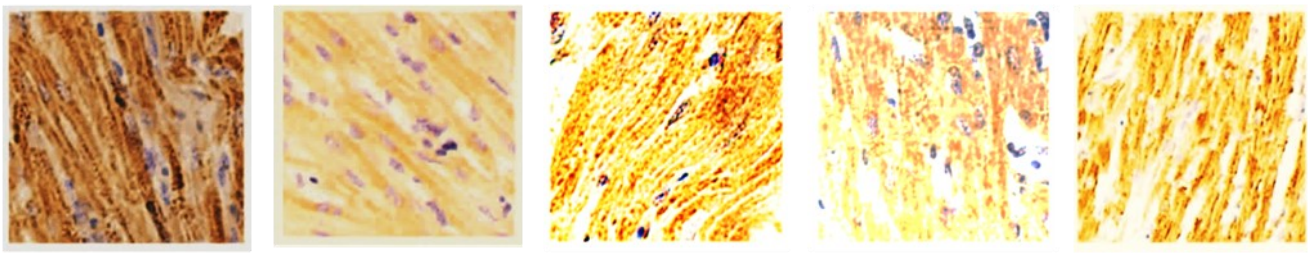
Localization and Density of HSP70 in Chicken Heart:

The immunohistochemistry results were consistent with the histopathological examination of chicken heart. HSP70 staining intensities were higher in HS group (Fig.23, A) and intensity significantly decreased in Zn and Se supplemented groups especially in organic minirals, Zn Methionine and Se Proteinate (Fig.23, B and D) than in the inorganic, Zn sulfate and Na selenite (Fig.23, C and E). These results are consistent with **(Xu *et al.,* 2016)** who reported that HSP70 is significantly induced in chicken myocardial cells under HS ,It important and highly conserved member of the HSP family, its cytoprotective function has been widely investigated; it senses oxidative damage and repairs unfolded or misfolded proteins. HSP70 can bind to and inhibit apoptotic proteins to regulate apoptosis under various environmental stresses. previous research confirmed that the cause of heat-induced sudden death was heart cell damage (cell necrosis and cell degeneration) **(Tang *et al.,* 2014)**. The heart is the most important organ and the thermal tolerance to heat stress is impaired during cardiovascular diseases **(Cui and Sinoway, 2014)**. The cellular damage thus caused by reactive oxygen species (ROS) accumulation is considered as a key factor in the activation of HSP genes. Cells when subjected to heat stress with increased lipid peroxidation accumulate HSP70, which might work as a tissue biomarker for potential damage caused by stress. Thus, the damage caused by a strong stress results in the high expression levels of HSP70**(Banu *et al.* 2009)**. On other hand, exogenous supplementation with antioxidants has shown to interfere with this adaptation due to synergetic effect of Zn and Se with probiotics under the natural heat, because (Se and Zn) are negatively correlated with high damage caused by oxidation in poultry reared under stress conditions **(Sahin *et al.* 2002)**.Thus leads to scavenge free radicals, which restrict the expression of HSP proteins and thus improve the cell survival. **(Kumbhar *et al* ., 2018).**



A **B** **C** **D** **E**

Fig (22). Histopathologicalchanges in different treatment groups. H&E.X400. HS gr:myocardial fiber fractures, karyopyknosis and acute degeneration were observed (fig22.A). Zn methionine group, l esions were relatively less severe with some fiber fracture and hemorrhage (fig22.B). Zn sulfate group, moderate degree of myocardial fiber fractures, hemorrhage and degeneration (fig22.C). Se proteinate group, normal basic structure with mild pyknosis and hemorrhage of cardiac muscle fibers (fig22.D).Naselenite group, showed karyopyknosis and degeneration of myocytes (fig22.E).



A B C D E

Fig (23): immunohistochemical analysis of heat shock protein 70 (HSP70) in myocardial fiber. Immunoperoxidase. Counter stain Mayer's hematoxylin, X400. HS. gr: showed higher brown staining intensity of HSP70(fig23. A). The intensity significantly decreased inorganic Zn &Se supplemented groups (fig23. B&D). Comparing toinorganic Zn & Se supplemented groups which slightly more staining than organic forms (fig23.C,E).

Effect of dietary (Zinc and Selenium) supplementation on the intestinal morphology of broiler chickens under heat stress .

As shown in Table (6), broilers in heat stress had shorter villus height, deeper crypt depth and lower villus height to crypt depth ratio than those of minerals supplemented groups. In organic Zn and Se supplemented groups, villus height, increased ,normal crypt depth and increased villus height to crypt depth ratio comparing to inorganic (Zn and Se) supplemented groups. These results are consistent with the findings of (**Song *et al.,* 2014)**, who found that greater blood flow to the myocardium, turbinates, nasal mucosa, and respiratory muscles during heat stress result in a decrease of gut blood flow. In addition, ischaemia of the enteric canal can cause epithelial shedding, leading to shortened villus height and deeper crypt depth. (**Hosseini *et al.,* 2016)** reported that villus height is an important indicator of the digestive health of chickens and directly related to the absorptive capacity of the mucous membrane. (**Ghazi *et al.,* 2012)** found that dietary Zn and Se supplementation stimulate the digestive and absorptive functions of broilers , increase intestinal integrity and may be helpful in explaining the improvement in the growth performance observed in this research.

Table (6): Effect of dietary (Zinc and Selenium) supplementation on the intestinal morphology of broiler chickens under heat stress .

Items	Control (heat stress group)	Zinc methionine (organic,40ppm)	Zinc sulfate (inorganic.40ppm)	Selenium proteinate (organic.0.15ppm)	Na selenite (inorganic.0.15ppm)
villi length (um)	685.63±54.55d	1115.37±44.96b	853.48±56.99bcd	986.42±148.21bc	844.39±115.42bcd
villi width (um)	116.46±40.24b	126.52 ±15.48b	141.25 ±30.07b	122.28±30.24b	115.16 ±16.37b
crypt depth (um)	133.60±20.50bc	162.34±20.70abc	163.08 ±45.91abc	221.54±41.48a	112±13.17c
villilength/ crypt depth	5.30±0.64c	7.32±o.72b	5.45±0,46c	4.54±0.94c	9.89.±0.62a

Values are means ± standard error. Means within the same row of different litters are significantly different at ($P < 0.05$).

Conclusion

Generally, from the results of the present study, it could be concluded that trace minerals Zn (40ppm) and Se (0.15ppm) supplementation to the diets of broiler chicks exposed to high ambient temperature and high relative humidity may be effective to alleviate a part of these stresses specially the organic form. Dietary supplementation with these elements improves growth performance data (body weight & feed conversion), immune response to Newcastle disease virus and minimize the incidence and severity of the pathological changes in the internal organs and helps to regulate concentration of serum metabolites, total protein, globulin, albumin, glucose, triglycerides and cholesterol, to maintain good health condition and improve productivity in local broiler farms, which always expose to several periods of high ambient temperature and high relative humidity during summer season

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