Effects of Early Age Feed Restriction and Heat Conditioning on Heat Shock Protein 70 Expression, Resistance to Infectious Bursal Disease, and Growth in Male Broiler Chickens Subjected to Heat Stress

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ABSTRACT The effects of early age feed restriction and heat conditioning on heat shock protein (HSP) 70 expression, antibody production, resistance to infectious bursal disease (IBD), and growth of heat-stressed male broiler chickens were investigated. Chicks were divided into 4 groups: 60% feed restriction on d 4, 5, and 6 (FR); exposure to 36 ± 1°C for 1 h from d 1 to 21 (HT); combination of FR and HT (FRHT); and control. From d 35 to 50, heat stress was induced by exposing birds to 38 ± 1°C and 80% RH for 2 h/d. On d 36, each bird was administered 10 times the normal dose of live IBD vaccine. After heat exposure, the FRHT birds had higher HSP 70 density (d 41) and weight gain (from d 35 to 49) and lower bursal histological score (BHS) (d 51) than their HT and control counterparts. The HSP 70 expression and BHS of FR birds were not significantly different from those of the other 3 groups during the heat exposure period. Heat shock protein 70 and BHS data were negatively correlated ($r = -0.33$, $P = 0.0008$). We concluded that FRHT could improve weight gain and resistance to IBD in male broiler chickens under heat stress conditions. The improved heat tolerance and disease resistance in FRHT birds could be attributed to better HSP 70 response.

(*Key words*: early age feed restriction, heat conditioning, heat shock protein 70, heat stress, infectious bursal disease)

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INTRODUCTION

There is an abundance of literature on possible techniques to alleviate the adverse effects of heat stress in broiler chickens. One of the practical approaches that has yielded promising results is altering birds' abilities to cope with high ambient temperatures through stimulation early in life. Converging evidence suggests that stressful experiences during the neonatal stage can have considerable impact on various facets of an animal's physiology and behavior. Exposure of 5-d-old broiler chicks to elevated temperature improved survivability in otherwise lethal heat treatment at the age of 42 d (Arjona et al., 1988, 1990; Yahav and Hurwitz, 1996; De Basilio et al., 2001). Previous studies (Zulkifli et al., 1994a,b, 2000) have consistently shown that early age feed restriction enhances the ability of chickens to withstand high ambient temperature as juveniles more than it does for those fed ad libitum throughout the experiment. Therefore, it is reasonable to hypothesize that subjecting chicks to early age fasting and heat conditioning can further enhance their tolerance to high ambient temperatures.

It is well documented that chronic stress response may impede antibody production and cell-mediated immunity and thereby increase susceptibility to viral infections (Siegel, 1995). However, stress due to early age feed restriction (Zulkifli et al., 1994a,b) can improve resistance to marble spleen disease in heat-stressed chickens when tested at older ages. Thus, it appears that stressful experiences during development have the potential to modify disease resistance in poultry. It is not known, however, whether heat conditioning leading to acquired improved thermotolerance can also alter disease resistance in chickens.

There is the question of how neonatal stresses can modify a bird's thermoregulatory system. Recent studies have shown that acquired enhanced heat tolerance resulting from early age fasting (Zulkifli et al., 2002) and thermal conditioning (Wang and Edens, 1998) in broiler chickens could be attributed to improved heat shock protein (HSP) 70 response. The HSP play a profound role in regulating protein folding and in coping with proteins affected by

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Abbreviation Key: BBR = bursa-to-body weight ratio; BHS = bursal histological score; FR = 60% feed restriction on d 4, 5, and 6; FRHT = combination of FR and HT; HSP = heat shock protein; HT = exposure to 36 ± 1°C for 1 h from d 1 to 21; IBD = infectious bursal disease; ND = Newcastle disease; PVDF = polyvinyledine difluoride.

heat and other stresses (Gething and Sanbrook, 1992). 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 (Chirico et al., 1988; Hartl, 1996).

Most research on HSP in poultry has emphasized its association with body temperature (Wang and Edens, 1993; Yahav et al., 1997; Givisiez et al., 1999). To the best of our knowledge, there is no information on the relationship between HSP 70 activity and disease resistance in poultry under stressful conditions. Because early age feed restriction may improve resistance to viral infections (Zulkifli et al., 1994a,b) and HSP 70 expression (Zulkifli et al., 2002), there is a possibility that both phenomena are interrelated. Thus, the aim of the present experiment was to evaluate the effects of early age feed restriction, heat conditioning, and their combinations on HSP 70 expression, antibody production, resistance to infectious bursal disease (IBD), and heat tolerance of male broiler chickens exposed to heat stress.

MATERIALS AND METHODS

Birds, Housing, and Diet

A total of 192 1-d-old commercial male broiler chicks (Cobb) were obtained from a local hatchery. Upon arrival, the chicks were individually wing-tagged, weighed, and randomly assigned in groups of 8 to 24 battery cages with wire floors in an environmentally controlled chamber. The chicks were raised at 32 ± 1°C, and then the temperature was gradually decreased until 24 ± 1°C was reached by d 21. All chicks were fed corn and soybean meal-based starter (crumble form; 21% CP and 2,950 kcal ME/kg) from d 1 to 21 and finisher (pellet form; 19% CP and 3,100 kcal ME/kg) from d 22 onward. Water and feed were supplied ad libitum except as noted.

Experimental Procedures

Commencing from d 1, equal numbers of chicks were subjected to 1 of the following 4 treatments: 60% feed restriction on d 4, 5, and 6 (FR); exposure to 36 ± 1°C and 50 to 60% RH for 1 h daily from d 1 to 21 (HT); 60% feed restriction on d 4, 5, and 6 and exposure to 36 ± 1°C and 50 to 60% RH for 1 h daily from d 1 to 21 (FRHT); ad libitum feeding and no subjection to heat conditioning (control). The feed restriction was 60% of the previous day's feed intake of the control group. During the daily heat conditioning from d 1 to 21, the HT and FRHT chicks were individually removed from their cages, placed in plastic crates, and transferred to another environmentally controlled chamber. Both environmentally controlled chambers were adjacent to each other and of similar dimensions and set up. The HT and FRHT birds remained in the crates throughout the heat conditioning period. To avoid the confounding effect of being removed and placed in plastic crates, from d 1 to 21, the control and the FR birds were also subjected to similar treatment, but they were not moved out of the chamber. Feed and water were not provided during the heat conditioning period. Immediately following the 1-h heat conditioning procedure, birds were returned to their home cages.

All birds were vaccinated[2] against Newcastle disease (ND) intraocularly on d 7 and 21. Prior to feeding, individual BW was determined on d 1, 7, 14, 21, 28, 35, 42, and 49. Relative weight gain from d 35 to 49 was determined [(BW d 49 − BW d 35)/(BW d 35)] × 100. Mortality was recorded daily.

Heat Challenge

To elicit heat stress from d 36 to 50, all chicks (at the same time) were exposed to ambient temperature of 38 ± 1°C and 80% RH for 2 h/d. The time taken for the ambient temperature to rise from 24 to 38°C was about 45 min. Feed and water were provided ad libitum throughout heat challenge.

Blood and Brain Samples

On d 36 (prior to heat challenge), 37, 39, 41, 44, and 51, ten birds from each group were chosen at random, and blood samples (2.0 mL) were obtained from the brachial vein using a 23-ga needle. Serum was separated from the blood samples and used for determining the IBD and ND antibodies with ELISA kits.[3] Immediately after each occasion of blood sampling, 5 birds from each treatment group were randomly chosen and killed by cervical dislocation, and brain samples were removed for detection of HSP 70 (Zulkifli et al., 2002). The samples were frozen quickly in liquid nitrogen and kept at –70°C for further analysis.

SDS-PAGE and Immunoblot Analysis

The brain samples (0.5 g) for detection of HSP 70 were homogenized in an Ultra-Turrax homogenizer, with 5 mL chilled Tris-HCl buffer (20 m*M* Tris, pH 7.5; 0.75 *M* NaCl; 2m*M* 2-mercaptoethanol) and centrifuged at 23,000*g* for 30 min at 4°C. Protein concentrations of the supernatants were quantified by the Bicinchoninic Acid Protein Assay Kit[4] (Procedure No. TRPO-562) with bovine serum albumin as a standard. Thirty micrograms of total protein was loaded and separated on 1.5 × 80 × 100-mm 12% polyacrylamide gels containing SDS (Laemmli, 1970), a Hoefer Mini Gel apparatus was used.[5] Gels were subjected to electrophoresis at 150 V until the tracking dye reached the base of the gel. The fractionated proteins were visualized by Coomasie blue staining or transferred to polyvinyledine difluoride (PVDF) membranes (Towbin et al., 1979). After electrophoretic transfer, the PVDF membranes were stained with 0.5

[2]TAD ND vac La Sota, Lohmann Animal Health GmbH & Co. KG, Heinz-Lohmann-Str. 4, Cuxhaven, Germany.
[3]IDEXX, Laboratory, Inc., Westbrook, ME.
[4]Sigma Chemical Co., St. Louis, MO.
[5]Hoefer Scientific Instruments, San Francisco, CA.

g/L Ponceau S in 10 g/L acetic acid solution to visualize and mark the positions of the proteins used as molecular weight standards. After washing away the excess Ponceau S with distilled water, the nonspecific binding sites were blocked using 10 mL of cold blocking buffer containing 10% nonfat milk and 0.05% sodium azide for 30 min. The membranes were incubated overnight (4°C) with 5 mL of blocking buffer containing antiserum[4] (mouse anti-chicken HSP 70) against HSP 70 in a 1:1,000 dilution. Following overnight incubation, the blots were washed 4 times (5 min each) with 10 mL of cold blocking buffer. The blots were then allowed to react with goat anti-mouse secondary antibody conjugated to alkaline phosphatase[4] for 1 h. After a rinse with cold PBS, the color reaction on the PVDF membrane was developed using commercially prepared BCIP/NBT.[4] Relative density of the HSP 70 was determined using a AlphaImager densitometer[6] with the aid of AlphaEase software.[6]

IBD Vaccine Challenge

On d 37, each chick was intraocularly administered 10 times the normal dose of live IBD vaccine.[7] The vaccine was packed in vials, with each vial recommended for administration to 1,000 chicks. In this experiment, each bird was administered 0.1 mL with the following dilution: 1 vial of vaccine to 10.0 mL of distilled water. Prior to IBD challenge (d 37), and 2 d (d 39), 4 d (d 41), 7 d (d 44), and 14 d (d 51) postchallenge, the bursas of Fabricius were removed from the same group of birds (5 birds/treatment group) that were killed for determination of HSP 70 in the brains. The weights of the bursas of Fabricius were obtained, and the gross pathological changes were observed and recorded. Individual bursa-to-body weight ratio (BBR) was calculated by dividing bursal weight by BW and multiplying by 1,000 (Giambrone and Closser, 1990). The bursas were then fixed in 10% buffered formalin, processed for histology, and stained with hematoxylin and eosin (Bancroft et al., 1996). Bursas were examined under a light microscope. The bursal histological lesions were scored from 0 to 5 according to the modified criteria described by Henry et al. (1980).

Statistical Analyses

Results were evaluated by analysis of variance. Differences between group means were analyzed by Duncan's multiple range test, using the general linear models procedure in the Statistical Analysis System (SAS, 1991). When interactions between main effects were significant, comparisons were made within each experimental variable. Prior to analyses, ND and IBD antibody titer data were transformed to common logarithm. All data presented in this paper are the actual untransformed data. Correlation was calculated on HSP 70 density and BHS to describe any relationship between these parameters. Mortality data were subjected to chi-squared analysis. Statistical significance is considered as $P \leq 0.05$ throughout the paper.

RESULTS

BW

Subjecting chicks to FR and FRHT resulted in suppression of BW from 7 to 28 d (Table 1). However, because of accelerated growth, the BW of the FRHT and FR birds were similar to controls by d 28 and 35, respectively. The HT procedure had negligible effect on growth throughout the duration of study. Although all the birds had similar BW on d 42 and 49 (period of heat exposure), the FR and FRHT birds had greater relative weight gain than controls during the heat treatment period (Figure 1). The relative weight gains of the HT chickens were not significantly different from those of the other 3 groups.

Mortality

During the heat exposure period, FRHT birds had significantly lower mortality than their HT counterparts (Figure 2). The mortality rates of FR and control birds were similar to those of HT and FRHT.

Antibody Titers

The ND and IBD antibody titers are presented in Table 2. Neither early age treatment nor age had significant effect on antibody titer against ND vaccinations. The IBD antibody titers increased significantly following IBD challenge, and peak response was noted at 51 d of age (14 d postheat challenge). The early age treatment had no significant effect on IBD antibody titer.

Heat Shock Protein 70 Density

There were interactions of early age treatment by age ($P = 0.043$) for HSP 70 density in the brain samples (Table 3). The interactions were observed because significant effect of early age treatment was noted only on d 41 and 44. On d 41, the FRHT birds had greater HSP 70 response than did HT and controls. The mean HSP 70 densities of FR birds on d 41 were not significantly different from those of control, HT or FRHT birds. The enhanced HSP 70 response of FRHT chicks was maintained on d 44. The mean HSP 70 densities of FR and HT birds were not significantly different from those of the other two groups. Except for HT and FRHT birds, there was no significant increase in HSP 70 expression following the heat exposure. Irrespective of early age treatment, HSP 70 density declined following 8 d of heat exposure (d 44).

BBR and BHS

Irrespective of treatment group, the BBR decreased with number of days following IBD challenge (Table 4). Early

[6]Alpha Innotech Corporation, San Leandro, CA.
[7]V877 strain, Malaysia Vaccines & Pharmaceuticals Sdn. Bhd., Kuala Lumpur, Malaysia.

TABLE 1. Mean (± SEM) BW (g) of broiler chickens by early age treatment at various ages

	Treatment[1]			
Age (d)	Control	HT	FR	FRHT
1	49 ± 0.42 (n = 72)	50 ± 0.47 (n = 72)	50 ± 0.49 (n = 72)	51 ± 0.47 (n = 72)
7	180 ± 1.89[a] (n = 72)	184 ± 1.69[a] (n = 72)	127 ± 1.13[b] (n = 71)	127 ± 1.36[b] (n = 72)
14	420 ± 4.57[a] (n = 70)	420 ± 4.59[a] (n = 72)	382 ± 5.53[b] (n = 70)	391 ± 3.93[b] (n = 72)
21	831 ± 8.34[a] (n = 69)	836 ± 7.51[a] (n = 71)	794 ± 8.45[b] (n = 70)	788 ± 6.53[b] (n = 71)
28	1,394 ± 14.07[a] (n = 69)	1,394 ± 13.78[a] (n = 71)	1,351 ± 13.47[b] (n = 69)	1,357 ± 12.00[ab] (n = 70)
35	1,959 ± 23.62 (n = 69)	1,943 ± 22.91 (n = 70)	1,928 ± 18.83 (n = 69)	1,928 ± 21.40 (n = 70)
42	2,493 ± 46.81 (n = 43)	2,389 ± 92.20 (n = 43)	2,419 ± 48.80 (n = 42)	2,474 ± 39.10 (n = 47)
49	2,926 ± 83.93 (n = 32)	2,968 ± 102.10 (n = 33)	2,868 ± 53.30 (n = 28)	3,022 ± 47.41 (n = 38)

[a,b]Means within a row with no common letters differ at $P < 0.05$.

[1]Control = fed ad libitum and no heat conditioning; HT = exposure to 36 ± 1°C for 1 h/d from d 1 to 21; FR = 60% feed restriction on d 4, 5, and 6; FRHT = exposure to 36 ± 1°C for 1 h/d from d 1 to 21, and 60% feed restriction on d 4, 5, and 6.

age treatment had no effect on BBR. There was an interaction of age by treatment ($P = 0.038$) for BHS (Table 5). A significant effect of early age treatment on BHS was noted only on d 51. The FRHT birds exhibited lower BHS than their control and HT counterparts on d 51. The BHS of FR birds were not significantly different from those of the other 3 groups.

HSP 70 Density and BHS Correlation

The relationship between HSP 70 density and BHS was negative (r = –0.33, $P = 0.0008$). Birds with greater HSP 70 response had lower BHS than did birds with lower HSP 70 density.

DISCUSSION

As expected, the 60% feed restriction on d 4, 5, and 6 retarded the growth rate of birds. The mean BW of the FR birds on d 7 were approximately 70% of those for birds fed ad libitum. The FR birds, however, exhibited accelerated growth, and complete growth compensation was attained on d 35. Similar findings have been reported in White Plymouth Rock and commercial female broiler chickens subjected to 60% feed restriction on d 4, 5, and 6 (Zulkifli et al., 1994a,b; 2000). It is interesting to note that the HT treatment had negligible effect on BW of the chicken. Yahav and Hurwitz (1996) reported that exposure to 36 ± 1°C and 70 to 80% RH at the age of 5 d, or 5 and 7 d, depressed BW of male broiler chickens. On the contrary, despite subjecting

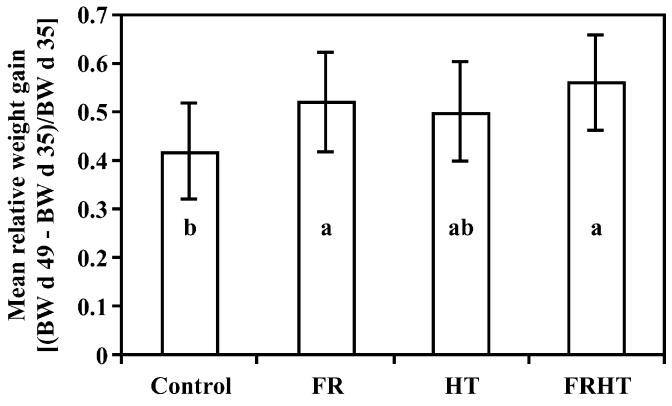

FIGURE 1. Mean relative weight gains [(BW d 49 – BW d 35)/BW d 35] of broiler chickens by early age treatment during heat exposure (d 36 to 50). [a,b]Means with no common letters differ at $P \leq 0.05$. Control = fed ad libitum and no heat conditioning; HT = exposure to 36 ± 1°C for 1 h/d from d 1 to 21; FR = 60% feed restriction on d 4, 5, and 6; and FRHT = exposure to 36 ± 1°C for 1 h/d from d 1 to 21, and 60% feed restriction on d 4, 5, and 6.

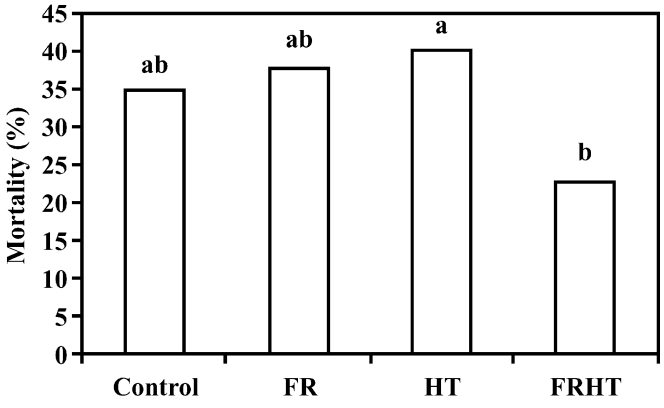

FIGURE 2. Mean (n dead/total: control, 24/69; HT, 28/70; FR, 26/69; FRHT, 16/70) mortality rates of broiler chickens by early age treatment during heat exposure (d 36 to 50). [a,b]Means with no common letters differ at $P \leq 0.05$. Control = fed ad libitum and no heat conditioning; HT = exposure to 36 ± 1°C for 1 h/d from d 1 to 21; FR = 60% feed restriction on d 4, 5, and 6; and FRHT = exposure to 36 ± 1°C for 1 h/d from d 1 to 21, and 60% feed restriction on d 4, 5, and 6.

TABLE 2. Mean (± SEM) Newcastle disease (ND) and infectious bursal disease (IBD) antibody titers by age and early age treatment

	Antibody titer[1]	
Item	ND	IBD
Age[2] (d)		
37	2,298 ± 242.4 (n = 40)	316 ± 27.4[c] (n = 40)
39	2,275 ± 318.7 (n = 40)	258 ± 36.2[c] (n = 40)
41	2,379 ± 440.5 (n = 40)	244 ± 45.3[c] (n = 40)
44	1,820 ± 215.1 (n = 40)	1,893 ± 175.7[b] (n = 40)
51	1,752 ± 272.7 (n = 40)	4,143 ± 251.1[a] (n = 40)
Treatment[3]		
Control	2,606 ± 227.4 (n = 50)	1,177 ± 205.3 (n = 50)
HT	2,124 ± 269.9 (n = 50)	991 ± 224.7 (n = 50)
FR	2,131 ± 280.4 (n = 50)	803 ± 161.6 (n = 50)
FRHT	1,839 ± 218.9 (n = 50)	1,265 ± 245.7 (n = 50)

[a–c]Means within a row with no common letters differ at $P \leq 0.05$.

[1]Antibody titers were measured by ELISA.

[2]From d 36 to 50, all chickens were exposed to 38 ± 1°C and 80% RH for 2 h/d. On d 37, each chick was administered 10 times the normal dose of live IBD vaccine.

[3]Control = ad libitum feeding and no heat conditioning; HT = exposure to 36 ± 1°C for 1 h/d from d 1 to 21; FR = 60% feed restriction on d 4, 5, and 6; FRHT = exposure to 36 ± 1°C for 1 h/d from d 1 to 21, and 60% feed restriction on d 4, 5, and 6.

birds to a similar heat conditioning procedure, Yahav and Plavnik (1999) did not observe significant reduction in BW as compared with controls. Thus, it appears that the effect of early age heat exposure on BW of broilers is inconsistent. The negligible effect of HT on BW was also reflected in the FRHT birds. The FRHT and FR birds had similar BW throughout the study.

It is well established that voluntary feed consumption is diminished in response to heat stress, and consequently weight gain will be adversely affected (Etches et al., 1995). In the present study, the FR and FRHT birds gained significantly more weight than controls during heat exposure (d 36 to 49). The noted improvement in BW gain of heat-stressed FR birds is compatible with previous findings (Zulkifli et al., 1994a,b; 2000). Thus, it appears that subjecting broilers to FR or FRHT may alleviate the detrimental influence of high ambient temperature on growth. Study by Arjona et al. (1988) and Yahav and Plavnik (1999) suggested that exposing chicks to 36 ± 1°C for 24 h at 5 d of age improved weight gains in response to subsequent heat challenge. In the present study, however, the weight gains of the HT birds were not significantly different from those of their control, FR and FRHT counterparts. The discrepancies between studies could stem from the differences in the protocol of heat conditioning. There is a possibility that the heat conditioning technique practiced in the present study was more severe than that of Arjona et al. (1988) or Yahav and Plavnik (1999). Zulkifli et al. (2002) indicated that the severity of the early age stress might have profound impact on the magnitude of improvement in heat tolerance later in life.

One of the most striking findings of the work by Zulkifli et al. (2000) is the observation that early age fasting markedly improved survivability of heat-stressed female broiler chickens. Although exposure to high ambient temperature resulted in high mortality among the controls (17%), none of the feed-restricted birds succumbed to the heat challenge. In the present study, however, the mortality rates of control (34.6%) and FR (37.7%) birds were not significantly different. Because different sex of birds and RH (80% vs. below 60%) were used in the present study, and it is well documented that raising male broiler chickens under high RH may exacerbate heat stress problems, inferences should be made with caution. Similarly, the HT procedure failed to enhance survivability of male broiler chickens under heat stress conditions. On the contrary, heat conditioning by exposure to 36 ± 1°C for 24 h at the age of 5 d has been

TABLE 3. Mean (± SEM) heat shock protein 70 densities where age × early age treatment interactions were significant

	Treatment[1]			
Age[1] (d)	Control	HT	FR	FRHT
36	25,980 ± 763.0[ab] (n = 5)	25,252 ± 1,008.9[c] (n = 5)	27,982 ± 938.0[a] (n = 5)	25,252 ± 1,008.9[c] (n = 5)
39	28,028 ± 1,362.0[a] (n = 5)	31,122 ± 1,008.9[a] (n = 5)	30,940 ± 1,186.5[a] (n = 5)	30,940 ± 1,602.2[b] (n = 5)
41	29,666 ± 2,451.9[ay] (n = 5)	27,118 ± 928.0[bcy] (n = 5)	30,576 ± 1,274.0[axy] (n = 5)	35,672 ± 928.0[ax] (n = 5)
44	26,390 ± 406.9[ay] (n = 5)	28,392 ± 1,013.3[abxy] (n = 5)	27,530 ± 781.5[axy] (n = 5)	29,666 ± 617.2[bx] (n = 5)
51	22,386 ± 364.0[b] (n = 5)	21,480 ± 813.9[d] (n = 5)	21,112 ± 1,013.3[b] (n = 5)	22,932 ± 971.6[c] (n = 5)

[a–c]Means within a column with no common letters differ at $P \leq 0.05$.

[x,y]Means within row with no common letters differ at $P \leq 0.05$.

[1]From d 36 to 50, all chickens were exposed to 38 ± 1°C and 80% RH for 2 h/d. On d 37, each chick was administered 10 times the normal dose of live IBD vaccine.

[2]Control = ad libitum feeding and no heat conditioning; HT = exposure to 36 ± 1°C for 1 h/d from d 1 to 21; FR = 60% feed restriction on d 4, 5, and 6; FRHT = exposure to 36 ± 1°C for 1 h/d from d 1 to 21, and 60% feed restriction on d 4, 5, and 6.

TABLE 4. Mean (± SEM) bursa-to-body weight ratios (BBR) by age and early age treatment

Item	BBR
Age[1] (d)	
37	1.65 ± 0.12[a] (n = 20)
39	1.60 ± 0.09[a] (n = 20)
41	1.68 ± 0.12[a] (n = 20)
44	0.94 ± 0.09[b] (n = 20)
51	0.64 ± 0.05[c] (n = 20)
Treatment[2]	
Control	1.26 ± 0.12 (n = 25)
HT	1.24 ± 0.11 (n = 25)
FR	1.34 ± 0.13 (n = 25)
FRHT	1.36 ± 0.13 (n = 25)

[a–c]Means within a column with no common letters differ at $P \leq 0.05$.

[1]From d 36 to 50, all chickens were exposed to 38 ± 1°C and 80% RH for 2 h/d. On d 37, each chick was administered 10 times the normal dose of live IBD vaccine.

[2]Control = ad libitum feeding and no heat conditioning; HT = exposure to 36 ± 1°C for 1 h/d from d 1 to 21; FR = 60% feed restriction on d 4, 5, and 6; FRHT = exposure to 36 ± 1°C for 1 h/d from d 1 to 21, and 60% feed restriction on d 4, 5, and 6.

reported to significantly reduce mortality of heat-stressed male broiler chickens (Arjona et al., 1988, 1990; Yahav and Plavnik, 1999). Thus, it appears that the HT protocol practiced in the present study is less effective than that of Arjona et al. (1988, 1990) or Yahav and Plavnik (1999) in improving survivability under heat stress conditions. Subjecting birds to FRHT did not significantly improve survivability of broilers during heat challenge as compared with the FR and control counterparts.

Disease resistance is complex and involves various factors of the individual, population, and host pathogen levels. There is considerable evidence to indicate that response and coping with environmental fluctuations can modify biological defense systems. Stressor may impede production of antibodies and effective cell-mediated immunity, thereby increasing susceptibility to viral diseases (Siegel, 1995). The present findings are in agreement with those of Zulkifli et al. (1994a,b, 2000) that early age feed restriction had a negligible effect on antibody production in heat stressed birds compared to those fed ad libitum. Following heat exposure, the ND and IBD antibody titers of FR and control birds were not significantly different. Similarly, control, HT and FRHT birds had similar ND and IBD antibody titers. Despite the failure to improve antibody production against IBD vaccine, it appeared that FRHT could improve resistance to IBD infection. On d 51, the FRHT birds had significantly lower BHS than controls. The results of this study confirm earlier findings (Zulkifli et al., 1994a,b) that stressful experiences during the early age could be beneficial in improving resistance to viral infections.

Zulkifli et al. (2002) showed that early age feed restriction, leading to acquired heat tolerance, enhanced the ability of broiler chickens to express HSP 70. HSP have been proven to play a profound role in regulating protein folding and in coping with proteins affected by heat and other stresses (Gething and Sanbrook, 1992). In the present study, the FRHT chicks had significantly greater HSP 70 density than controls following 5 d of heat treatment. The HSP 70 response of FR birds, however, was not significantly different from those of control and FRHT groups.

Irrespective of treatment group, it is apparent that the overall HSP 70 response to the heat exposure was relatively lower as compared with previous studies (Zulkifli et al., 2002; Wang and Edens, 1998). A likely explanation for the

TABLE 5. Mean (± SEM) bursal histological scores[1] where age × early age treatment interactions were significant

	Treatment[3]			
Age[2] (d)	Control	HT	FR	FRHT
37	1.20 ± 0.49[b] (n = 5)	0.60 ± 0.40[b] (n = 5)	1.20 ± 0.97[b] (n = 5)	0.60 ± 0.60[c] (n = 5)
39	0.80 ± 0.37[b] (n = 5)	1.20 ± 0.37[b] (n = 5)	2.40 ± 0.51[ab] (n = 5)	1.60 ± 0.40[bc] (n = 5)
41	3.20 ± 0.37[a] (n = 5)	3.40 ± 0.51[a] (n = 5)	2.80 ± 0.20[ab] (n = 5)	2.20 ± 0.37[b] (n = 5)
44	4.20 ± 0.20[a] (n = 5)	4.00 ± 0.32[a] (n = 5)	3.80 ± 0.37[a] (n = 5)	3.60 ± 0.24[a] (n = 5)
51	3.60 ± 0.60[ax] (n = 5)	3.40 ± 0.24[ax] (n = 5)	2.80 ± 0.37[abxy] (n = 5)	2.00 ± 0.32[by] (n = 5)

[a–c]Means within a column with no common letters differ at $P \leq 0.05$.

[x,y]Means within row with no common letters differ at $P \leq 0.05$.

[1]The bursal histological lesions were scored from 0 to 5 according to the modified criteria described by Henry et al. (1980).

[2]From d 36 to 50, all chickens were exposed to 38 ± 1°C and 80% RH for 2 h/d. On d 37, each chick was administered 10 times the normal dose of live IBD vaccine.

[3]Control = ad libitum feeding and no heat conditioning; HT = exposure to 36 ± 1°C for 1 h/d from d 1 to 21; FR = 60% feed restriction on d 4, 5, and 6; FRHT = exposure to 36 ± 1°C for 1 h/d from d 1 to 21, and 60% feed restriction on d 4, 5, and 6.

lack of marked HSP 70 response in the present study during the heat challenge could be associated with the IBD challenge. Infection of mammalian cells with picornavirus leads to shutdown of host cell protein synthesis by inactivation of the cap binding protein complex (Trachsel et al., 1980). According to Moore et al. (1981) and Reavy et al. (1983), this inhibition of protein synthesis extends to the synthesis of HSP, which is virtually abolished. In view of this finding, it can be suggested that response to viral infection could be detrimental to HSP 70 activity and heat tolerance, consequently.

Previous studies have shown that HSP response will be augmented because of nonthermal stressors, such as nutrient deprivation, oxygen starvation, or the presence of heavy metals, oxygen radicals, or alcohol (Morimoto, 1993). However, to the best of our knowledge, its association with disease resistance in poultry has not been previously studied. The negative correlation between HSP 70 density and BHS suggests that HSP 70 may influence resistance to IBD. The FRHT birds had greater HSP 70 response and lower BHS than controls. Hence, there is a possibility that HSP 70 can protect the bursa of Fabricius from being damaged by IBD vaccine virus.

In conclusion, these data suggest that the FRHT combination can enhance weight gain and resistance to IBD in heat-stressed male broiler chickens by eliciting greater HSP 70 response. As measured by weight gain, mortality rate, HSP 70 density, and resistance to IBD, the FR and HT birds showed similar response to the heat challenge. Under conditions of this experiment, HSP 70 response appears to be beneficial in enhancing resistance to IBD in heat-stressed broiler chickens.

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