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Effect of Dietary Zinc-Oxide or Nano-Zinc Oxide on Growth Performance, Oxidative Stress, and Immunity of Growing Rabbits under Hot Climate Conditions

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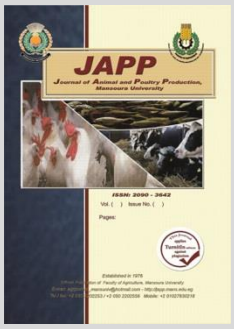

ABSTRACT

The aim of the present study was to evaluate growth performance parameters, antioxidant capacity, immune response, and hepatic and renal functions of APRI growing rabbits fed diets supplemented with zinc-oxide (ZnO) or nano-zinc oxide (ZnO-NP). A number of 60 weaned rabbits (5 wk of age) were divided into three experimental groups were fed a basal diet supplemented with 0 (G1), 50 mg ZnO (G2), and 30 mg ZnO-NP (G3) per kg during the growing period (5 to 13 wk of age). Live body weight, feed consumption, weight gain, feed conversion ratio, performance index and mortality rate were recorded. Biochemical parameters, antioxidant and immunity status were determined at 13 wk of age. Results show that dietary ZnO or ZnO-NP addition increased (P<0.05) growth performance parameters, serum high-density lipoproteins, glutathione, glutathione S-transferase and superoxide dismutase, immunoglobulins. Concentrations of triglycerides and MDA in blood serum reduced (P<0.05) in treatment groups. In conclusion, dietary supplementation with ZnO or ZnO-NP can enhance growth performance, lipid profile, immunity and antioxidant status of growing rabbits under heat stress conditions.

Keywords: rabbits, zinc-oxide, nano-particles, growth performance, antioxidant status, immunity response.

INTRODUCTION

In majority of tropical countries, heat stress is one of the most important stressors negatively affecting poultry industry leading to a great economic loss each year. Higher ambient temperature reduces live weight gain, feed intake, feed efficiency, total mineral retention, and immune response of chicken broilers (El-Deep *et al*., 2016;Saleh *et al*., 2018). Heat stress may promote formation of reactive oxygen species (Mujahid *et al*., 2005), which damages proteins, DNA, cell phospholipid membranes and other vital macromolecules causing lipid peroxidation (Ebeid *et al*. 2013). Such damage is associated with apoptosis and various diseases (El-Deep *et al*., 2016). Therefore, a balance between reactive oxygen species production and the antioxidant defense system must be established to maintain immune function, health and productivity (Suraï, 2002).

Several methods are available to alleviate the negative effects of heat stress on animals. In this respect, trace mineral like zinc reducing the negative effects of heat stress of poultry (Saleh *et al*., 2018) through participating in many metabolic processes and physiological functions (Hassan *et al*., 2017). Zinc is the second most abundant trace element in the animal body (Swain *et al*., 2016). Zinc is an important mineral with diverse physiological functions in mammals and birds for normal growth performance, regulation of the immune system, oxygen free radical scavenging and antibacterial activity (Fathi *et al*., 2016). The secretion of thymulin from thymus gland to stimulate T-cell production was affected by zinc. Thus, zinc deficiency resulted in thymus malfunctioning, which severely affects normal immune function (Mocchegiani *et al*., 1998). Moreover, Zinc are involved more than 300 enzymes that participate in the synthesis and/or breaking down of proteins, carbohydrates, lipids, and nucleic acids (Liu *et al*., 2011).

Natural antioxidants have important mechanisms which help in the cells from reactive oxygen species protection by reducing free radicals and preventing the peroxidation of lipids (Grashorn, 2007). The antioxidant system includes numerous antioxidant enzymes, such as glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (Suraï, 2002). In this line, zinc necessary component of SOD enzyme, which has a vital role in the antioxidant defense system (Powell, 2000). In addition, zinc deficiency in animals lead to low circulating levels of growth hormone (GH) and insulin-like growth factor-I and decreased hepatic production of insulin-like growth factor-I, GH receptor and GH-binding protein (Chrastinová *et al*., 2018).

Recently, the development of nanotechnology and its related products has rapidly progressed in different scientific areas (Fathi *et al*., 2016). In the animal body, nano-minerals interact more effectively with organic and inorganic substances due to their larger surface area, high catalytic efficiency, and strong adsorbing ability (Wijnhoven *et al*.,2009; Zaboli *et al*., 2013). Nano-minerals have the ability to cross the small intestine and further distribute into the blood and the internal body

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organs (Hillyer and Albrecht, 2001). So, these nano-minerals are expected to be effective in small doses, offer better bioavailability and have stable interaction with other components when fed as an alternative to the traditional sources (Hassan *et al*., 2017). In this regard, the zinc oxide nano-particles (ZnO-NP) were mainly found to be retained in the liver after 14-day sub-acute exposure (Sharma *et al*., 2012), and permeability of ZnO-NP can help prevent adverse gastrointestinal reactions and improve the absorption of medicine (Zhao *et al*., 2014). Datary supplementation of ZnO (Selim *et al*., 2012; Chrastinová *et al*., 2015) or ZnO-NP (Hassan *et al*., 2017) was found to enhance the productive performance of growing rabbits. Furthermore, heat stress conditions increase Zn requirements of rabbits. So far, little evidence is yet available regarding the evaluation the potential beneficial role of ZnO-NP compared with zinc oxide in growth parameters and physiological status of heat stressed rabbits. Also, data on zinc concentration in blood, liver and muscles of heat-stressed rabbit treated ZnO-NP are still limited. Therefore, the objective of the present study was to compare the effects of two different zinc sources (zinc oxide nano-particles or zinc oxide) on growth performance, blood biochemical, antioxidative status, lipid peroxidation, immune function in growing rabbit reared under summer condition in Egypt.

MATERIALS AND METHODS

The present study was carried out at a private rabbit farm, while laboratorial work was carried out in the laboratory of Physiology and Biotechnology, Department of Animal Production, Faculty of Agriculture, Mansoura University, Egypt, during the period from May 2018 to July 2018.

Rabbits were housed in semi-open building with electric exhausted fans. Average of air temperature (AT) and relative humidity (RH %) inside the rabbitry were estimated weekly by digital thermo-hygrometer. The maximal and minimal AT, RH and the calculated temperature-humidity index (THI) during the experimental weeks are presented in Table 1. The THI was computed based on Marai *et al.* (2001) equation.

$$THI = db\ ^oC - [(0.31 - 0.31 \times RH) \times (db\ ^oC - 14.4)].$$

Where

db ºC = dry bulb temperature. According to this equation, THI values of less than 27.8 mention to the absence of heat stress (HS), THI values (27.8-28.8) mention to mild HS; THI values (28.9-29.9) mention to severe HS, while THI more than 30.0 mention to very severe HS.

Table 1. Maximal values of air temperature, relative humidity and THI during the experimental weeks. (Mean ± standard error)

Experimental week	Air temperature (°C)	Relative humidity (%)	THI value
1st	29±0.38	56.57±0.97	27.03±0.37
2nd	29.14±0.34	60.86±0.51	27.35±0.31
3rd	29.57±0.37	62.57±1.19	27.81±0.31
4th	30.29±0.42	70±0.49	28.81±0.36
5th	30.71±0.47	70.71±0.29	29.23±0.43
6th	31.57±0.37	71.14±0.50	30.04±0.34
7th	31.86±0.40	71.29±0.42	30.30±0.39
8th	31.71±0.29	71.86±0.51	30.20±0.28
Overall mean	30.48±0.29	66.88±0.51	28.83±0.28

A number of 60 weaned male APRI rabbits (Egyptian line) having five wk of age and weighing 702.40±1.25 g as an initial body weight were used in this study. They were divided based on their weights into 3 experimental groups, 20 rabbits in each,. During the experimental period, rabbits were individually housed in galvanized wire cages (35 × 60 × 35 cm) under similar hygienic and managerial conditions. Faeces and urine were removed from the rabbitry floor every morning. The rabbits were adapted to the basal diet over a 2-week period. Feed and clean water were offered *ad libitum* and refilled at 7:00 am and 2:00 pm daily until 13 weeks of age. All groups were fed a commercial pelleted diet formulated to cover all essential nutrient requirements of growing rabbits (Table 2). The dietary supplementation was 0 (G1), 50 mg ZnO/kg (G2) and 30 mg of ZnO-NP (G3).

Table 2. Composition and chemical analysis of the control diet used for feeding rabbits.

Ingredient	(%)	Chemical composition	(%)
Berseem hay	30.3	Ether extract	2.23
Barley grain	25.0	Nitrogen free extract	57.27
Wheat brain	25.0	Ash	8.75
Soybean meal (44%)	15.0	Organic matter	91.25
Molasses	3.00	Crude protein	18.25
Di-calcium phosphate	0.20	Crude fiber	13.50
Limestone	0.90	Total	100
DL-Methionine	0.20	-	-
NaCl	0.30	-	-
Premix[1]	0.30	-	
Total	100	-	-

[1] Each on kg contains 150, 000 UI, 100 mg, 10 mg, 21 mg, 40 mg, 15 mg, and 0.1 mg of vitamins A, E, B_1, K_3, B_2, B_6, and B_{12}; 100 mg pantothenic acid, 200 mg niacin, 0.5 mg biotin, 10 mg folic acid, 5000 mg, choline chloride, 800 mg manganese, 600mg zinc, 300 mg iron, 40 mg copper, 500 mg iodine, 100 mg selenium, and 100 mg cobalt.

Throughout an experimental period of 8 wk, growth performance parameters including LBW and feed consumption were recorded, then average daily weight gain (ADG) and feed conversion ratio (FCR) were calculated at 5-9, 9-13 and 5-13 wk as age intervals. Also, number of live rabbits at birth and weaning was recorded, and total viability rate (VR) was calculated. Performance index [*PI = (Final LBW (kg)/FCR) x100]* was calculated during the growing period (5-13 wk).

Blood samples were collected at the end of the experimental period (13 wk of age), blood samples (n = 5 per group) were collected from the ear vein into sterile tubes without anticoagulant for biochemical parameters in blood serum. The samples were left for 20 min at room temperature to coagulate and then centrifuged at 1075 × g in a refrigerated centrifuge (BOECO centrifuge C-28 A, Hamburg, Germany) for 10 min, which was stored at -20ºC till assayed.

In blood serum of rabbits, total proteins, albumin, glucose, cholesterol, total lipids, triglycerides, high density lipoproteins (HDL), and low density lipoproteins (LDL) concentrations were determined using spectrophotometer and commercial kits (Bio-Merieux, Laboratory Reagents and Products, France). Globulin concentration was computed by the difference between TP and AL concentration. In addition, glutathione content, GSH

(Beutler *et al*., 1963), glutathione peroxidase, GPx, superoxide dismutase, SOD and MDA were assayed in blood serum using commercially available kits (Bio-diagnostic Co., Recycling Crusher-SBM®). Concentration of immunoglobulin (IgG and IgM) was assayed in blood serum using commercially available kits (Spinreact Co., Santa Coloma, Spain).

All data was statistically analyzed by one-way ANOVA design using a software package (SAS, 2002). Completely randomized design was used based on the following model:

$$Y_{ij} = \mu + G_i + e_{ij}$$

Where

μ = the overall mean, G_i = groups (1…..3), and e_{ij} = residual error.

Mortality percentage was statistically analyzed using Chi-Square test. Duncan's multiple range test was used to test the significant differences at P<0.05 among the experimental groups.

RESULTS AND DISCUSSION

Productive performance:

Growth performance, including LBW of rabbits significantly (P<0.05) increased in treatment groups (G2 and G3) as compared to control (G1), being significantly (P<0.05) the heaviest in G3 at 9 and 13 wk of age. Also, average daily gain significantly (P<0.05) increased in G2 and G3 in comparing G1, being the highest in G3 at all age intervals. Although feed intake was not affected by treatment, feed conversion ratio at all age intervals and performance index were significant (P<0.05) improve in G2 and G3 as compared to in G1. However, the effect of zinc oxide and nano-Zinc oxide on mortality rate was not significant (Table 3). These results indicated beneficial effects of dietary supplementation of nano-zinc oxide on growth performance of growing rabbits.

Table 3. Growth performance parameters of growing rabbits in the experimental groups at different ages.

Item	G1 (Control)	G2 (50 mg ZnO/ kg diet)	G3 (30 mg ZnO-NP/ kg diet)	SEM	P-value
Average live body weight (g)					
At 5 wk (Initial)	705.45	699.31	702.43	3.715	0.0415
At 9 wk	1385.55^{c}	1416.23^{b}	1432.50^{a}	5.206	<.0001
At 13 wk (Final)	2090.64^{c}	2155.54^{b}	2185.2^{a}	4.828	<.0001
Average daily gain (g)					
At 5~9 wk	24.29^{b}	25.60^{a}	26.07^{a}	0.165	<.0001
At 9~13wk	25.18^{c}	26.40^{b}	26.88^{a}	0.039	<.0001
At 5~13 wk	24.74^{c}	26.00^{b}	26.48^{a}	0.076	<.0001
Average daily feed intake (g)					
At 5~9 wk	67.45	66.46	65.71	0.715	0.2478
At 9~13wk	109.09	108.08	108.43	1.625	0.9105
At 5~13 wk	88.27	87.27	87.07	0.949	0.6512
Feed conversion ratio (g feed/g gain)					
At 5~9 wk	2.78^{a}	2.59^{b}	2.52^{b}	0.035	<.0001
At 9~13 wk	4.33^{a}	4.09^{b}	4.03^{b}	0.062	0.0048
At 5~13 wk	3.57^{a}	3.36^{b}	3.29^{b}	0.040	<.0001
Performance index	58.71^{b}	64.33^{a}	66.53^{a}	0.822	<.0001
Mortality (%)*	0.73	0.87	0.93	-	

a, b and c: Significant group differences at P<0.05. *Chi-square test.

Blood serum biochemicals:

Data in Table 4 revealed that concentrations of total protein and their fractions were not affected by treatment. Glucose concentration significantly (P<0.05) increased in treatment groups as compared to control, being the highest in G3. Rabbits in G3 showed significantly (P<0.05) the highest serum triglycerides and HDL concentrations, while there were insignificant differences in serum cholesterol and LDL concentrations (Table 4).

Table 4. Serum biochemicals of growing rabbits in the experimental groups at 13 wk of age.

Parameter	G1 (Control)	G2 (50 mg ZnO/ kg diet)	G3 (30 mg ZnO-NP/ kg diet)	SEM	P-value
Protein fractions (g/dl):					
Total proteins	6.23	6.26	6.27	0.016	0.2159
Albumin	3.55	3.56	3.60	0.017	0.0971
Globulin	2.68	2.70	2.68	0.023	0.6700
Albumin / Globulin ratio	1.32	1.32	1.35	0.017	0.4043
Carbohydrate metabolism (mg/dl):					
Glucose	64.80^{c}	78.20^{b}	92.60^{a}	1.322	0.0001
Lipid metabolism (mg/dl):					
Cholesterol	132.60	129.80	125.20	2.390	0.1288
Triglycerides	120.80^{c}	92.60^{b}	79.80^{a}	1.575	0.0001
HDL	51.00^{b}	56.40^{a}	58.20^{a}	1.058	0.0313
LDL	47.40	48.00	48.40	1.381	0.8769

a, b and c: Significant group differences at P<0.05.

Antioxidants capacity and immunity:

Results in Table 5 show that glutathione (GSH), glutathione S-transferase (GST) and superoxide dismutase (SOD) were significantly (P<0.05) higher, while MDA concentration was significantly (P<0.05) lower in blood serum of rabbits in treatment groups than the control one,

being the best (P<0.05) in G3. Also, concentrations of immunoglobulins (IgG and IgM) significantly (P<0.05) increased in treatment as compaered to control, being the highest in G3. These results reflect higher immune response of G2 and G3 than in G1, being the best (P<0.05) in G3.

Table 5. Antioxidant enzymes, lipid peroxidation and immunoglobulins concentrations in blood serum of growing rabbits in the experimental groups.

Parameter	G1 (Control)	G2 (50 mg ZnO/ kg diet)	G3 (30 mg ZnO-NP/ kg diet)	SEM	P-value
Antioxidant enzymes (IU/ml)					
GSH	11.73^{c}	14.58^{b}	15.93^{a}	0.184	<.0001
GPx	3.14^{c}	4.02^{b}	4.24^{a}	0.018	<.0001
SOD	6.75^{c}	6.96^{b}	7.23^{a}	0.017	<.0001
Lipid peroxidation (nmol/ml)					
MDA	7.25^{a}	6.37^{b}	5.99^{c}	0.014	<.0001
Immunity status (mg/dl):					
IgG	2.11^{c}	3.25^{b}	3.51^{a}	0.017	<.0001
IgM	3.96^{c}	4.35^{b}	5.16^{a}	0.028	<.0001

a, b and c: Significant group differences at P<0.05.

Discussion

The most challenge of animal production is heat stress (Daader *et al*., 2018) as indicated for growing rabbits in our study. The recorded average THI value during the experimental period (THI = 28.83) in Table 1 indicated that growing rabbits in all treatments were kept under moderate heat stress (Marai *et al*., 2001). Occurrence of OS under HS condition means increasing ROS generation and decreasing the antioxidant capacity (Peña *et al*., 2019), causing loss of animal welfare and productivity (El-Ratel *et al*., 2020). Natural antioxidants play vital roles in protecting cells from ROS by reducing free radicals and preventing the peroxidation of lipids (Grashorn, 2007). Biologically, zinc has antioxidative properties because it is considered the main element in Cu/Zn superoxide dismutase (the antioxidant enzyme). Zinc play an important role in upregulation of synthesis of proteins proteins (e.g. thioneinm) which have capacity of oxidant scavenging (Oteiza, 2012). Increasing the interest of nano-technology application for improving the dietary efficiency of trace elements (Al-Beitawi *et al*., 2017), thus using Zn-NP could be used as a substitute to conventional sources of zinc in animal feeding (Ramiah *et al*., 2019). In the current study, the supplementation of rabbit diets with ZnONP at 30mg/kg diet enhanced growth performance when compared to the control and ZnO diets, suggesting a better absorption and higher bioavailability of nano-zinc, which is consistent with the previous studies (Ahmadi *et al*., 2013; Zhao *et al*., 2014). In comparing with inorganic zinc sources, the dietary supplementation of ZnO-NP could modulate the mineral deposition given their high bioavailability (Ibrahim *et al*., 2017). The dietary zinc is important to proper physiological functions of the animal, such as growth (Case and Carlson, 2002), synthesis of DNA and cell division (Prasad, 1991), improving the immunity of the animal body (Zhao *et al*., 2014). Also, Zn is a component of the free radicals scavengers which are produced during different physiological processes (Zhao *et al*., 2014). These results are in the same line with Siddhartha *et al*. (2016) who stated that birds fed diet supplemented with nano-Zn/kg had greater weight gains and better feed conversion ratio than the control. Also, El-Katcha *et al*. (2017) found that final body weight, BWG and FCR, of broiler chicken were improved by nano-zinc supplementation (30,45 and 60 mg/kg) than the control.

The present study improving carbohydrate metabolism and lipid metabolism as affected by zinc treatment may indicated enhancement of healthy status of rabbits in G2 and G3, in terms of reducing serum triglycerides and increasing levels of glucose and HDL. These results are in agreement with Borah *et al.* (2013) reported that serum albumin level in growing pigs was not affected by different supplemented level of zinc compared with control ones. However, other studies showed that insignificant effect of ZnO on level of serum total proteins and globulin of piglets at weaning (Wang *et al*., 2013; Cho *et al*., 2015). The dietary nano-zinc supplementation increased HDL than the control of broiler chicks (El-Katcha *et al*., 2017). Also, reducing of triglycerides levels in blood serum in this study may be due to the zinc's role in enzyme action which an integral part of several enzymes (metalloenzymes) that are severed in lipid digestion and absorption (Hazim *et al*., 2011). High ambient temperature significantly depresses growth performance of broiler chickens (El-Deep *et al*., 2014), while supplementation with antioxidants such trace elements has proven beneficial in alleviating the adverse effects of high ambient temperature challenge (Sahin *et al*., 2009). It has been shown that dietary Zn supplementation improves antioxidant status by activating SOD in piglets (Wang *et al*., 2012) and rabbits (Alissa *et al*., 2004). In addition, Zhao *et al.* (2014) found that the concentration of Cu-Zn-SOD was significantly increased in serum of broilers after feeding 60 or 100 mg/kg nZnO compared with ZnO group. In our study, nano-ZnO at applied levels (30 mg/kg) were associated with significantly increased in SOD, GSH and GPx concentrations in serum and significantly decreased MDA as a lipid peroxidation. This observation confirmed the role of zinc in antioxidant defense system. Farombi *et al*. (2004) reported that SOD, GPx and MDA levels are markers of oxidative stress in the antioxidant defense system and lipid peroxidation, respectively. The Zn is considered as the most important antioxidant containing thiol group was found to regulate metabolism of GSH, (Oteiza, 2012). The mechanisms of zinc as an antioxidant may be related to its antioxidant gen upregulation, and Nrf2 may be a major mechanism controlling zinc antioxidant action (Cortese *et al*., 2008). As shown in our results, significant variation was noticed in the serum IgG and IgM levels and obtained consistently higher values in

30 mg nano-ZnO group compared with other treatments. These results were in agreement with the previous report that nano-ZnO supplementation of weanling piglet diets increased γ–globulin and IgG levels compared to piglets fed on ZnO (Li *et al*., 2016). The importance of zinc in animal feeding may be related to competence of animal immunity (Suttle, 2010), but the balance of ROS generation with the antioxidant system must be achieved for maintaining the immunity function, health status and productive performance of the animal (Chand *et al*. 2016; Saleh *et al*. 2017). Several authors indicated that zinc is an important element for improving immune response in heat stress conditions (Chand *et al*. 2014; Abudabos *et al*. 2017). In this respect, the supplementation of dietary Zn was reported to increase weights of lymphoid organs, the responses to primary and secondary antibodies, phagocytic activity of macrophages, antibody titres of IgM and IgG in male broilers raised in high environmental temperature (Bartlet and Smith, 2003). It could be concluded that dietary ZnO-NP may improve the immunity and health status of growing rabbit under high ambient temperature.

CONCLUSION

The administration of zinc nano-particles to growing rabbits as an alternative to conventional zinc sources could elicit favorable influences on growth performance, serum parameters and oxidative stress and immunity under heat stress.

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تاثير الاضافة الغذائية لأوكسيد الزنك أو نانو أكسيد الزنك على خصائص النمو والاجهاد التاكسدي والمناعة للارانب النامية تحت ظروف الاجهاد الحراري

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تمت دراسة تأثير الأضافه الغذائيه لأكسيد الزنك أو أكسيد النانو الزنك على أداء النمو، حالة مضادات الأكسدة، المناعة، وظائف الكبد والكلى للأرانب النامية (APRI). تم تقسيم إجمالي 60 أرنباً إلى 3 مجموعات تم تغذيتها على العلف الأساسي بدون (G1) أو بأضافة 50 مجم أكسيد الزنك (G2) أو 30 مجم أو أكسيد النانو الزنك (G3) لكل كيلوجرام من العلف من عمر 5-13 أسبوعًا على التوالي. تم تسجيل وزن الجسم الحي، العلف المتناول اليومي، وحساب الزيادة اليومية فى الوزن والتحويل الغذائي للأرانب و مؤشر الأداء ومعدل الوفيات. تم تحديد الخصائص البيوكيميائية ومضادات الأكسدة وحالة المناعة فى الدم عند عمر 13 أسبوعًا. أظهرت النتائج أن الأضافة الغذائية من أكسيد الزنك أو أكسيد النانو الزنك زادت (P <0.05) من معاملات أداء النمو، والبروتينات الدهنية عالية الكثافة في الدم، والجلوتاثيون والجلوتاثيون S-ترانسفيراز، وأنزيم سوبر أوكسيد ديسميوتاز ، والجلوبيولينات المناعية. أنخفض تركيز الدهون الثلاثية و المالون داى الدهيد (MDA) في مصل الدم (P <0.05) فى المجموعات المعامله مقارنه بالكونترول. في الختام ، يمكن للمكملات الغذائية التي تحتوي على أكسيد الزنك أو أكسيد النانو الزنك أن تعزز أداء النمو، ومظهر الدهون، والمناعة وحالة مضادات الأكسدة للأرانب النامية تحت ظروف الإجهاد الحراري.