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Study of impact of different season on HSP-70 and cortisol level of broiler chicken of different agro-climatic zone of Assam

M Sarma, P Deka, A Deka, KP Kalita, D Sapkota, JD Mahanta, BN Bhattacharyya and S Deori

Abstract

Purpose: The study on HSP-70 and cortisol level of broiler chicken in different season of Assam is of great value in regard to stress management and diseases control regime of chicken. The aim of this study was to evaluate the HSP-70 and Cortisol level of broiler chicken in different climatic condition of Assam.

Materials and Methods: The proposed study was carried out on Cobb 400 broilers with a view to find out the seasonal impact on heat shock protein 70 and cortisol level of broiler chicken at five different agro-climatic zones prevalent in Assam *viz.* Upper Brahmaputra Valley (Z₁), Hill Plateau (Z₂), North Bank Plain (Z₃), Central Brahmaputra Valley (Z₄) and Lower Brahmaputra Valley (Z₅) from March 2017 to February 2018 which had different temperature, rainfall and relative humidity values. The stress response of the experimental birds was examined at 14th, 28th and 42nd day of age. Fifty birds were selected randomly in each season from selected farms under study for collection of blood. The blood for serum sampling were collected by puncturing the wing vein of randomly selected experimental birds (n= 50 each season) under sterile condition in clot-activated vacutainer tubes, and transported in water proof containers containing crushed or dry ice to the laboratory. Clotted blood samples were centrifuged for 20 min at 1500 rpm and serum was isolated and stored at -20 °C for determination of HSP-70 and level of cortisol. Concentrations of HSP-70 in serum samples collected in the present study were measured using Chicken Heat Shock Protein 70 (HSP-70) ELISA kit. For the quantitative determination of cortisol in chicken sera collected in the present study, Chicken Cortisol ELISA kit was used. All the data obtained in the present experiment were statistically analysed with Two way ANOVA using SAS System, 'Local' X 64_7 PRO software. Statements of statistical significance were based on $p < 0.05$ except for HSP and Cortisol which were analysed with one way ANOVA.

Result: The season wise mean serum HSP-70 level (ng/ml) of broiler chicken at 14th, 28th and 42nd day of age under different agro-climatic zones have been presented in Table. 1. The mean serum HSP-70 (ng/ml) of broiler chicken at 14th day of age, during pre-monsoon, monsoon, post-monsoon and winter seasons was 1.74±0.07, 2.16±0.09, 1.75±0.05 and 4.39±0.08 respectively. The analysis of variance indicated that serum HSP-70 level (ng/ml) of broiler chicken at 14th day differed significantly ($p \leq 0.05$) among different seasons of Assam, where the HSP-70 level of broiler chicks in winter season was significantly ($p \leq 0.05$) higher followed by monsoon, pre-monsoon and post-monsoon seasons. However, the HSP-70 level of broiler chicks in pre-monsoon and post-monsoon season did not differ significantly ($p > 0.05$). At 28th day of age, the mean serum HSP-70 level (ng/ml) of broiler chicken during pre-monsoon, monsoon, post-monsoon and winter seasons was 1.81±0.03, 4.16±0.07, 1.83±0.04 and 3.52±0.07 respectively.

Conclusion: In current investigation, it was observed that the HSP-70 and Cortisol level in blood was found to be higher in adult broiler (42 days) during monsoon season due to high environmental temperature and consequent thermal stress on birds.

Keywords: Study, impact, season, HSP-70, cortisol level, broiler, chicken, agro-climatic, zone, Assam

Introduction

The Indian poultry sector is one of the most vibrant, fastest growing, agro-based, techno-commercial industry which is characterized by its industrialization, faster growth in consumption and trade than any other major agricultural sectors in the world. In spite of the tremendous growth and figures of production in Indian poultry sector, there are several constraints affecting growth of the poultry industry, among which temperature-associated environmental challenges, especially adverse environmental conditions (hot and cold climate) imposes severe stress on birds and leads to reduced performance. The State of Assam lies in the regime of monsoon climate of the sub-tropical belt.

The climate of the state is characterized by hot-humid summer and dry and cool winter. The periodic change of climate in Assam reflects four distinct seasons: pre-monsoon, monsoon, post-monsoon and winter. (Annonymous, 2006) [2]. Pre-monsoon season: The pre-monsoon season is associated with occasional thunder storm and heavy shower towards early March. Such a condition continues up to the end of May. Monsoon season: The monsoon season starts in Assam in late June and continues to late September. The period is characterized by high temperature, heavy rainfall and high relative humidity. Post- monsoon season: The post- monsoon period starts with fair weather and morning fog in late September and continues up to the month of November. Winter season: The winter period starts from the later part of November and the horizon becomes foggy in the morning and evening hours. The situation remains more or less the same up to the end of February. Based on rainfall, terrain and soil characteristics, the State of Assam has been broadly delineated into the six agro-climatic zones *viz.*, North Bank Plain (Darrang, Sonitpur, Lakhimpur and Dhemaji district), Upper Brahmaputra Valley (Jorhat, Sivasagar, Dibrugarh and Tinsukia district), Central Brahmaputra Valley (Nagaon and Morigaon district), Lower Brahmaputra Valley (Nalbari, Kamrup, Goalpara, Barpeta, Kokrajhar, and Dhuburi district), Barak Valley (Silchar, Hailakandi and Karimganja district) and Hills (Karbi-Anglong and North Cachar district). In Assam presently around 16-17 thousand youth are engaged in broiler sector. Majority of them (85%) are running their farm in small scale of their own and rest (15%) are under integration system. Due to poor economic condition of the majority of the farmer, the broiler sheds are kuccha, made up with locally available materials without any sophistication in open house system of management thereby, highly vulnerable to a wide range of seasonal variations prevailing in Assam which greatly influence the productivity of broilers and is a key threat for broiler farmers of Assam. The production performances of broiler chicken are greatly affected due to adverse ambient temperatures (Al-Fataftah *et al.*, 2007, Zahraa *et al.*, 2008) [1, 14]. Thermal discomfort may result in improper expression of genetic potential in birds (Kataria *et al.*, 2008) [10]. Feed intake, feed conversion efficiency and growth rate in broilers are affected as a results of fluctuation in ambient temperature. Till now no detailed study has been carried out on impact of seasons on HSP-70 and Cortisol level of broiler chicken of Assam. Hence, to provide valuable guideline to the broiler farmers, the present study was designed to determine the seasonal effect on HSP-70 and Cortisol level of broiler chicken in five agro-climatic zones of Assam.

Aims and Objectives

The study on HSP-70 and cortisol level of broiler chicken in different season of Assam is of great value in regard to stress management and diseases control regime of chicken. The aim of this study was to evaluate the HSP-70 and Cortisol level of broiler chicken in different climatic condition of Assam.

Materials and Methods

The proposed study was carried out on Cobb 400 broilers with a view to find out the seasonal impact on heat shock protein 70 and cortisol level of broiler chicken at five different agro-climatic zones prevalent in Assam *viz.* Upper Brahmaputra Valley (Z₁), Hill Plateau (Z₂), North Bank Plain (Z₃), Central Brahmaputra Valley (Z₄) and Lower Brahmaputra Valley (Z₅)

from March 2017 to February 2018 which had different temperature, rainfall and relative humidity values. The farmers were assisted to obtain the quality chicks from the local dealer that was sourced to the same hatchery. The local dealer was influenced to procure the chicks from the particular hatchery. One of the most important aspects of broiler producers is to minimize stress during production. The stress response of the experimental birds was examined at 14th, 28th and 42nd day of age. Fifty birds were selected randomly in each season from selected farms under study for collection of blood. In order to minimize handling stress, each bird captured was isolated for five minutes before blood collection. The blood for serum sampling were collected by puncturing the wing vein of randomly selected experimental birds (n= 50 each season) under sterile condition in clot-activated vacutainer (BD Vacutainer serum) tubes, and transported in water proof containers containing crushed or dry ice to the laboratory. Clotted blood samples were centrifuged for 20 min at 1500 rpm and serum was isolated and stored at -20 °C for determination of HSP-70 and level of cortisol. Concentrations of HSP-70 in serum samples collected in the present study were measured using Chicken Heat Shock Protein 70 (HSP-70) ELISA kit. For the quantitative determination of cortisol in chicken sera collected in the present study, Chicken Cortisol ELISA kit was used. All the data obtained in the present experiment were statistically analysed with Two way ANOVA using SAS System, 'Local' X 64_ 7 PRO software. Statements of statistical significance were based on $p < 0.05$ except for HSP and Cortisol which were analysed with one way ANOVA.

Results

HSP estimation: The season wise mean serum HSP-70 level (ng/ml) of broiler chicken at 14th, 28th and 42nd day of age under different agro-climatic zones have been presented in Table. 1. The mean serum HSP-70 (ng/ml) of broiler chicken at 14th day of age, during pre-monsoon, monsoon, post-monsoon and winter seasons was 1.74 ± 0.07 , 2.16 ± 0.09 , 1.75 ± 0.05 and 4.39 ± 0.08 respectively. The analysis of variance indicated that serum HSP-70 level (ng/ml) of broiler chicken at 14th day differed significantly ($p \leq 0.05$) among different seasons of Assam, where the HSP-70 level of broiler chicks in winter season was significantly ($p \leq 0.05$) higher followed by monsoon, pre-monsoon and post-monsoon seasons. However, the HSP-70 level of broiler chicks in pre-monsoon and post-monsoon season did not differ significantly ($p > 0.05$). At 28th day of age, the mean serum HSP-70 level (ng/ml) of broiler chicken during pre-monsoon, monsoon, post-monsoon and winter seasons was 1.81 ± 0.03 , 4.16 ± 0.07 , 1.83 ± 0.04 and 3.52 ± 0.07 respectively. The statistical analysis showed that the mean serum HSP-70 level of broilers among different seasons differed significantly wherein significantly ($p \leq 0.05$) higher serum HSP-70 level was recorded during monsoon followed by winter, pre-monsoon and post-monsoon seasons. However, no significant ($p > 0.05$) differences were recorded between pre-monsoon and post-monsoon season. At 42nd day of age, the mean serum HSP-70 level (ng/ml) of broiler chicken during pre-monsoon, monsoon, post-monsoon and winter seasons was 2.16 ± 0.06 , 7.12 ± 0.03 , 2.17 ± 0.06 and 2.48 ± 0.05 respectively. The statistical analysis showed that the mean serum HSP-70 level of broilers among different seasons differed significantly ($p \leq 0.05$) with higher serum HSP-70 level during monsoon season. However, no significant ($p > 0.05$) differences were recorded between pre-

monsoon, post-monsoon and winter seasons. During this season the highest temperature and humidity inside the broiler shed (i.e. micro-climate) was recorded as 36.39 °C and 85.37 per cent respectively and the highest outside (macro-climate) temperature and relative humidity recorded was 40.7 °C and 86 per cent respectively, where the birds might have failed to cope up with the hot and humid environment. HSPs are required for performance of functions of normal cells and also are required to withstand the toxic effects of fixed in temperature. The most important of these with respect to thermal stress (heat and cold stress) is heat shock protein 70 kDa (HSP-70) and was identified to be the ideal biological marker for thermal stress in chicken. Generally, HSPs are involved in the assembly/disassembly of protein/ protein-containing complexes during life and death of a normal cell. Under heat stress, there is impairment of transcription, RNA processing, translation, post-translational processing, oxidative metabolism, membrane structure and function, cytoskeletal structure and function etc. When environmental temperature is high, the body synthesises this protein (HSP) to adjust the body with it or prevent some body reaction that increases the body temperature. Response of cells/organism to heat-shock is extremely rapid, but transient; the response includes redistribution of pre-formed HSPs within the cell as well as immediate translation of preformed mRNA to HSPs. During monsoon the temperature changed abruptly and crosses 35 °C which was far beyond the thermo-neutral zone resulting in stress which is evident by rise in serum HSP-70 in adult broiler chicken. Requirement of heat is normally high during brooding period of the chicks (35 °C in first week). However, it was found that during winter the environmental temperature fell below (19.95 - 25.10 °C) the bearable range for the chicks. This condition, even further worsened by power-cut leading to severe cold stress is indicated by rise in serum HSP-70 in the suffered chicks. The level of HSP increased during winter particularly in chicks (below 14 days) to preserve the body temp or to maintain the body temperature. However, no change in HSP-70 was recorded in adult bird. The growth of feathers is complete in adult broiler chicken that helps in preserving the body temperature against the fall of environmental temperature which might explain the stable HSP-70 level at this age.

Cortisol estimation: The season wise mean serum cortisol level (ng/ml) of broiler chicken at 14th, 28th and 42nd day of age under different agro-climatic zones have been presented in Table. 2. The mean serum cortisol level (ng/ml) of broiler chicken at 14th day of age, during pre-monsoon, monsoon, post-monsoon and winter seasons was 1.05±0.002, 1.73±0.004, 1.05±0.003 and 1.76±0.006 respectively. The analysis of variance indicated that serum cortisol level (ng/ml) of broiler chicken at 14th day differed significantly ($p \leq 0.05$) among different seasons of Assam, where the serum cortisol level of broiler chicks in winter season was significantly ($p \leq 0.05$) higher followed by monsoon, pre-monsoon and post-monsoon seasons. However, the cortisol level in pre-monsoon and post-monsoon season did not differ significantly ($p > 0.05$). At 28th day of age, the mean serum cortisol level (ng/ml) of broiler chicken during pre-monsoon, monsoon, post-monsoon and winter seasons was 1.08±0.003, 2.40±0.008, 1.07±0.003 and 1.07±0.009 respectively. The statistical analysis showed that the mean serum cortisol level of broilers among different seasons differed significantly ($p \leq 0.05$) with higher serum cortisol level during monsoon

season. However, no significant ($p > 0.05$) differences were recorded between pre-monsoon, post-monsoon and winter seasons. At 42nd day of age, the mean serum cortisol level (ng/ml) of broiler chicken during pre-monsoon, monsoon, post-monsoon and winter seasons was 1.10±0.003, 2.92±0.004, 1.10±0.002 and 1.83±0.003 respectively. The statistical analysis revealed that the mean serum cortisol level of broilers among different seasons differed significantly, wherein significantly ($p \leq 0.05$) higher serum cortisol level were found during monsoon season. However, no significant ($p > 0.05$) differences were recorded between pre-monsoon post-monsoon and winter season.

Discussion

HSP estimation: The season wise mean serum HSP-70 level (ng/ml) of broiler chicken at 14th, 28th and 42nd day of age under different agro-climatic zones. The mean serum HSP-70 (ng/ml) of broiler chicken at 14th day of age, during pre-monsoon, monsoon, post-monsoon and winter seasons was 1.74±0.07, 2.16±0.09, 1.75±0.05 and 4.39±0.08 respectively. The analysis of variance indicated that serum HSP-70 level (ng/ml) of broiler chicken at 14th day differed significantly ($p \leq 0.05$) among different seasons of Assam, where the HSP-70 level of broiler chicks in winter season was significantly ($p \leq 0.05$) higher followed by monsoon, pre-monsoon and post-monsoon seasons. However, the HSP-70 level of broiler chicks in pre-monsoon and post-monsoon season did not differ significantly ($p > 0.05$). At 28th day of age, the mean serum HSP-70 level (ng/ml) of broiler chicken during pre-monsoon, monsoon, post-monsoon and winter seasons was 1.81±0.03, 4.16±0.07, 1.83±0.04 and 3.52±0.07 respectively. The statistical analysis showed that the mean serum HSP-70 level of broilers among different seasons differed significantly wherein significantly ($p \leq 0.05$) higher serum HSP-70 level was recorded during monsoon followed by winter, pre-monsoon and post-monsoon seasons. However, no significant ($p > 0.05$) differences were recorded between pre-monsoon and post-monsoon season. At 42nd day of age, the mean serum HSP-70 level (ng/ml) of broiler chicken during pre-monsoon, monsoon, post-monsoon and winter seasons was 2.16±0.06, 7.12±0.03, 2.17±0.06 and 2.48±0.05 respectively. The statistical analysis showed that the mean serum HSP-70 level of broilers among different seasons differed significantly ($p \leq 0.05$) with higher serum HSP-70 level during monsoon season. However, no significant ($p > 0.05$) differences were recorded between pre-monsoon, post-monsoon and winter seasons. These findings supported the observation of Sahin *et al.* (2009) [12] who reported that serum HSP-70 levels increased in poultry subjected to heat stress. Interestingly, Banerjee *et al.* (2014) [6] opined that that both heat and cold stress induced the over expression of HSP - 70 genes. The higher level of HSP-70 in monsoon season might be due to the higher in-house temperature. During this season the highest temperature and humidity inside the broiler shed (i.e. micro-climate) was recorded as 36.39 °C and 85.37 per cent respectively and the highest outside (macro-climate) temperature and relative humidity recorded was 40.7 °C and 86 per cent respectively, where the birds might have failed to cope up with the hot and humid environment. Heat tolerance in birds helps to increase the level of heat shock protein (HSP) (Liew *et al.*, 2003) [11] as HSP modify protein folding and aids the body to handle with proteins affected by heat and other stressors (Gething & Sambrook, 1992) [8]. HSPs are required for performance of functions of normal cells and also

are required to withstand the toxic effects of fixed in temperature (Anonymous¹⁰). The most important of these with respect to thermal stress (heat and cold stress) is heat shock protein 70 kDa (HSP-70) (Kamboh *et al.*, 2013)^[9] and was identified to be the ideal biological marker for thermal stress in chicken (Archana *et al.* 2017)^[5]. Generally, HSPs are involved in the assembly/disassembly of protein/ protein-containing complexes during life and death of a normal cell. Under heat stress, there is impairment of transcription, RNA processing, translation, post-translational processing, oxidative metabolism, membrane structure and function, cytoskeletal structure and function etc. When environmental temperature is high, the body synthesises this protein (HSP) to adjust the body with it or prevent somebody reaction that increases the body temperature. Response of cells/organism to heat-shock is extremely rapid, but transient; the response includes redistribution of pre-formed HSPs within the cell as well as immediate translation of preformed mRNA to HSPs. Increase in HSP-70 in serum indicates that the birds were under thermal stress, either heat or cold (Banerjee *et al.* 2014)^[6]. During monsoon the temperature changed abruptly and crosses 35 °C which was far beyond the thermo-neutral zone resulting in stress which is evident by rise in serum HSP-70 in adult broiler chicken. Requirement of heat is normally high during brooding period of the chicks (35 °C in first week). However, it was found that during winter the environmental temperature fell below (19.95 - 25.10 °C) the bearable range for the chicks. This condition, even further worsened by power-cut leading to severe cold stress is indicated by rise in serum HSP-70 in the suffered chicks. The level of HSP increased during winter particularly in chicks (below 14 days) to preserve the body temp or to maintain the body temperature. However, no change in HSP-70 was recorded in adult bird. The growth of feathers is complete in adult broiler chicken that helps in preserving the body temperature against the fall of environmental temperature which might explain the stable HSP-70 level at this age.

Cortisol Estimation: The season wise mean serum cortisol level (ng/ml) of broiler chicken at 14th, 28th and 42nd day of age under different agro-climatic zones have been presented in Table 4.26. The mean serum cortisol level (ng/ml) of broiler chicken at 14th day of age, during pre-monsoon, monsoon, post-monsoon and winter seasons was 1.05±0.002,

1.73±0.004, 1.05±0.003 and 1.76±0.006 respectively. The analysis of variance indicated that serum cortisol level (ng/ml) of broiler chicken at 14th day differed significantly ($p \leq 0.05$) among different seasons of Assam, where the serum cortisol level of broiler chicks in winter season was significantly ($p \leq 0.05$) higher followed by monsoon, pre-monsoon and post-monsoon seasons. However, the cortisol level in pre-monsoon and post-monsoon season did not differ significantly ($p > 0.05$). At 28th day of age, the mean serum cortisol level (ng/ml) of broiler chicken during pre-monsoon, monsoon, post-monsoon and winter seasons was 1.08±0.003, 2.40±0.008, 1.07±0.003 and 1.07±0.009 respectively. The statistical analysis showed that the mean serum cortisol level of broilers among different seasons differed significantly ($p \leq 0.05$) with higher serum cortisol level during monsoon season. However, no significant ($p > 0.05$) differences were recorded between pre-monsoon, post-monsoon and winter seasons. At 42nd day of age, the mean serum cortisol level (ng/ml) of broiler chicken during pre-monsoon, monsoon, post-monsoon and winter seasons was 1.10±0.003, 2.92±0.004, 1.10±0.002 and 1.83±0.003 respectively. The statistical analysis revealed that the mean serum cortisol level of broilers among different seasons differed significantly, wherein significantly ($p \leq 0.05$) higher serum cortisol level were found during monsoon season. However, no significant ($p > 0.05$) differences were recorded between pre-monsoon post-monsoon and winter season. These findings were in accordance with the observations of Sohail *et al.* (2010)^[13] who opined that heat stress significantly increased ($p \leq 0.05$) the concentration of cortisol as compared to broilers kept in thermo-neutral zone. Further, the results also corroborated well with the findings of Bahrami *et al.* (2012)^[7] who kept broiler chicken under a heat stress temperature (33±3 °C) and noticed increased cortisol level (ng/mL) on 28th (2.58) and 42nd day (2.72) of age. During heat stress body organs require to do extra functions which need extra energy. Cortisol, apart from using blood glucose involves in production of additional glucose from non carbohydrate sources of the body, particularly through catabolism of muscle proteins i.e. gluconeogenesis. To combat with the extra requirement of energy the hypothalamus-cortex system starts functioning which is evident by release of ACTHRH and consequent release of cortisol into the blood.

Table 1: Season wise mean serum hsp-70 level (ng/ml) of broiler chicken at 14th, 28th and 42nd day of age under different agro-climatic zones of Assam

Season \ Age	14 th Day	28 th Day	42 nd Day
Pre-monsoon (March to May)	1.74 ^A ±0.07	1.81 ^A ±0.03	2.16 ^A ±0.06
Monsoon (June to September)	2.16 ^A ±0.09	4.16 ^B ±0.07	7.12 ^B ±0.03
Post-monsoon (October to November)	1.75 ^A ±0.05	1.83 ^A ±0.04	2.17 ^A ±0.06
Winter (December to February)	4.39 ^B ±0.08	3.52 ^C ±0.07	2.48 ^A ±0.05

Means bearing different capital letters among rows within a column differ significantly ($p \leq 0.05$).

Table 2: Season wise mean serum cortisol level (ng/ml) of broiler chicken at 14th, 28th and 42nd day of age under different agro-climatic zones of Assam

Season \ Age	14 th Day	28 th Day	42 nd Day
Pre-monsoon (March to May)	1.05 ^{Aa} ±0.002	1.08 ^{Aa} ±0.003	1.10 ^{Aa} ±0.003
Monsoon (June to September)	1.73 ^{Ba} ±0.004	2.40 ^{Ba} ±0.008	2.92 ^{Ba} ±0.004
Post-monsoon (October to November)	1.05 ^{Aa} ±0.003	1.07 ^{Aa} ±0.003	1.10 ^{Aa} ±0.002
Winter (December to February)	1.76 ^{Ba} ±0.006	1.07 ^{Aa} ±0.009	1.83 ^{Aa} ±0.003

Means bearing different capital letters among rows within a column differ significantly ($p \leq 0.05$). Means bearing different capital letters among rows within a column differ significantly ($p \leq 0.05$).

Conclusion

In current investigation, it was observed that the HSP-70 and Cortisol level in blood was found to be higher in adult broiler (42 days) during monsoon season due to high environmental temperature and consequent thermal stress on birds.

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