REVIEW AND PERSPECTIVE

DIETARY TREATMENT OF HEAT SHOCK PROTEINS WITH SELECTED FEED ADDITIVES TO ENHANCE THERMOTOLERANCE IN POULTRY UNDER EGYPTIAN DESERT CONDITIONS: REVIEW AND PERSPECTIVE

R. E. Khidr[1], A. S. Morsy[2]

ABSTRACT

Egypt, located in northeastern Africa and southwestern Asia on the coordinates 27° E and 30° N is usually hot and dry in the summer (June-September), where the peak of outdoor ambient temperature converge at a sizzling 45°C,which is higher than the critical temperature (35°C), with a relative humidity of 61%. The meteorological factors, particularly high ambient temperature and high relative humidity, result in severe heat stress in fowls, culminating in exertion of harmful effects on thermoregulation reactions, hematological parameters, immune responses, and egg production in hens and semen characteristics in cocks. There have been several research efforts to enhance thermotolerance for minimization of heat-related mortality and maintenance of productivity in fowls. These researches include heat acclimation, nutritional manipulation, and inclusion of feed additives, particularly heat shock proteins, alkalis (e.g., Sodium bicarbonate) and vitamins (Vitamin C) in diet and water. However, efficiencies of most of the interventions were variable or inconsistent. More recently, two methods have been explored for early-life conditioning, i.e., perinatal heat acclimation and genetic selection of breeds with enhanced heat tolerance capacity for coping with heat stress conditions, with exploration on boosting of heat shock proteins (HSP) in cells and tissues, under both unstressed and stressed conditions. Environmental and pathological stresses induce HSPs, especially the inducible forms of HSP70 and HSP72, which act as chaperone, and play a critical role in protecting cells against the adverse effects of hyperthermia, and improve bird's productive performance and survival ability during exposure to heat stress at later ages. It has also been observed that thermo-respiratory responses (rectal temperature and respiration rate) decreases in hens exposed to HSPs, which means that they can be physiologically manipulated to boost heat stress tolerance by thermal conditioning. HSPs improve immunity of heat stressed hens leading to higher survival rate, besides improving their productivity. Studies have also shown that supplementation with 250 mg vitamin C and 1.5% sodium bicarbonate ($NaHCO_3$) singly or conjointly enhances productivity of hens. Vitamin C is a powerful antioxidant, while $NaHCO_3$ is an electrolyte that causes respiratory alkalosis. HSPs also improve ejaculate volume, sperm concentration, total sperm output, sperm motility, total motile sperms in cocks. The perspective of the study highlights the prospects of integrating HSPs with vitamin C and Sodium bicarbonate singly or conjointly to heat stressed fowls for sustenance of egg production in hens and improvement in semen quality of cocks as a valuable breeding protocol for poultry production under heat stress conditions in Egyptian deserts.

KEY WORDS

Egg production, Feed additives, Heat stress, Poultry, Semen characteristics

Author attribution:[1] Professor of Poultry Nutrition,[2]Professor of Poultry Physiology, Animal and Poultry Production Division, Desert Research Center, Cairo, Egypt. Receipt: 07-Aug-2020, Acceptance: 18-Sept-2020. Corresponding author: (Email: raafatkhidr@yahoo.com). pp. 12-24.

INTRODUCTION

Egypt, located under tropical and sub-tropical regions, suffers from heat stress conditions where the peak of outdoor ambient temperature reaches a sizzling 45^{o}C and temperature higher than the critical temperature (35^{o}C) is being recorded more regularly during summer (June-September) months (Faisal et al., 2008).

In Egypt, meteorological factors exert a significant influence on domestic birds. Direct meteorological factors affecting birds include high ambient temperature and high relative humidity, resulting in severe heat stress. Heat stress leads to harmful effects on thermoregulation responses, hematological parameters, immune responses, egg production and semen quality characteristics (Mashaly et al., 2004). Many researchers have indicated that increased thermo-tolerance lead to minimization of heat-related mortality coupled with maintenance of higher productivity (Edens, 1997).

These researches include heat acclimation, feed intake manipulation and feed deprivation and use of electrolytes and vitamins in drinking water. Strategies to deal with heat stress conditions in poultry farms included environmental management, nutritional manipulation, inclusion of feed additives in the diet and water. However, efficiencies of most of the interventions are either variable or inconsistent. More recently, two methods have been explored, including early-life conditioning (i.e., perinatal heat acclimation) and genetic selection of breeds with increased capacity of coping with heat stress conditions (i.e., increased heat tolerance).

Important feed additives for easing heat stress: There are many important feed additives used to alleviate the negative effects of heat stress in laying hens which include vitamin C and sodium bicarbonate (Daghir, 2008; Attia et al., 2009; Morsy, 2018). Vitamin C is a water-soluble vitamin with anti-oxidant activity, protecting animals under stress, body temperature regulation and boosting immunity (Khan et al., 2012). Vitamin C, also known as L-ascorbic acid, a water-soluble vitamin is a very useful dietary supplement for poultry, particularly during heat stress (Abdulrashid et al., 2010).

In Egypt, poultry industry suffers from heat stress conditions. Heat stress is one of the most important environmental stressors challenging poultry production worldwide. Negative effects of heat stress on laying hens range from reduced growth and egg production to decreased egg quality and safety.

Many researchers have reported the damaging effects of heat stress during summer months on the productive performance; decrease in welfare and egg quality of laying hens. Summer months often cause laying hens to produce thin and soft-shelled eggs and extreme losses in income from marketable eggs can occur. Heat stress causes 1% reduction in egg weight, decrease in egg shell thickness and egg production (Morsy, 2018).

Also, exposure of hens to heat stress conditions cause decrease in feed intake, disturbances of hormones and blood constituents and depressed immune function (Emam, 2013). Heat stress conditions are also associated with peak mortality in laying hens and 28% increase in mortality rate in laying hens reared in arid weather conditions of Egypt (Nagwa et al., 2012).

Heat shock proteins are present in every cell type and tissue, under both unstressed and stressed conditions. Environmental and pathological stresses induce HSPs, especially the inducible form of Hsp70 and Hsp72. These proteins improve bird's productive performance and survival ability during exposure to heat stress at later ages. However, the degree of induction depends on the level and duration of exposure to stress (Kiang and Tsokos, 1998).

Members of HSP70 protein family act as chaperone, which assist in the folding, transport and assembly of protein in cytoplasm, mitochondria and endoplasmic reticulum or appears to play a critical role in protecting cells against the adverse effects of hyperthermia and help in the formation of newly synthesized folded proteins (Morimoto et al., 1990).

Importance of feed additives during heat stress: Many conventional processes are used to alleviate the negative effects of heat stress on laying hens, such as administration of vitamin C and sodium bicarbonate ($NaHCO_3$) along with diet (Attia et al., 2009; Daghir, 2008; Morsy, 2018).

Vitamin C is a water-soluble vitamin with anti-oxidant activity, which protects animals under stress, regulates body temperature and boosts immunity (Khan et al., 2012). Vitamin C has been demonstrated to be a powerful antioxidant that acts through formation of an ascorbate radical that destroys free radicals and inhibits other oxidation reactions (Strong, 2014).

Additionally, improvements in the average feed consumption, feed conversion, egg production, egg weight and egg shell thickness were also observed with supplementation of vitamin C in drinking water or supplementation at 200–400 mg/ kg diet in laying hens (Strong, 2014; Ciftçi et al., 2005). Moreover, vitamin C is known to decrease the utilization of corticosteroids released during stress (Sahin et al., 2003), thus playing an important role in response to stress.

On the other hand, the acid-base balance of the birds is negatively affected by heat stress. Under heat stress, birds regulate heat loss by evaporation of water from their lungs. As a result of hyperventilation, levels of plasma CO2 and HCO3 are reduced and respiratory alkalosis occurs.

Laying hens administered with an electrolyte, e.g.; sodium bicarbonate (Trefz et al., 2017) at the level of 0.4% produce significantly fewer number of rough-shelled eggs (Enst et al., 1975), with improvement in eggshell quality (Odom et al., 1985), increase in egg production potential (Altan et al., 2000), decrease in mortality rate and improvement in feed conversion ratio and feed intake (Morsy, 2018; Yörük et al., 2004) at high temperatures.

Approaches, such as heat shock have been developed to increase thermo-tolerance leading to reduction in heat-related mortality and to maintenance of productivity in poultry (De Basilio et al., 2002). Poultry can acclimatize by exposure to repeated, short and daily heat shock (Narongsak, 2004), especially, if this exposure is applied at early growth phases (Yahav et al., 2004).

It is thus possible to improve survivability, growth and feed efficiency of heat-stressed chickens by prior heat exposure through controlling thermal stressors (Yalcin et al., 2001).

Many heat shock programs have been applied to enhance performance of poultry under heat stress. These include early heat shock at 3-5 days of age for 4 hours at 38°C or 43°C in broilers (Rahimi, 2005) and at 3 days of age for 24h at 37-38°C in ducks (Nagwa et al., 2004), in laying hens (Emam, 2013), and in cocks (Morsy, 2013).These programs improved bird's final productive performance and survival ability during exposure to heat stress at later ages.

Effect of heat shock program (HSP) on thermo-respiratory responses: Thermo-respiratory responses (Rectal temperature and Respiration rate) decrease significantly in the hens (Figure 1) exposed to heat shock programs, i.e., heat exposure at 3 days, 8 days and 16 weeks of age compared with control group (Emam, 2013).

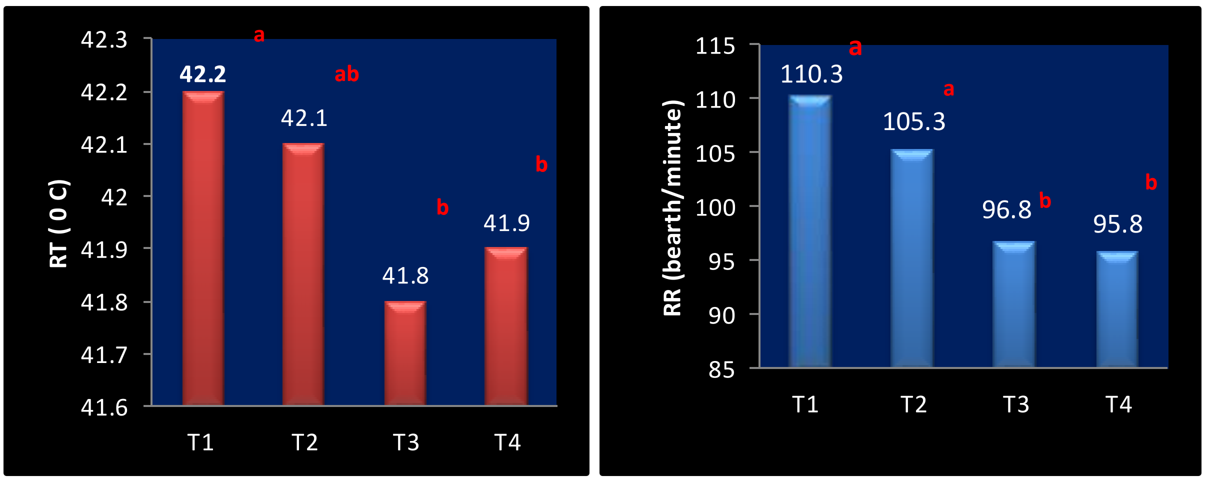

Figure 1: Rectal temperature and respiration rate of laying hens under hot conditions as affected by heat shock programs. RT = rectal temperature; RR=respiration rate. T1= Control; T2= Heat exposure at 3 days of age; T3= Heat exposure at 3 days and 8 weeks of age; T4= Heat exposure at 3 days, 8 and 16 weeks of age. a, b. Means with different superscripts between columns are significantly different (P<0.05).

Early heat exposure at 3-5 days of age and at 8 wks or at 8 and 16 weeks of age may enhance thermo-tolerance of laying hens that would face heat stress in advance (Yahav and McMurtry, 2001). This assumption might explain the results that hens exposed to heat shock programs are physiologically manipulated to better tolerate heat stress by thermal conditioning. Previous studies have also confirmed that heat acclimated broiler hens (exposed to heat shock programs) had lower rectal temperature and respiration rate than un-acclimated birds (non-heat exposure) when both exposed to high environmental temperature at later age (Yahav and Plavnik, 1999).

Effect of HSP on immunity responses: Improvement in immunity has been reported in hens, exposed to heat shock programs (Emam, 2013) due to significant increase of globulin concentration, which is an indicator of immune responses and source of antibodies production (El-Kaiaty and Hassan, 2004).

These results are in agreement with other reports (Mashaly et al., 2004; Nagwa et al., 2012), where immunity has increased in chicken with pre-conditioning and later exposed to heat stress at later age. It is also reported that primary response for antibody titer against sheep red blood cells was enhanced ($P<0.05$) in the cocks exposed to heat shock programs by 62% - 75% as compared to control group (Morsy, 2013).

Heat shock exposures lead to increase in HSP70 which in turn act to inhibit the release of cytokines, oxygen free radicals and nitric oxide resulting in increased immunity responses (Polla and Cossarizza, 1996). On the other hand, heat stress causes reduction in antibody synthesis (Zulkifi et al., 2000; Zulkifi et al., 2009) due to an increase in inflammatory cytokines under heat stress (Ogle et al., 1997), which stimulates corticosterone production (Sapolsky et al., 1987). Corticosterone inhibits antibody production (Gross, 1992). In addition, heat stress is known to decrease T-helper 2 cytokines (Wang et al., 2001), which are important for antibody production (Lebman and Coffman, 1988).

Effect of HSP on Productive performance: Egg weight, egg number and egg mass in the hens exposed to heat shock programs (Table 1) have shown significant ($P<0.05$) increase compared to the non exposed hens to heat shock (Emam, 2013).This increase in egg weight and number may be due to the increase of body weight at sexual maturity, and this reflected on increased egg mass.

It is worth mentioning that the chickens of heat shock treatments had better values of feed conversion by 10.5%, 7.5% and 4.9% compared to control group. These results might be attributed to improvement of performance traits as a result of heat shock programs (Franco-Jimenez et al., 2007).

Mortality rate has also decreased ($P<0.05$) in the hens exposed to heat shock programs by 35.7 - 42.8 % compared to that of control group (Emam, 2013). This demonstrated that heat shock programs lead to decrease in mortality, which might be due to enhanced thermo-tolerance of the hens. Increasing mortality rate may be attributed to inability to regulate body temperature under heat stress conditions (Yahav et al., 1997).

There is reported improvement in egg shell thickness in the hens exposed to heat shock programs compared to the control group, due to reduction in respiration rate and increment of feed intake due to acclimation to high temperatures, which prevents disturbance of several temperature-induced physiological processes, including thermoregulation and acid-base balance (Morsy, 2018).

Effect of EHSP with Feed additives on Productive performance: Final body weight and body weight changes in the control and treatment groups of hens exposed to early heat shock programs (EHSP) along with feed additives (Table 2) were comparable, while there were significant ($P<0.05$) increases in the mean values of egg weight, egg number and egg mass in the hens of T2, T3 and T5 groups as compared to the control group (T1). Moreover, hens of T4 group had yielded significantly higher egg number (14.18%) and egg mass values (19.78%) compared to the hens in the control group (Morsy, 2018).

Table 1: Performance of laying hens under hot conditions as affected by heat shock programs

Traits	T1	T2	T3	T4
SM	151.3±1.8	147.2±1.7	144.8±1.7	143.2±1.9
BWSM	1260.2±77.5	1222.6±53.1	1283.4±63.6	1320.2±70.0
FBW	1282.3±56.9	1250.7±29.9	1316.0±35.1	1340.1±28.1
EW (g)	35.4^{b}±0.38	36.7^{ab}±0.56	37.8^{a}±0.45	37.3^{a}±0.56
EN	34.2^{b}±1.4	34.5^{b}±1.6	35.8^{ab}±1.6	38.7^{a}±1.5
DFI (g)	59.9±2.4	58.6±2.5	62.7±1.9	64.5±1.5
TFI (g)	5392.3±216.1	5247.7±225.3	5645.7±179.4	5807.6±141.5
EM (g)	1214.5^{b}±56.5	1267.7^{ab}±63.2	1356.7^{ab}±64.1	1449.2^{a}±71.7
FC	4.48±0.20	4.26±0.27	4.28±0.31	4.13±0.29

SM= age at sexual maturity; BWSM= body weight at sexual maturity; FBW= final body weight; EW= egg weight;; EN= egg number; DFI= daily feed intake; TFI= total feed intake; EM= egg mass;; FC= feed conversion; T1= control; T2= heat exposure at 3 days of age; T3= heat exposure at 3 days and 8 weeks of age; T4= heat exposure at 3 days, 8 and 16 weeks of age.

a, b Means with different superscripts within row are significantly different (P<0.05)

Egg number and egg mass were significantly (P<0.05) higher in the group of hens treated with vitamin C and sodium bicarbonate (T5) as compared to their counterparts in other groups. Daily feed intakes were significantly (P<0.05) lower in the hens of T1 group by 10.93% and 9.12% respectively as compared to the hens of T2 and T5 groups (Morsy, 2018).

These results indicated that the hens of T3, T4 and T5 (EHSP with addition of vitamin C, sodium bicarbonate singly and in combination) had significantly (P<0.05) improved values of feed conversion by 14.64%, 13.05% and 19.74%, respectively as compared to the hens of the control group (T1).

It has been reported that the hens of T2 showed marginal improvement (P<0.05) in feed conversion (9.55%) compared to the hens in T1 (Morsy, 2018). The decrease in productivity of hens in T1 (control) group could be attributed to negative effects of heat stress, which is associated with compromised performance and productivity through a decline in feed intake, nutrient utilization, egg production, egg quality, feed conversion and reduced antioxidant status and subsequently, increased oxidative stress (Khan et al., 2012).

Table 2: Effect of early heat exposure program (EHSP) and feed additives on productive performance of laying hens under heat stress conditions.

Traits	T1	T2	T3	T4	T5	± SE
IBW	1646.0	1645.3	1645.2	1646.8	1646.2	55.0
FBW	1859.6	1940.7	1969.7	1940.5	1974.0	64.7
BWC	213.6	295.3	324.5	293.7	327.7	54.5
EW (g)	43.4^{b}	47.7^{a}	47.1^{a}	45.5^{ab}	48.0^{a}	1.1
EN	63.9^{c}	72.0^{b}	72.5^{b}	72.9^{b}	79.1^{a}	0.49
DFI (g)	96.6^{c}	108.4^{a}	100.9^{bc}	100.2^{bc}	106.3^{ab}	2.3
TFI (g)	8697.1^{c}	9764.4^{a}	9084.9^{bc}	9019.9^{bc}	9570.0^{ab}	210.4
EM (g)	2777.7^{c}	3433.1^{b}	3416.0^{b}	3327.4^{b}	3800.1^{a}	78.4
FC	3.1^{a}	2.8^{ab}	2.6^{b}	2.7^{b}	2.5^{b}	0.10

IBW= initial body weight; FBW= final body weight; BWC= body weight change; EW= egg weight; EN= egg number; DFI= daily feed intake; TFI= total feed intake; EM= egg mass; FC= feed conversion.
T1= control; T2= EHSP; T3= EHSP + additive 250 mg vitamin C / kg diet during production period; T4= EHSP + additive 1.5 % sodium bicarbonate during production period; T5= EHSP +additive 250 mg vitamin C/ kg diet +1.5 % sodium bicarbonate during production period.
$^{a-c}$ Means with different superscripts within row are significantly different (P<0.05)

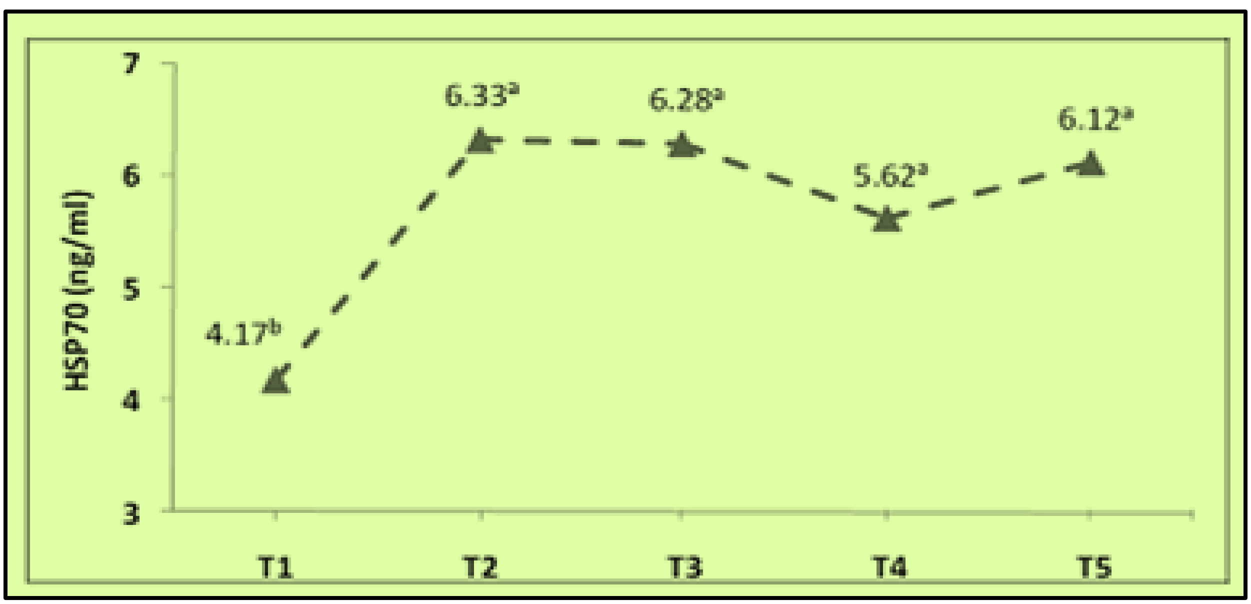

Figure 2 (Above): Effect of early heat exposure program (EHSP) and some feed additives on heat shock protein 70 expression (HSP70) of laying hens under heat stress conditions.

T1= control; T2= EHSP; T3= EHSP + additive 250 mg vitamin C / kg diet during production period; T4= EHSP + additive 1.5 % sodium bicarbonate during production period; T5= EHSP +additive 250 mg vitamin C/ kg diet +1.5 % sodium bicarbonate during production period. [a-b] Means with different superscripts in a row are significantly different (P<0.05).

Moreover, it is observed that exposure of hens to severe heat stress lead to decreased feed intake with consequent decline in the functions of endocrine system, function of internal organs and disturbance of acid–base balance. These adverse effects influence the process of egg formation in ovary and reproductive tract along with interruption of the process of ovulation and oviposition (Oguntunji and Alabi, 2010).

Improvement in hens' performance traits due to treatments might be attributed to heat exposure program during early growth period (Franco-Jimenez et al., 2007; Emam, 2013), since heat exposure program might enhance thermo-tolerance of laying hens that would be exposed to severe heat stress in advanced or later ages (Yahav and McMurtry, 2001; Morsy, 2018). These results agree with earlier reports on use of heat shock exposure programs to acclimate birds to heat stress leading to enhancement of physiological responses (Faisal et al., 2008; El-Badry et al., 2009; Zulkifli et al., 2009; El-Moniary et al., 2010).The positive impact was achieved through increasing HSP70 gene expression (Figure 2) and enhancing immunity responses by increasing primary antibody titer and globulin concentration and decreasing H/L ratio (Emam, 2013; Morsy, 2013; Morsy, 2018).

Effect of HSP on semen quality: It is reported (Morsy, 2013) that semen quality is improved (P<0.05) in cocks exposed to heat shock program (heat shock exposure at 3 days, and at 8 and 16 weeks of age) as compared to control group (non-heat shock exposure only during growth period). Cocks showed the highest values of ejaculate volume, sperm concentration, total sperm output, sperm motility, total motile sperm and semen quality factor. Cocks also recorded the lowest values of dead spermatozoa and sperm abnormalities (Table 3).

Heat stress affects all phases of semen production in chicken cocks (Banks et al., 2005). The improvement in semen quality characteristics may be explained by heat shock exposure leading to increased expression of HSP70, which stimulates testicular growth in the early phase and promotes increased semen volume and sperm concentration.

In addition, HSP70 protects the seminiferous epithelial cell differentiation against heat stress damage, which is demonstrated with increase in semen quality characteristics and/or maintenance of homeostasis under the stress conditions (Obidi et al., 2008). Likewise, heat shock exposure might also be associated with sperm motility through activation of nitric oxide synthase (NOS), which is beneficial for sperm motility (Garcia-Cardena et al., 1998).

Heat shock protein 70 (HSP70) is a stress-induced protein. It is an important part of the cell's machinery for protein folding. High levels can be produced by cells in response to hyperthermia. The protein acts as a molecular chaperone by binding to other cellular proteins, assisting intracellular transport and folding into the proper secondary structures, thus preventing aggregation of protein during stress (Hartl, 1996).

Table 3: Semen quality of White Leghorn cocks under hot conditions as affected by heat shock exposures.

Heat shock exposures (HSE)				
Traits	**Control**	**HSE1**	**HSE2**	**HSE3**
EV (ml)	$0.22^{b}\pm0.01$	$0.26^{ab}\pm0.02$	$0.25^{ab}\pm0.02$	$0.28^{a}\pm0.02$
SC ($\times10^{6}$ml)	$630.00^{b}\pm106.22$	$702.22^{b}\pm147.54$	$761.42^{ab}\pm88.11$	$1104.44^{a}\pm144.21$
TSO ($\times10^{6}$)	$138.60^{b}\pm30.67$	$182.57^{b}\pm41.18$	$190.35^{ab}\pm35.67$	$309.24^{a}\pm58.76$
SM (%)	$60.00^{b}\pm5.35$	$67.77^{ab}\pm8.29$	$77.14^{ab}\pm9.93$	$80.55^{a}\pm4.28$
TMS ($\times10^{6}$)	$83.16^{b}\pm11.01$	$123.72^{b}\pm36.31$	$146.83^{ab}\pm43.88$	$249.09^{a}\pm62.33$
LS (%)	$62.54^{c}\pm3.60$	$73.88^{b}\pm2.97$	$73.14^{b}\pm1.93$	$78.00^{a}\pm1.65$
DS (%)	$37.45^{a}\pm3.60$	$26.11^{b}\pm2.97$	$26.85^{b}\pm1.93$	$22.00^{c}\pm1.65$
SA (%)	$17.45^{a}\pm0.93$	$14.22^{ab}\pm1.22$	$11.14^{b}\pm2.34$	$10.44^{b}\pm1.59$
SQF	$86.68^{b}\pm16.62$	$134.88^{b}\pm31.59$	$139.22^{b}\pm25.88$	$241.20^{a}\pm45.48$
pH	7.90 ± 0.11	7.66 ± 0.11	7.78 ± 0.14	7.72 ± 0.08

EV, ejaculate volume; SC, sperm concentration; TSO, total sperm output; SM, sperm motility; TMS, total motile sperm; LS, live spermatozoa; DS, dead spermatozoa; SA, sperm abnormalities; SQF, semen quality factor; pH, hydrogen ion.

Control, non heat exposure during growth period; HSE1, heat exposure at 3 days of age; HSE2, heat exposure at 3 days and 8 weeks of age; HSE3, heat exposure at 3 days, 8 and 16 weeks of age.

a, b, c. Means with different superscript in the different columns are significant differences ($P<0.05$).

CONCLUSION

It could be concluded that exposure of hens to heat shock programs with addition of some feed additives such as vitamin C and sodium bicarbonate may alleviate some of negative effects of heat stress on thermo-respiratory responses, productive performance and semen quality of birds reared under Egyptian hot desert conditions.

FUTURE PERSPECTIVES

Thermal stress in avian species has been causing heavy economic losses to the poultry industry. Under desert and harsh condition, the recent approaches of biotechnology must be taken into consideration. This mean that the interrelationships of heat shock protein with poultry adaptability with regard to productive performance must be a tool for poultry breeding programs in the future. The encoding genes of HSP, particularly for our local strains are in a great need for further studies. Also, there is a need for further investigations to determine the mechanisms by which HSP is regulated in avian species.

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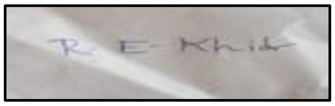
UNDERTAKING

It is certified that the paper '**DIETARY TREATMENT OF HEAT SHOCK PROTEINS WITH SELECTED FEED ADDITIVES TO ENHANCE THERMOTOLERANCE IN POULTRY UNDER EGYPTIAN DESERT CONDITIONS: REVIEW AND PERSPECTIVE'** is an original work carried out by the authors in the Animal and Poultry Production Division, Desert Research Center, Cairo, Egypt. I/We have duly acknowledged all the sources from which the ideas and excerpts have been drawn. The project is free from plagiarism. It has neither been published nor contemplated for publication elsewhere.



Professor (Dr) R. E. Khidr

CORRESPONDING AUTHOR