EFFECT OF HEAT ACCLIMATION ON BODY FLUIDS AND PLASMA PROTEINS OF BROILERS EXPOSED TO ACUTE HEAT STRESS IN SUMMER

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SUMMARY

The present study was carried out during summer at the Poultry Experimental Station of the Animal Production Department Faculty of Agriculture, Cairo University, Giza, Egypt. The mean ambient temperature and relative humidity during the experimentation period was 34±4°C and 47±12%, respectively. Sixty one-day-old Arbor-Acres hybrid broiler chicks were raised for 7 weeks with feed and water supplied *ad-libitum*. Chicks were divided into 4 equal groups (each comprised 15 birds): Group 1 (control group, C), group 2 (unacclimated heat stressed group, HS), group 3 (early heat acclimated group exposed to a constant temperature of 38°C for 3h during the first 3 days of age, EHA) and group 4 (biweekly heat acclimated group, BHA). At 7 weeks of age, birds of groups 2, 3 and 4 were exposed to heat stress (38°C for 3h). Plasma total proteins (TPP), albumin (A), globulin (G), A/G ratio and body fluid compartments (total body water TBW, extra cellular fluids ECF, interstitial fluids ISF, plasma volume PV and intracellular fluids ICF) were determined at 7 weeks of age in all groups (after heat stress in groups 2, 3 and 4).

Biweekly heat acclimation decreased significantly final body weight as compared to the other three groups. However, early heat acclimated group had almost similar final body weight to the mean of the two unacclimated groups (groups 1 & 2). Heat stress (38°C for 3 h) at 7 weeks of age increased significantly TBW% in acclimated and unacclimated groups causing a significantly lower TBS% in HS, EHA and BHA groups than in the control group. In unacclimated group (HS), the significant increase in TBW% was accompanied with a significant increase in ECF% and ISF%, while a significant decrease in ICF% and PV% occurred. Similar trends of TBW% and ECF% were found in heat acclimated groups, meanwhile no significant changes occurred in ICF% and PV%. Consequently, the main effect of heat stress on broilers was the occurrence of interstitial edema (shifts of fluid from ICF and PV to ISF) may be due to the reduction in plasma osmolality (low plasma albumin concentration) which was sustained by either early or biweekly heat acclimation.

Keywords: Broilers, heat stress, body fluids, plasma proteins, heat acclimation

INTRODUCTION

Acute (heat waves) or chronic (seasonal variation) heat stress occurs when ambient temperature exceeds the upper critical temperature of broilers which was found to be about 33°C by Boone (1968) and Boone and Hughes (1971). Eberhart and Washburn (1993) stated that mortality and morbidity during acute or chronic heat stress continues to be a problem in the poultry industry.

Heat stress decreases food consumption through its direct effect on thermoregulation center in hypothalamus causing a significant reduction in daily gain (Yahav *et al.*, 1995 & 1996 and Hacina *et al.*, 1996). Heat waves (acute heat stress) increases mortality rate through its effect on heat balance, protein requirement and acid-base balance. The predisposing cause of death has been identified as a cardiovascular failure in birds undergoing thermal stress (Whittow *et al.*, 1964).

Daghir (1995) stated that the normal functions of tissues are dependent upon the stability of the total osmolality of intracellular and extracellular fluids. Studies concerning the effect of chronic and acute heat stresses on body fluid compartments are conflicting. Meanwhile Yahav et al. (1997) found no significant effect of acute heat stress on blood and plasma volume, Khalifa and Shoukry (1997) found that acute heat stress (40 °C) caused a significant reduction in broiler plasma volume which was not attributed to low plasma Na concentration. Chronic heat stress (broilers reared under hot season vs. those reared under cold one) caused a significant reduction in plasma volume at 6 weeks of age or more (Khalifa *et al.*, 1994 and Ahmed Nagwa *et al.*, 1998). The latter explained that this reduction in plasma volume was due to the interstitial edema caused by lower plasma albumin concentration, which might interrupt the general homeostasis mechanism leading to high mortality. The reduction in plasma proteins or albumin concentration during heat stress was also found by Faltas *et al.* (1987) and Hussien

(1994). Meanwhile, Squibb *et al.* (1959) concluded that constant environmental temperature of 99°F (37.2°C) had no significant effect on serum level of total protein of New Hampshire.

Thermotolerance can be improved by acclimation to heat stress (Hillman *et al.*, 1985). During heat stress, heat acclimated broilers had lower body temperature (May and Lott, 1992), feed consumption and body weight gain (Francis *et al.*, 1991, Teeter *et al.*, 1992, Smith, 1993, Yahav and Hurwitz, 1996) and mortality rate (Lott, 1991). El-Tantawy *et al.* (1998) demonstrated that early heat acclimation improved carcass characteristics during heat stress. Dawoud (1998) found that early heat acclimation prevented the loss in plasma volume during chronic heat stress. The preliminary results of unpublished data (Khalifa, *et al.*, 2000) indicated that early heat acclimation alleviated the effect of acute heat stress (50°C) on mortality. Khalifa (1999) concluded that acclimation to heat stress can be considered as the best method to alleviate mortality under heat stress, may be through the production of heat shock proteins.

The objective of the present study was to elucidate physiological mechanism involved in the induction of homeostasis by an early-life thermal exposure (acclimation), in attempt to alleviate the harmful effect of heat stress on broilers.

MATERIALS AND METHODS

The present study was carried out during summer (August to September, 1996) at the Poultry Experimental Station of the Animal Production Department Faculty of Agriculture, Cairo University, Giza, Egypt.

The experiment comprised 60 one-day-old Arbor-Acres hybrid broiler chicks. Broilers were raised for 7 weeks with feed and water supplied *ad-libitum*. All birds were healthy and clinically free from diseases. The average ambient temperature and relative humidity were 34±4°C and 47±12%, respectively.

Chicks were assigned in 4 equal groups: Group 1 (control group, C) was kept under normal ambient temperature of summer during the whole period. Group 2 (heat stressed group, HS) was exposed to 38°C ambient temperature for 3h at 7 weeks of age. Group 3 (early heat acclimated, EHA) chicks were exposed to a constant temperature of 38°C for 3h during the first 3 days of age. Group 4 (biweekly heat acclimated, BHA) chicks were exposed to a constant ambient temperature of 38°C for 3h during the first 3 days of age and biweekly thereafter until the end of experiment (7 weeks of age).

Physiological parameters were measured at 7 weeks of age in all groups (after heat stress in groups 2,3 and 4). Blood samples of 5 birds from each group were withdrawn from the wing vein before dyes injection as a blank sample in which plasma total proteins (TPP) and albumin (A) were determined. Total plasma proteins was measured as described by Weichselbaun (1946). Plasma albumin was measured as described by Doumas *et al.* (1971). Plasma globulin (G) was calculated by subtraction and therefore A/G ratio was calculated. Cocktail dyes prepared freshly were injected for the determination of total body water, extracellular fluids and plasma volume (Mishra *et al.*, 1983). Total body water (TBW) was measured using antipyrine method as described by Weiss (1958). The plasma volume (PV) was estimated by Evan's blue method as described by Kennedy and Millikan (1938). Intracellular fluid (ICF) was calculated by subtracting extracellular fluids (ECF) from TBW and interstitial fluid (ISF) was calculated by subtracting PV from ECF.

Data was subjected to standard analysis of variance according to SAS program (SAS, 1988) and Duncan's Multiple Range Test ($P<0.05$).

RESULTS AND DISCUSSION

Biweekly heat acclimation decreased significantly final body weight as compared to the other three groups (Table 1). Meanwhile, early heat acclimated group had almost similar final body weight to the mean of the control and HS groups (1575 g vs. 1581 g for the EHA and mean of C and HS groups, respectively). This lower growth rate in BHA may be due to the direct effect of repeated heat stress during the growing period (Hacina *et al.*, 1996) or to the reduction in feed intake during heat stress (Francis *et al.*, 1991, Teeter *et al.*, 1992 and Yahav and Hurwitz, 1996). Edwards (1979) demonstrated that hyperthermia affects cells during mitosis. Thus organs with rapid cellular proliferation are sensitive to heat.

As percentage from body weight (Table 1), heat stress (38°C for 3 h) at 7 weeks of age increased significantly TBW% in unacclimated (HS) and acclimated (EHA & BHA) groups. Consequently, TBS% was significantly lower in HS, EHA and BHA groups than in the control group. Khalifa *et al.* (1994) found that at 7 weeks of age, TBW% of broilers was significantly higher in summer than in winter which was suggested by Abdel-Razik *et al.* (1985) to be due to high water turnover rate during hot season. Whittow (1968) stated that the higher TBW% in hot season is essential to compensate the loss of body water through high evaporative cooling. Intracellular fluids (ICF) was significantly higher in the control (C) and heat acclimated groups (EHA and BHA) than in heat stressed group (HS) (Table 1). However, this higher ICF% in the control group was accompanied by lower TBW% causing a significantly lower ECF% in this group than in the other groups. Meanwhile, in HS group ICF% was significantly lower due to the significantly higher ECF% than in other groups indicating shifts of body fluids from ICF to ECF. Heat acclimated groups (EHA and BHA) had significantly higher ECF% than the control group although their ICF% was almost unchanged indicating the increase in body water retention (shifts of fluids from gut and renal tubular to ECF). The effect was more pronounced in BHA group than in EHA.

The significantly higher ECF% in unacclimated heat stressed group than in the control one was due to significantly higher ISF%, while a significantly lower PV% was found in HS than in the control group (2.61 Vs 4.62). It is clear that heat stress in unacclimated birds (HS) resulted in shifts of fluids from ICF and PV to ISF (interstitial edema) may be due to the reduction in plasma osmolality as indicated by low plasma albumin concentration (Table 3). Similar trends were found during chronic heat stress during hot season by Khalifa et al (1994). Yahav *et al.* (1998) found that broilers exposed to cyclic temperature of 15 to 35 °C for 12h:12h cycle had significantly lower PV than those kept under constant temperature of 15°C. In heat acclimated groups (EHA and BHA), the higher ECF% as compared to control birds was due to higher ISF% meanwhile PV% was almost unchanged (Table 1). It can be concluded that acclimation to heat stress sustained the reduction in ICF % and PV% during heat stress which keeps adequate blood volume to maintain blood circulation and internal heat conduction which in turn will minimize mortality rate during heat stress.

As percentage from TBW (Table 2), HS had significantly higher ECF% and ISF% and lower ICF% and PV% than the control or heat acclimated groups. These results confirms the above results, when fluid compartments expressed as percentage from body weight, that acute heat stress during hot season (HS group) caused interstitial edema in 7-week-old broilers (shifts of fluids from ICF and PV to ISF). Heat acclimation either early or biweekly sustained the reduction in PV% and all body fluid compartments were almost similar in the control and heat acclimated groups. The significantly higher ECF and ISF as percentage from body weight in heat acclimated groups than in the control (Table 1) disappeared when they were expressed as percentage from TBW (Table 2) indicating that the increase in TBW during heat stress in EHA and BHA was due to the increase in ISF and ECF. As a result, the main effect of heat acclimation by exposure to 38°C for 3 h in the first three days of age (EHA), was the prevention of the loss in ICF and PV during heat stress at 7 weeks of age as compared to HS group to compensate the increase in water loss through evaporative cooling (Dawoud, 1998). Some effects occurred when biweekly acclimation was used; however it reduced growth rate causing lower body weight and TBS.

Table 1. Effect of heat stress and acclimation on body fluid compartments as percentage from body weight of broilers at 7 weeks of age

Traits	Control	Heat stressed	Early heat-acclimated	Biweekly heat-acclimated
Body weight	1500 [c]	1663 [a]	1575 [b]	1366 [d]
SE	40.1	33.7	43.3	37.3
Total body solids	44.99 [a]	42.18 [b]	41.58 [b]	41.63 [b]
SE	0.44	0.21	0.46	0.38
Total body water	55.01 [b]	57.82 [a]	58.42 [a]	58.40 [a]
SE	0.44	0.21	0.46	0.38
Intracellular fluids	22.65 [a]	18.70 [b]	23.71 [a]	22.19 [a]
SE	0.73	0.26	0.60	0.41
Extra cellular fluids	32.35 [d]	39.12 [a]	34.71 [c]	36.18 [b]
SE	0.46	0.11	0.33	0.32
Interstitial fluids	27.71 [c]	37.15 [a]	29.80 [b]	30.37 [b]
SE	0.62	0.80	0.36	0.38
Plasma volume	4.62 [b]	2.69 [c]	4.91 [b]	5.81 [a]
SE	0.34	0.15	0.21	0.11

[a,b,c,d] Means having different superscripts within rows are significantly different ($P<0.05$)

At 7 weeks of age when broilers exposed to 38°C for 3 h, total proteins was significantly lower in HS and BHA than in C and EHA groups. This reduction in TPP was due to a significant reduction in plasma albumin concentration of HS group, while it was due to the significant reduction in G of BHA group (Table 3). Although TTP of EHA was almost equal to the control group, However, albumin and

A/G ratio were significantly higher and globulin was significantly lower than control group. As a result, A/G ratio was significantly lower in HS than in the control group, while heat acclimation caused a significant increase in A/G ratio. This effect of early and biweekly heat acclimation in maintaining high albumin and A/G ratio will prevent the reduction in colloid pressure, consequently the reduction in plasma volume. The high albumin and low globulin in EHA and BHA groups may be due to the production of heat-shock protein (HSP). The resistance of broilers to heat depends on the amount of HSP that synthesized following heat-shock, HSP may bind to cellular proteins that have been denatured by the heat and may prevent their catastrophic precipitation (Finely *et al.*, 1984 and Siegel, 1995).

It can be concluded from the present study that early heat acclimation (exposure of broilers to 38°C for 3 h at 3 days of age) is one of the best physiological process to alleviate the effect of heat stress on broilers.

Table 2. Effect of heat stress and acclimation on body fluid compartments as percentage from total body water of broilers at 7 weeks of age

Traits	Control	Heat stressed	Early heat-acclimated	Biweekly heat-acclimated
Intracellular fluids	41.15 [a]	32.33 [c]	40.56 [a]	38.01 [b]
SE	1.08	0.36	0.79	0.56
Extra cellular fluids	58.85 [c]	67.67 [a]	59.45 [c]	61.99 [b]
SE	1.08	0.35	0.79	0.56
Interstitial fluids	50.42 [b]	64.27 [a]	51.04 [b]	52.05 [b]
SE	1.30	1.53	0.82	0.68
Plasma volume	8.41 [b]	4.51 [c]	8.40 [b]	9.95 [a]
SE	0.62	0.26	0.35	0.18

[a,b,c] Means having different superscripts within rows are significantly different ($P < 0.05$)

Table 3. Effect of heat stress and acclimation on plasma proteins (g/dl) of broilers at 7 weeks of age

Traits	Control	Heat stressed	Early heat acclimated	Biweekly heat acclimated
Albumin	1.89 [b]	1.15 [c]	2.30 [a]	1.93 [b]
SE	0.02	0.01	0.05	0.14
Globulin	1.28 [a]	1.34 [a]	0.92 [b]	0.85 [b]
SE	0.13	0.09	0.06	0.05
TPP	3.17 [a]	2.48 [b]	3.22 [a]	2.77 [b]
SE	0.12	0.09	0.08	0.15
A/G ratio	1.61 [b]	0.90 [c]	2.53 [a]	2.35 [a]
SE	0.19	0.09	0.17	0.25

[a,b,c] Means having different superscripts within rows are significantly different ($P < 0.05$)

REFERENCE

Abdel-Razik, M.A., Y.A., Katab and G.M. Gebriel, 1985. Effect of heat stress on body fluids and heat tolerance coefficient of White Giza and Buscat buck rabbits. Egypt. J. Anim. Prod., 25: 165-172.

Ahmed, Nagwa A.; H. H. Khalifa; S. M. T. El-Tantawy; M. A. Kicka and A. M. Dawoud, 1998. Response of broilers body fluids and some physiological traits to changes in environmental conditions. Egypt. J. Anim. Prod., 35, Suppl. Issue, Dec. (1998): 125-139.

Boone, M. A., 1968. Temperature at six different locations in the fowl's body as affected by ambient temperatures. Poult. Sci., 47:1961-1962.

Boone, M. A. and B. L. Hughes, 1971. Wind velocity as it affects body temperature, water consumption and feed consumption during heat stress of roosters. Poult. Sci., 50:1535-1537.

Daghir, N. J., 1995. Poultry production in hot climates. CAB International, UK.

Dawoud, A.M.I., 1998. Effect of environmental conditions on body compartments of broilers. Ph.D. Thesis, Fac. Agric. Cairo University.

Doumas, T. Basil; Ard W. Wtson, and Homer, G. Biggs, 1971. Albumin standards and the measurement of serum albumin with Bromocresol Green". Clin. Chim. Acta. 34: 87-96.

Eberhart, D. E. and K. W. Washburn, 1993. Variation in body temperature response to naked neck and normally feathered chickens to heat stress. Poult. Sci., 72:1385-1390.

Edwards, M.J., 1979. Is hypothermia a human teratogen? Am. Hearty. 98: 277-280.

El-Tantawy, S.M.T., Khalifa, H.H., Ahmed, A.A., Kicka, M.A. and Dawoud, A.M. 1998. Effect of season, dietary NaCl and heat acclimation on performance and carcass quality of broiler chicken. Egypt. J. Anim. Prod., 35, Suppl. Issue, Dec. (1998): 141-154.

Faltas A.A., A.K.I. Abd Elmoty and Kh. A. Mohammed, 1987. The effect of exposure at 40°C on the physiological response of the domestic fowl maintaining at 16°C. Minia. J. Agric. Res.& Dev. 9:383-398.

Finely, D., A. Ciechanover and A. Varshavsky, 1984. Thermoability of ubiquitin-activating enzyme from the mammalian cell cycle mutant ts85, Cell 37, 43-55.

Francis, C.A., M.G. Macleod and J.E. M. Anderson, 1991. Alleviation of acute heat stress by food withdrawal or darkness. British Poultry Sci. 32: 219-225.

Hacina, A.B., P.A. Geraert, J.C.F. Padilha and S. Gwllaumin, 1996. Chronic heat exposure enhances fat deposition and modifies muscle and fat partition in broiler carcass. Poultry Sci. 75:505-513.

Hillman, P.E., N.R. Scott, and A. Van Tienhoven, 1985. Physiological responses and adaptations to hot and cold environments pages 27-28 in: Stress physiology in Livestock. CRC Press, Inc., M.K. Yousef, ed. Boca Raton, F.L.

Hussien, A.A., 1994. The effect of some modifications in managemental and environmental condition on heat production of chickens. MSC. Poultry Prod. Fac. Agric. Cairo. Univ.

Kennedy, J. A. and G. A. Millikan, 1938. A micro blood volume method using a blue dye and photocell. J. Physiol., 93:276-284.

Khalifa, H.H. 1999. Evaluation of some managerial manipulation to alleviate the effect of heat stress on broiler chickens. Proceeding of International Congress of Biometeorology and International Conference on Urban Climatology, 8-12 Nov. 1999, Sydney, Australia. (In press).

Khalifa, H.H. and Shoukry, M.S. 1997. Effect of heat stress on plasma volume and some physiological variables in broiler chicks. Proceeding of Earth-Ocean-Atmosphere Forces for Change, Melbourne, Australia, 1-9 July, 1997.

Khalifa, H. H., N. F. Abdel-Hakim and A. M. I. Dawoud, 1994. Effect of growing season on growth rate and body fluid compartments of broiler. Egypt. J. Anim. Prod., 31, 403-418.

Khalifa, H.H., Khalil, M.H., Younes, T.M. and Abdel-Tawab, F. 2000. Comparative study on some methods to alleviate the effect of heat stress on broilers. Unpublished data.

Lott, B. D., 1991. The effect of feed intake on body temperature and water consumption of male broilers during heat exposure. Poult. Sci., 70:756-759.

May, J.D. and B.D. Lott, 1992. Feed and water consumption patterns of Broilers at high environmental temperatures. Poultry. Sci. 71: 331-336.

Mishra, V. K. and S. K. Saxena, 1983. Effect of season on body water distribution in three genotypes of crossbred calves. Indian. J. Dairy Sci., 36:21-23.

SAS, 1988. Statistical Analysis System, SAS User's Guide: Statistics, SAS Inst. Inc., Cary, NC.

Siegel, H. S., 1995. Stress, strains and resistance. Gordon Memorial Lecture, Br. Poult. Sci., 36:3-22.

Smith, M.O., 1993. Parts, yield of broilers reared under cycling high temperatures. Poultry Sci. 72: 1146-1150.

Squibb, R.L., M.A. Guyman. and N.S. Scrimshaw, 1959. Growth and blood constituents of immature New Hampshire fowl exposed to a constant temperature of 99° f for 7days. Poultry. Sci. 38: 220-221.

Teeter, R.G., M.O. Smith, and C.J. Wiernusa, 1992. Broiler acclimation to heat distress and feed intake effects on body temperature in birds exposed to thermoneutral and high ambient temperatures. Poultry. Sci. 71: 1101-1104.

Weichselbaun, T.E. Captain, 1946: An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. Am. J. Clin. Path. 10: 40-49.

Weiss, H. S., 1958. Application to the fowl of the antipyrine dilution technique for the estimation of body composition. Poult. Sci., 37: 484-489.

Whittow, G.C. 1968. Body fluid regulation. In: Adaptation of domestic animals. Hafez, E.S.E. (ed.), Lea & Fediger Publ., Philadelphia, pp. 119.

Whittow, G.C., P.D. Sturkie, and G. Stein., 1964. Cardiovascular changes associated with thermal polypnea in the chicken. Am. J. Physiol. 207: 1349-1353.

Yahav, S. and S. Hurwitz, 1996. Induction of thermotolerance in male broiler chickens by temperature conditioning at an early age. Poult. Sci., 75:402-406.

Yahav, S.,S. Goldfeld, I. Plavnik, and S.Hurwitz, 1995. Physiological responses to chickens and turkeys to relative humidity during exposure to high ambient temperature. Therm. Biol. 20:245-253.

Yahav, S., A. Straschnow, L. Plavnik and S. Hurwitz, 1997. Blood system response of chicken to changes in environmental temperature. Poult. Sci., 76:627-633.