Effect of Feed Withdrawal and Heat Acclimatization on Stress Responses of Male Broiler and Layer-type Chickens (*Gallus gallus domesticus*)

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ABSTRACT : This experiment was conducted to evaluate the effect of feed withdrawal (F) and heat acclimatization (A) on male-broiler and -layer chickens responses to acute heat stress (AHS) at four weeks of age. Totals of ninety male chicks of broiler or layer type were randomly allocated into 30 pens of grower batteries with raised wire floors. Chicks were subjected to F and A three times a week through the first three weeks of age. At each time, feed withdrawal and heat acclimatization (T = 35°C) lasted for six and four hours, respectively. Feed consumption (FC), body weight (BW), and feed conversion ratio (FCR) were recorded weekly for broiler type chickens only. At four weeks of age, all groups of chickens were exposed to AHS (T = 39±1°C) for three hours. Before and after AHS challenge, body temperature (Tb), heterophil (H), and lymphocyte (L) counts were recorded, and H/L ratio was calculated. Antibody (Ab) response to sheep red blood cells (SRBC) was assessed from all treatments without being exposed to AHS. Group F of broiler-type chickens weighed less ($p<0.05$) compared to control group. Also, both A and F groups of broiler-type chickens consumed less ($p<0.05$) feed when compared to control group. Acute heat stress elevated Tb of all treatment groups, however the increase was more profound ($p<0.001$) in broiler chicks. Broiler chicks of both A and F groups showed a tendency to have higher ($p = 0.08$) Tb when compared to control group. Acute heat stress elevated ($p<0.001$) H/L ratio in both types of chickens. Broiler chicks maintained higher ($p<0.001$) H/L ratio. Both F and A groups reduced ($p<0.01$) the level of elevation in H/L ratio compared to control groups of both types of chickens. Neither A nor F group affected the Ab production in response to SRBC. However, there was a tendency towards higher Ab responses in F group when compared to other groups in both types of chickens. Results of the present study demonstrate that previous history of feed withdrawal or episodes of heat exposures improved chicks' physiological withstanding of AHS and a tendency to improved humoral immune response. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 10 : 1445-1450)***

Key Words : Feed Withdrawal, Acclimatization, Body Temperature, Chicken, H/L, Heat Stress

INTRODUCTION

Although recent poultry crosses improved in all production aspects, environmental challenges still affirmed to exert major impact on meat and egg production. Over the past two decades, the effect of temperature received a greater attention from researchers all over the world to maximize the productivity of poultry meat, eggs, and reproduction output. The major effects of heat stress are increased mortality, reduced feed intake and lower weight gain or egg production and a decrease in profitability (Dale and Fuller, 1980; Teeter et al., 1985; Cahaner and Leenstra, 1992). Most of the proposed approaches to increase thermotolerance in poultry were directed towards ameliorating poultry macro-environment of housing, diet manipulation program (Bollengier-Lee et al., 1999; Kubikova et al., 2001; Mahmoud and Edens, 2003; Zulkifli et al., 2004), and heat conditioning (Davis et al., 1991; Yahav et al., 1997; Givisiez et al., 1999; Zulkifli et al., 2003).

Genetic variation in heat tolerance is known to exist within species. In poultry, for example, certain breeds or strains appear to survive heat stress more successfully than others, and chickens that are acclimatized to elevated temperatures are more elastic than those experiencing a swift heat shock (Eberhart and Washburn, 1993a, b; El-Gendy and Washburn, 1995). For instance, Jungle fowl survive heat stress more successfully than do normal commercial strains of chicken, as do Bedouin fowl in the Sinai desert (Marder, 1973). Subsequently, a cross between Leghorn and Bedouin fowl produced offspring with improved heat tolerance relative to the Leghorn parents (Arad et al., 1975), indicating a genetic component in this tolerance. Therefore, genotype and acclimatization are considered as factors that attenuate the adverse effect of high environmental temperature although genotype factor always compromise chicken meat and egg production. However, we think that acclimatization might be very rewarding in an attempt to magnify poultry production upon the exposure to high ambient temperature without compromising productivity. Most of the literatures associated with heat-stress responses of chickens are drawn from a diverse pool in which there are no direct connections because there were methodological differences in the experimental designs. Therefore, results from those studies can lead to different conclusions. The bases for such diverse conclusions stem from variations in body weight, age,

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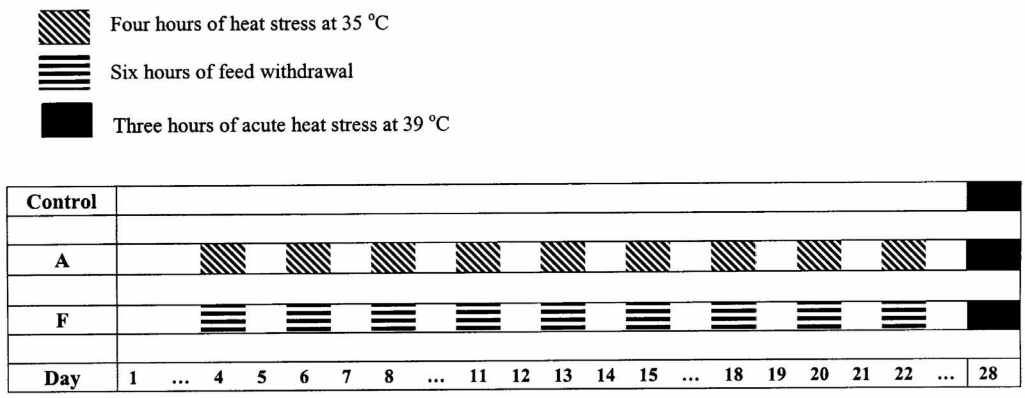

Figure 1. Outline of heat acclimatization (A) and feed withdrawal (F) dates for both types of chickens. Control group of chickens had *ad libitum* access to feed under normal rearing conditions. At 28 days of age, all groups were subjected to acute heat stress.

genetic backgrounds, genotypes, length and magnitude of the heat challenge. In herein study, measurements of different leukocyte counts [Heterophil (H) and lymphocyte (L)] and their ratio (H/L) and body temperature (Tb) were evaluated to investigate whether the exposure to a severe heat-stress of broiler or layer chicks can be attenuated through heat acclimatization or feed withdrawal. Also, the effect of different rearing regimens on antibody (Ab) titers to sheep red blood cells (SRBC), body weight (BW), feed consumption (FC) and feed conversion ratio (FCR) was evaluated.

MATERIALS AND METHODS

Experimental animals, design, and husbandry

Ninety day-old hatchling male-broiler and a similar quantity of male-layer hatchling were purchased from local hatcheries. Chicks were individually numbered with plastic transdermal tages then placed randomly in 30 pens of grower batteries with raised wire floors. Broiler and layer chicks were held in the same room but in different pens. Batteries were housed in the Animal House Center at Jordan University of Science and Technology with controlled light, temperature, and ventilation. Brooding temperature was set at 32±1°C, and then incrementally decreased to 23±1°C by the time chicks were 21-day old. Lightcycle was 23 h light and 1 h dark with a relative humidity of 50%. Corn-soybean based diets were formulated to meet all nutrient requirements of broiler or layer chicks that satisfy the recommendations of National Research Council (NRC, 1994). Chicks were kept under the same conditions throughout the first three days of age. Thereafter, three rearing regimens were applied for each type of chicks through the first three weeks of age as shown in Figure 1. Group 1 served as control (C) and reared under standard brooding conditions throughout the experiment. Group 2 (feed withdrawal: F) was subjected to six hours (10:00-16:00) of feed withdrawal three times a week. Group 3 (heat acclimatization; A) was subjected to heat stress (35°C) for four hours (10:00-14:00) three times a week in a different room with *ad libtum* access to feed and water. Total feed consumption (FC) and body weight (BW) was recorded and feed conversion ratio (FCR) was calculated for each rearing regimen of broiler chicks only. At 28 days of age, ten chicks from each rearing regimen were exposed to acute heat-stress (AHS; 39±1°C) for three hours with *ad libtum* access to feed and water.

Before and after AHS, body temperature (Tb) was recorded by deep clocal insertion of thermostat probe [Cooper Instrument Corporation (TM99A and TC100A series)] attached to a calibrated electronic thermometer. The thermometer reading was recorded when it stopped deviating for 30 sec. Also, blood smears were prepared from ten chicks per rearing regimen then heterophils (H) and lymphocytes (L) were counted as described by Gross and Siegel (1983). Total of a hundred cells per slide were counted using oil immersion microscopy at 100X magnification, then H and L were classified and the H/L ratios were calculated.

Antibody response to sheep red blood cells

At 29 days of age, ten chicks from each treatment, which were not exposed to AHS, were injected by 0.25 ml of 10% suspension of sheep red blood cells (SRBC) in each thigh. Four and seven days post injection, they were bled via the brachial vein into tubes primed with EDTA, and plasma was collected and stored at -20°C for later determination of total antibody titers by hemagglutination method (Munns and Lamont, 1991). Titers were expressed as the $\log_2$ of the reciprocal of the highest dilution giving visible hemagglutination.

Statistical analysis

Data were analyzed as a factorial arrangement of type of chicks (broiler or layer) and three different rearing regimens (Control, A, or F) as main effects in a completely

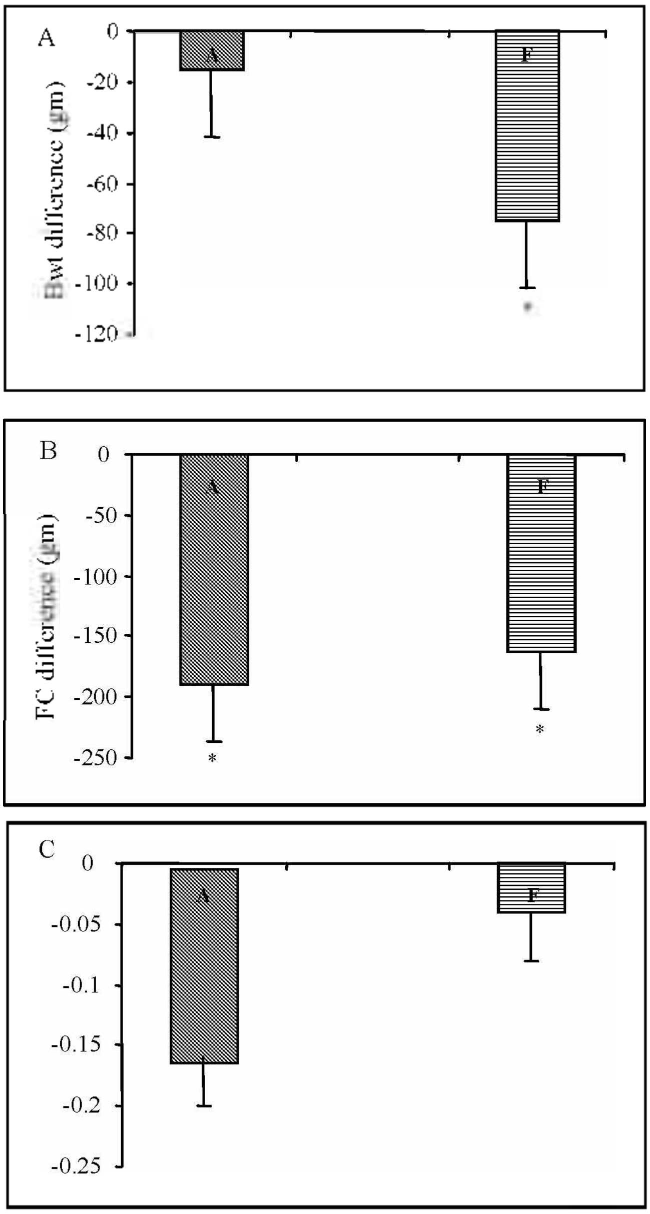

Figure 2. The difference in body weight (Panel A), feed consumption (Panel B), and feed conversion ratio (Panel C) between heat acclimated (A) and feed withdrawn (F) groups and control reared group of broiler chickens. Significant differences are indicated by the inclusion of asterisk. * $p<0.05$.

randomized design. The repeated measures of each experimental unit were analyzed as a split plot to test the effect of AHS. The statistical analysis was attained by the General Linear Model procedure of SAS (SAS Institute, 1990) and each source of variation was tested against the suitable mean square of errors for significance. The following pre-planned contrasts were requested by General Linear Model procedure of SAS: (1) control versus heat acclimatized and feed withdrawal within broiler (C vs. A&F/broiler) and layer (C vs. A&F/layer); (2) heat acclimatized versus feed withdrawal within broiler (A vs. F/broiler) and layer (A vs. F/layer). A probability level of $p\leq0.05$ or as described in the text was considered to be statistically significant.

RESULTS

The differences in production parameters (BW, FC and FCR) between broiler- and layer-type chicken were not of main interest to this research project. However, the effect of feed withdrawal or heat acclimatization on the production parameters of broiler type chicken was of main concern to this project. Figure 2 abstracts the difference in BW, FC, and FCR between A or F groups of broiler-type chickens and their control group. Group F of broiler-type chickens weighed less ($p<0.05$) when compared to control group while the reduction of weight in group A was only numerical when compared to control group. Also, broiler-type chickens of both A and F groups consumed less ($p<0.05$) feed when compared to the control chicks.

Body temperature of layer-type chickens was constantly higher when compared to broiler-type chickens (Table 1) before AHS challenge. Also, both A and F conditioned chickens maintained numerically higher Tb when compared to their respective control chicks prior to AHS in both types of chickens. However, contrasting Tb of A and F groups with the control groups in both type of chickens didn't reveal significant differences. Heat challenge elevated ($p<0.05$) Tb of all experimental groups except for layer-type A and F groups. Also, the rate of increase in Tb due to AHS challenge was more pronounced in broiler-type chicks. Nonetheless, it is worth mentioning that broiler chicks of A and F groups tended to upsurge ($p = 0.08$) their Tb as they were subjected to heat challenge compared to their respective control chicks.

Cell counts of H and L as well as their ratio was also abstracted in Table 1. Broiler chickens maintained higher H counts and lower L counts in all experimental groups when compared to their counterparts of layer chickens before and after AHS. Although heat stress increased H counts across all groups of chicks and decreased L counts, the significant changes were observed only in the control groups of both types of chickens. Heterophil and L counts of A and F chickens differed ($p<0.01$) compared to the control groups of both types of chickens when subjected to AHS. In both types of chickens, the most remarkable increase, after AHS, in H counts coincides with the highest decrease in L counts of control groups. Heat challenge resulted in a significant increase in H/L ratio in all experimental treatments. The H/L ratios were constantly higher in broiler compared to layers before and after AHS. The most profound increase ($p<0.01$) was observed in the control groups of both types of chickens when compared to A and F groups.

Antibody titers against SRBC at day four and seven of immunization are presented in Table 2. Anti-SRBC titers

Table 1. Effect of acute heat stress (AHS) on body temperature (Tb), heterophil (H), lymphocyte (L) and H/L ratio of different types of chicken acclimatized to heat stress (A) or subjected to feed withdrawal (F) through the first three weeks of age

Type of chicken	Treatment	Variable							
		Tb		H		L		H/L	
		Pre-AHS	Post-AHS	Pre-AHS	Post-AHS	Pre-HS	Post-AHS	Pre-AHS	Post-AHS
Broiler	Control	41.39*	42.58	25.1*	36.2	74.9*	63.7	0.358*	0.581
	A	41.50*	42.88	27.8	31.8	72.2	70.2	0.395	0.456
	F	41.60*	42.94	24.5	25.8	75.5	74.3	0.332	0.352
Layer	Control	41.80*	42.20	14.0*	28.8	86.0*	71.5	0.169*	0.406
	A	42.00	42.12	16.1	19.6	83.9	80.4	0.195	0.247
	F	41.94	42.28	16.1	22.0	83.9	78.6	0.206	0.281
SEM		0.118	0.204	3.36	2.68	3.36	3.05	0.062	0.055
Pre-planned comparisons									
Control vs. A and F/broiler		NS	0.08	NS	**	NS	**	NS	**
Control vs. A and F/layer		NS	NS	NS	**	NS	**	NS	**
A vs. F/broiler		NS	NS	NS	NS	NS	NS	NS	NS
A vs. F/layer		NS	NS	NS	NS	NS	NS	NS	NS

* Means in the same row within a variable differ significantly ($p<0.05$).
NS: Not significant. ** $p<0.01$.

Table 2. Antibody titers of sheep red blood cell (SRBC) of different types of chicken adapted to heat stress (A) or subjected to feed withdrawal (F) through the first three week of age

Type of chicken	Treatment	Days after Immunization	
		4	7
Broiler	Control	0.61	1.50
	A	0.56	1.65
	F	0.65*	2.30
Layer	Control	0.50*	2.15
	A	0.50*	2.05
	F	0.61*	2.90
SEM		0.077	0.60
Pre-planned comparisons			
Control vs. A and F/broiler		NS	NS
Control vs. A and F/layer		NS	NS
A vs. F/broiler		NS	NS
A vs. F/layer		NS	NS

* Means in the same row differ significantly ($p<0.05$).
NS: Not significant.

were elevated ($p<0.001$) at day seven of immunization when compared to day four in all experimental groups. The statistical analysis of this variable exhibited effect of neither type of chickens or kind of conditioning. Nevertheless, layer chicks sustained numerically higher anti-SRBC titers compared to broiler chicks. Also, F groups of both types of chickens maintained numerically higher titer of anti-SRBC.

DISCUSSION

Acclimatization is a term used to designate or to describe the increase in bird's ability to cope with stressor(s) as a result of previous exposure to episodes of stress from the same or different kind. Feed consumption, FCR, Tb, survival rate, H/L ratio, and glucose metabolism are among the production and physiological parameters used in the literature to asses the manifestation in tolerance to heat in birds subjected to previous exposure of feed withdrawal and/or heat stress. The present findings are consistent with those of Dale and Fuller (1980), Henken et al. (1983), and Teeter et al. (1992). It is remarkable to point that FC was reduced by 10.6% and 9.7% in A and F groups, respectively when compared to control group; however, BW reduction was 1.5% and 7.3% in A and F groups, respectively compared to control group. These results suggest that heat acclimatized chicks showed a higher feed efficiency, which may explain the numerical reduction in FCR.

There is an agreement among several previous studies regarding that heat acclimatized birds were able to maintain lower Tb compared to those of non-acclimated birds following heat challenge (May et al., 1987; Teeter et al., 1992). Contrarily, other researchers failed to signify a difference in Tb of acclimated and non-acclimated birds (Arjona et al., 1990; Yalcin et al., 2001). Recently, Zulkifli et al. (2003) reported that early age feed restriction or heat acclimation was numerically hyperthermic when compared to control group upon exposure to acute heat stress later in life. But when feed restriction and heat acclimation were experienced together at early age, Tb increased ($p<0.05$) after six hour of heat exposure. Our findings, on the other hand, showed that Tb prior to AHS of both A and F groups recorded similar readings when compared to their respective controls of broiler- and layer-type chicks. However, after AHS challenge Tb of all groups increased ($p<0.001$). The Tb of both A and F groups were higher compared to control groups of broiler chicks. Although there is an apparent inconsistency between our results and those in the literature, we think that the feed intake data of the experimental groups of the herein study may provide an explanation. Wiernusuz and Teeter (1996) observed that the increased heat production associated with feed metabolism is more pronounced during heat stress. Teeter et al. (1992)

reported that bird's rectal temperature during heat stress is influenced by both feed consumption level and previous heat stress exposures. They also reported that the lack of interaction between previous heat exposures and feed intake level suggests that their effect on Tb are additive. Therefore, acclimation response magnitude may differ in fed and starved birds. When broiler chicks are affronted with heat stress, any factor elevating heat production, such as feed metabolism will increase the birds' heat load (Wiernusz and Teeter, 1996). In our study, both heat acclimatization and feed withdrawal treatments were terminated by day 22 of age; thereafter, they were maintained at thermoneutral ambient temperature and *ad libitum* feed access. The compensatory growth (data not shown) during the forth week may provide an explanation which is well documented by Ballay et al. (1992). They reported that growth compensation of the restricted group of chicks increased at four weeks of age as indicated by higher feed intake and weight gain. Consequently, heat production from excess feed intake elevated the birds' heat load which may lead to a higher Tb of feed withdrawal followed by heat-acclimatized and then by control groups.

The response measurement of stress-induced immune alteration to heat exposure by the use of H and L as well as their ratio (H/L) is well documented (Zulkifli et al., 1994, 2000, 2003; Shini, 2003). The H/L ratio has been used as a sensitive hematological indicator of stress response among chicken populations (Gross and Siegel, 1983). Our results ascertained the presence of relationship between heat stress and the increase in the non-specific immune reactive cells, H cells; and the ability of previous heat acclimatization or feed withdrawal to ameliorate their response. Maxwell and Robertson (1998) described H cells as a non-specific immunological defense provider and emergence of H cells into the circulation comes as a consequent event to any stressful condition. Our findings are consistent with those reported in the literature and showed that chickens experienced either heat acclimatization or feed withdrawal reduced ($p<0.001$) H/L ratio in response to high environmental temperature in both broiler- and layer-type chickens. However, this does not indicate that their immune response is also modified. Broiler chickens maintained a higher ($p<0.001$) H/L ratio prior and after AHS exposure. This could be an indicative of a higher stress level, which could be due to the intensive growth rate selection. Furthermore, this study revealed a positive ($p<0.05$) correlation between H count and H/L and Tb, and a negative relationship ($p<0.05$) between Tb and L cell counts. These results are consistent with Zulkifli et al. (1994, 2000, 2003) although the application of feed restriction was at earlier age compared to the circumstances of our trial. In contrast, Plavnik and Yahav (1998) failed to report an effect of early feed restriction on birds' response to high ambient temperature later in life.

Neither conditioning type affected the Ab production in response to SRBC. However, there was a tendency towards higher Ab response in F group when compared to other groups in both types of chickens which is consistent with Zulkifli et al. (2000) finding. A comparable conclusion was drawn by a more recent work by Shini (2003). He reported that housing conditions and social stress might have a great effect on H/L ratio while humoral response seems unaltered although a negative correlation was observed between H/L ratio and Ab production. In conclusion, our results distinctly suggest that both feed withdrawal and heat acclimatization, in both broiler and layer chickens, further attenuate physiological response to heat stress, as indicated by lower H/L ratio. Layer chickens maintained a higher Tb when compared to broiler chickens before AHS, however it was the other way around after AHS. Broiler chickens sustained higher H/L ratios when compared to layer chickens before and after AHS. Feed withdrawal in broiler has a tendency to lower the physiological response to heat stress more than heat acclimatization.

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