Enhancing heat tolerance, heat shock protein 70 response and disease resistance in broiler chickens through early age fasting and thermal conditioning

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Introduction

A great deal of literature on laboratory animals suggests that stressful experiences during the neonatal stage may improve their ability to cope with stressors later in life. It has been noted that infantile stimulation through handling evoked long lasting modifications of the hypothalamic-pituitary-adrenal axis in rodents. On becoming adults, the handled rats had lower basal corticosterone concentrations and a faster recovery to baseline at the end of stress than those that were not handled. Its implications in the context of veterinary science and animal agriculture have been of major interest. There has, however, been little work investigating the phenomenon in poultry. Stresses that occur early in life, while many systems of the chicks are still developing, may have long-lasting impact and could possible modify the expression of their genetic potential. It has been reported that early age feed restriction ameliorated heat-stress related responses in White Rock chickens at a later age. Previous studies suggest that female broiler chicks subjected to 60% feed restriction at 4, 5 and 6 days of age were better able to withstand high ambient temperatures as juveniles than those that were not fasted or were food restricted to a lesser extend. The question, however, as to how early age feed restriction can have a profound impact on thermoregulation later in life has yet to be answered. The acquired improved thermotolerance resulting from prior exposure to controlled thermal stressors in poultry has been associated with enhanced heat shock protein (hsp) 70 response. When living organisms are exposed to thermal and other stressors, the synthesis of most proteins is retarded but a group of highly conserved proteins known as hsp are rapidly synthesized. Heat shock proteins have been proven to play a key role in regulating protein folding and in coping with proteins affected by heat and other stresses. In a heat-shocked cell, the hsp may bind to heat-sensitive proteins and protect from degradation, or may prevent damaged proteins from immediately precipitating and permanently affecting cell viability. It is well documented that one of the most important functions of hsp is to protect organisms from the toxic effects of heating. Most research on hsp in poultry has emphasized its association with body temperature. To the best of our knowledge there is no information on the relationship between hsp activity and disease resistance in poultry under stressful conditions.

Materials and Methods

Expt 1: Day-old commercial broiler chicks were brooded for 3 weeks and then maintained at 24 C. On day (d) 1, chicks were assigned to one of four feeding regimens; (i) ad libitum feeding (ALF), (ii) 40% feed restriction on d 4, 5 and 6 (F40), (iii) 60% feed restriction on d 4, 5 and 6 (F60), (iv) 80% feed restriction on d 4, 5 and 6 (F80). From d 35 to 41, all chicks were exposed to 38 C for 2h/d. Traits measured were body weight, survivability rate, heterophil/lymphocyte ratios (HLR) (stress index), serum concentrations of glucose and cholesterol, and heat shock protein (hsp) 70 density in the brain. Heat shock protein 70 was determined by SDS-PAGE and Western blotting, and the relative density was measured using a densitometer.

Expt. II: Broiler chicks were subjected to (1) 60% feed restriction on day 4, 5 and 6 (FR); (2) exposure to 36 C for 1 h from day 1 to 21 (HT); (3) both FR and HT; (4) or control. On day 35, birds were exposed to 39 C for 6 h. Traits measured were HLR, rectal temperature and hsp 70 density in the brain. Heat shock protein 70 was determined by SDS-PAGE and Western blotting, and the relative density was measured using a densitometer.

Expt. III: Broiler chicks were subjected to (1) FR; (2) HT; (3) FRHT; (4) or control. From d 35 to 50, all chicks were exposed to 38 C for 2h/d. On d 36, each bird was administered with 10 times the normal dose of live infectious bursal disease (IBD) vaccine. Bursa samples were collected on d 44, 49 and 51 for determination of bursal histological score (BHS). Heat shock protein 70 was determined by SDS-PAGE and Western blotting, and the relative density was measured using a densitometer

Results and Discussion

Expt. I: Serum concentrations of glucose were elevated by the heat challenge, but were not affected by the feeding regimen. The heat treatment resulted in hypocholesteremia among ALF and F80 chicks, whereas the concentrations increased and remained constant in the F60 and F40m birds, respectively. Subjecting chicks to F60 improved growth and survivability and reduced HLR in response to heat treatment as compared with the ALF and F80 regimens. The survivability rate and HLR of F40 chicks were similar to those attained by chicks on other regimens. It appears that the F60 regimen is beneficial for alleviating the detrimental effects of heat stress in female broiler chickens. Prior to the heat challenge, all chicks had similar hsp 70 response. Irrespective of feeding regimen, there was a marked increase in hsp 70 expression after four days of heat treatment. Following seven days of heat challenge, except for the F60 birds, the augmented hsp 70 expression in the brains of

ALF, F80 and F40 birds was not maintained. Enhancement of hsp 70 expression was noted in birds subjected to F60, but not ALF, F80 or F40, throughout the period of heat exposure. The most striking finding of the current work is the observation that the F60 treatment enhanced hsp 70 expression in the brain throughout the period of heat exposure. The hsp 70 response was more pronounced in the F60 birds with increasing duration of heat treatment while the marked increases in hsp 70 induction following four days of heat exposure in ALF, F40 and F80 chickes were not maintained thereafter. Hence it appears that hsp 70 paly a key role in eliciting thermotolerance by F60. In the present study, it appeared that F60 may improve hsp 70 response of heat-stressed birds four weeks following the cessation of feed restriction. There is a possibility early-age fasting may have evoked hsps mRNA transcription but the RNA may have been 'sequestered' and not translated until exposure to heat challenge later in life.

Expt. II: Subjecting chicks to FR, HT, and FRHT reduced HLR response to th heat challenge. The FR and FRHT birds had improved hsp 70 response and the latter were more hyperthermic than controls during the heat exposure. In this study we showed that subjecting birds to FR and HT reduced HLR response to high ambient temperature, suggesting better ability to cope with heat stress. Our results also suggest that HT has no profound impact on induction of hsp 70 response as compared to controls. Significant induction of hsp 70 was detected at hyperthermia of 43.7 C or higher. The hsp 70 response is elicited after a to a lethal temperature, in which case, non-hsp-synthesis is shut off and cells produce hsps at maximal rates as long as protein synthesis continue.

Expt. III Following 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) (day 51) than their HT and control counterparts. The hsp 70 expression and BHS of FR birds were not significantly different from the other three groups during the heat exposure period. Heat shock protein 70 and BHS data were negatively correlated (r = -0.33, P = 0.0008). Previous studies have shown that HSP response will be augmented because of non-thermal stressors, such as nutrient deprivation, oxygen starvation or the presence of heavy metals, oxygen radicals or alcohol. 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. We concluded that FRHT can 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.

Conclusions

This study demonstrates that subjecting to 60% feed restriction at 4, 5 and 6 days of age leads to improved heat tolerance later in life, by enhancing the ability to express hsp 70 in the brain. These data also 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.

Benefits from the study

The findings have important implications to veterinary science and animal agriculture and have opened new avenues in the study of stress biology in poultry. The research team has successfully developed practical, effective and economical strategies to improve the tolerance of poultry to stresses. We have also demonstrated that hsp 70 response may be used as an analytical tool to better understand an animal's response to heat stress and viral infection on a molecular basis. A thorough molecular characterisation of the heat-shock response in a range of poultry would provide the basis for future genetic manipulation of the heat-shock response in a way that has not been possible before.

Patent(s), if applicable:

Nil

Stage of Commercialization, if applicable :

Nil

Project Publications in Refereed Journals

Zulkifli, I., M.T. Che Norma, D.A. Israf and A.R. Omar .2002. The effects of early-age food restriction on heat shock protein 70 response in heat-stressed female broiler chickens. *British Poultry Science*, 43:141-145.

Zulkifli, I., P.K. Liew, D.A. Israf, D.A., A.R. Omar and M. Hair-Bejo. 2003. Effects of early age feed restriction and thermal conditioning on heterophil/lymphocyte ratio, heat shock protein 70 and body temperature of male broiler chickens subjected to acute heat stress. *Journal of Thermal Biology*, 28:217-222.

Liew, P.K., **I. Zulkifli**, M. Hair-Bejo, A.R. Omar and D.A. Israf. 2003. Effects of early age feed restriction and thermal conditioning on heat shock protein 70 expression, resistance to infectious bursal disease and growth in male broiler chickens subjected to chronic heat stress. *Poultry Science*, (in press).

Zulkifli, I., B. Norbaiyah and A. Siti Nor Azah. 2003. Growth performance, mortality and immune response of two commercial broiler strains subjected to early age feed restriction and heat conditioning under hot, humid tropical environment. *Archive für Geflügelkunde* (in press).

Project Publications in Conference Proceedings

Liew, P.K., **I. Zulkifli**, M. Hair-Bejo, D.A. Israf and A.R. Omar (2002). Effects of early age feed restriction and thermal conditioning on heat shock protein 70 expression, disease resistance and heat stress in male broiler chickens. *Proceedings of the 24th Malaysian Society of Animal Production Annual Conference*, Penang, Malaysia, pp. 133-134.

Graduate Research

Name of Graduate	Research Topic	Field of Expertise	Degree Awarded	Graduation Year
Che Norma Mat Taib	Heat shock protein 70 and heat tolerance in early-age feed restricted broiler chickens	Poultry Physiology	2000	2000
Liew Pit Kang	Modifying the response of male broiler chickens to heat stress through early age feed restriction and thermal conditioning	Poultry Physiology	2002	2002

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