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Characterization of Hilly Chickens in Consideration of Climate Change Factors: Light and Heat

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ABSTRACT

Hilly chickens were characterized from January 2015 to September 2016 considering climate factors (light and heat). The experimental birds were divided into three groups (heat stress; light and control). The heat was generated in the chicken's shelter by a black shaded light (Lantern) for two to three hours more after sunset. A lighting device (Lantern) was kept in the chicken's shelter for the same period for increasing daylight duration. The individual chicken's egg production, egg weight and mature live weight were studied from onset of egg production. It was observed that reddish brown hilly type chicken produced more (100.80 no/year/chicken) and larger sized (43.21g) eggs than spotted chicken (83.4 no/year/chicken and 40.46g). Among the three treatments, the lighting group produced 7.71 and 5.60 more eggs than the control and heat stress group, and one hour lighting lengths increased egg production 4 to 5.8%. Fifty-five blood samples were collected, and DNA was isolated from whole blood. For genetic characterization, 10 microsatellites markers from FAO recommendation list were used in this study. PCR amplification was performed in an MJ PTC-200 Peltier Thermal Cycler. The gene flow among breeds and genetic differentiation was assessed by computing between-breed genetic parameters: molecular co-ancestry (fij). It was found that genetic diversity of these two types of chickens was moderate. Results indicated that they were originated from the same ancestor. Therefore, priority should be given for implementation of appropriate breeding programme and strategies are necessary to avoid loss of genetic diversity.

INTRODUCTION

The effects of heat stress on broilers and laying hens are reduced growth and egg production with decreased poultry meat and egg quality. Locus *et al.,* 2013; and Star *et al.* (2008) reported a reduction of 31.6% in feed conversion, 36.4% in egg production, and 3.41% in egg weight in laying hens subjected to heat stress. Similar findings were reported by Deng *et al.* (2012) for heat stress on laying hen. Daylight has positive effects on feed intake and egg formation, which leads to the increase live weight and more eggs (Nayak et al., 2015). However, in Bangladesh, there are still some remote areas where no electricity supply is available.

The phenotypic performance studies (Khan *et al.,* 2004, Khan *et al.,* 2007, Faruque *et al.,* 2010) suggested that the hilly chickens are comparatively better than other native chickens. Although the genetic characterization study using microsatellite marker of different native chicken's populations are available in the literature (Fonteque *et al.,* 2014, Nedup *et al.,* 2012, Zanetti *et al.,* 2010) however, these literatures lack information on hilly chickens of Bangladesh.

The genetic diversity of chicken genetic resources provides the basis for genetic improvement in order to increase productivity (Tixier-Boichard *et al.,* 2009, Boettcher *et al.,* 2010). Moreover, the understanding of the constitution of breeds, effective management and traceability of breed origin is needed for potential utilization of the genetic resources (Dalvit *et al.,* 2007, Nakamura *et al.,* 2010). Therefore, the current study was conducted with the objectives to characterize hilly chickens in consideration of climate change factors and to select the best genotypes of hilly chickens for further genetic improvement.

MATERIALS AND METHODS

The research work was conducted at the Animal Genetics/Poultry Research and Training Centre (PRTC) laboratory at Chittagong Veterinary and Animal Sciences University and the Chittagong Hill Tract (CHT), Bandarban district of Bangladesh from January 2015 to September 2016. The chickens and farmers were categorized per the phenotypic and morphological features of the chickens. The chosen hilly chickens were identified and kept in the farmers` households. The selected chickens were maintained by providing supplementations of concentrate feed (50g/chicken/day) containing 2700 kcal/kg and 15.5 percent crude protein. The feed ingredients used were broken corn (38%), broken wheat (26.50), rice polish (16%), soybean meal (14%), oyster shell (5%) and salt (0.5%). These feed ingredients were selected by allowing the chickens to scavenge the surrounding of the farmer's house where they pickup their required feed. If there were any deficiencies of energy, protein, calcium and phosphorus that were filled up from the supplements. The supplementary feeds were supplied to the farmers twice in a month, from the commencement up to the end of the study. The farmers offered these feed ingredients twice a day (morning and afternoon) to the experimental chickens in a temporary bamboo made fenced guard. For the remaining period the chickens were allowed to scavenge the surroundings of the farmer's house. The selected farmers were trained for poultry rearing in two months interval.

The selected chickens were divided into three treatment groups (heat stress, light and control). The egg production potential under these treatments were studied from April to September. Fifteen farmers were selected for each treatment group and each treatment having 30 layer chickens. The average temperature during April to September was 27°C from April to September. The heat was generated in the chicken's shelter by providing a black shaded light (Lantern) for 2 to 3 hours more after sunset and the temperature was increased by 2°C. A lighting device (Lantern) was kept in the chicken's shelter for 2 to 3 hours more after sunset in order to increase the duration of daylight. The control group was maintained for posterior comparison of the effects of heat stress and light. For light and control group the normal ambient temperature was maintained.

For all treatment groups, from the onset of egg production, the egg production and other traits of the individual chickens were recorded and monthly egg production, yearly egg production, mature live weight, egg weight and clutch size were calculated. More than 40 weeks aged chickens were considered as mature chicken.

For genetic characterization, 40 blood samples from both hilly type chickens under three treatment groups (control, light and heat stress) were collected from the jugular vein in a microtube containing 0.5 M EDTA (as an anticoagulant). DNA was isolated followed by a protocol of PureLink® Genomic DNA Kit from whole blood samples. Extracted DNA samples were stored at -20°C until subsequent use as a template for Polymerase Chain Reaction (PCR).

Ten microsatellite markers from the list of recommended microsatellites for chicken by the ISAG/FAO were used to amplify the microsatellites regions in the chickens' genome (Table 1). A total of 25 µl polymerase chain reactions (PCR) composed of 12.5 µl Supermix, 2.5 µlM primers (forward and reverse), buffer 5 µl and 2.5 µl DNA template were prepared (Invitrogen™). The PCR amplification was conducted in an MJ PTC-200Peltier Thermal Cycler or a Bio-Rad C 1000 Thermal Cycler with initial denaturation at 94°C for 5 minutes followed by 40 cycles of denaturation at 94°C for 30 s, annealing for 30 s at the optimized temperature (Amie Marini *et al.,* 2014), and extension at 72°C for 30 s, and a final extension at 72°C for 10 minutes. The PCR products were electrophoresed on 2% agarose gel (Lonza USA) at 90 V for 1.5 to 2 h and stained with ethidium bromide. The microsatellite banding patterns were visualized on a UV transilluminator and photographed.

Statistical analysis

The total number of alleles, average number of alleles per locus across breeds and genetic diversity of the studied breeds was assessed by computing the observed (H_o) and expected (H_E) heterozygosity within population between breeds. Furthermore, the diversity

Table 1 – Microsatellite markers and their sequence with corresponding fragments size, average number of alleles and annealing temperature/sec.

Sl. No.	Primer	Sequence	Fragments size (bp)	Number of alleles	Annealing Temp.(°C)
1	ADL0112	GGCTTAAGCTGACCCATTAT	120 to 134	1	60
2	ADL0268	CTCCACCCCTCTCAGAACTA	102 to 121	2	58
3	MCW0014	TATTGGCTCTAGGAACTGTC	166 to 189	2	62
4	MCW0034	TGCACGCACTTACATACTTAGAGA	212 to246	2	62
5	MCW0069	GCACTCGAGAAAACTTCCTGCG	158 to 176	1	60
6	MCW0081	GTTGCTGAGAGCCTGGTGCAG	143 to 155	2	60
7	LEI0166	CTCCTGCCCTTAGCTACGCA	251 to 261	1	58
8	LEI0094	GATCTCACCAGTATGAGCTGC	251 to 283	2	58
9	MCW0248	GTTGTTCAAAAGAAGATGCATG	213 to 245	1	62
10	MCW0295	ATCACTACAGAACACCCTCTC	86 to 102	2	58
Average				1.6±0.52	59.8±1.75

Source: (FAO, 2007; Nassiri, 2007 and Zanniti et al. 2010).

was calculated by the polymorphic information content (PIC) according to Botstein *et al*. (1980). The gene flow among breeds and genetic differentiation was assessed as Caballero and Toro, (2002) by computing the following between-breed genetic parameters: molecular co-ancestry (fij).

Least square means were estimated for all the parameters according to treatments and chicken types were analyzed by Proc GLM of SAS (2008). The mean value was compared using the Duncan Multiple Range Test (DMRT) at 5% level of significance.

RESULTS AND DISCUSSION

Phenotypic performance of hilly chickens

The performance of hilly chickens in three different treatment groups is presented in Table 2. Reddish brown hilly type chicken laid longer period (clutch size) and larger sized egg than the spotted hilly type chicken in all treatments. In addition, egg production, body weight and egg weight of reddish brown hilly were better for artificial lighting group than heat stress and control group. Similar findings were observed by Lucas *et al*. (2013) and Deng *et al.* (2012). Egg weight of both type of hilly chickens was ranged from 40.46 to 43.85g. The mature live weight of reddish brown type chicken was higher under lighting condition than spotted hilly type chicken. These findings were similar to Khan *et al.* (2007, 2017).

The mortality of the chickens was 13 to 23 percent. The mortality of birds was higher in heat stress group than others (Table 2), the extra percentage of mortality compared to the other group was due to heat stress. In addition to heat stress, the causes of mortality in all groups were the outbreak of diseases: Fowl pox and predator attack in chicken rearing. The hilly people did not treat their diseased chickens. Once in a year the chickens in hilly areas are vaccinated against New castle disease by the Department of Livestock Services (DLS) of the Government of Bangladesh and also by the village poultry workers but this is not regular. Predator problems are common as the housing is near or within the hills and most of the hills are succumbed with predators like fox, wild cat, mongoose etc. Both types of chicken showed broodiness and nesting behavior and hatched their chicks.

Table 2 – Performances of different types of hilly chickens under three treatments.

Parameters	Treatment group						SEM	*p* value
	Control		Heat stress		Light			
	RB	SBW	RB	SBW	RB	SBW		
Egg production (no/Mo/bird)	7.89^{a}	6.52^{b}	7.96^{a}	6.69^{b}	8.56^{a}	6.83^{b}	0.354	0.047
Egg production (no/year)	94.66^{a}	78.24^{b}	95.52^{a}	80.28^{b}	102.72^{a}	81.96^{b}	4.315	0.021
Egg weight (g)	41.52^{b}	41.63^{b}	40.46^{c}	41.77^{ab}	43.19^{a}	43.21^{a}	0.531	0.001
Live weight (g)	1454.58^{b}	1366.15^{bc}	1409.99^{b}	1392.26^{b}	1483.13^{a}	1412.55^{c}	22.45	0.016
Clutch Size (d)	11.07^{ab}	12.03^{a}	10.87^{ab}	8.99^{c}	11.76^{a}	9.98^{b}	1.307	0.001
Mortality (%)	18.70^{b}	15.15^{b}	19.80^{a}	22.87^{a}	17.93^{b}	13.20^{b}	2.562	0.025

Legends: RB= Reddish Brown; and SBW= Spotted Black and White

Means with different superscripts are different at 5% level of significance (in column)

SEM= Standard error of mean.

Breed variability and differentiation

The genetic variability of each type of hilly chicken under the three treatments observed (H_O) and expected (H_E) heterozygosity, molecular coancestry (fij) and polymorphism information content (PIC) are shown in Table 3. The Ho and H_E values of Reddish brown as ranged from 0.67 (light) to 0.81(control) and 0.43 (Heat) to 0.57 (Light), respectively. For the spotted black and white the Ho and H_E values were as 0.75(Heat) to 0.88 (Control) and 0.33(Control) to 0.55 (Heat), respectively. From this study, it can be concluded that the observed values were higher than expected for all treatments and chicken types. The high number of monomorphic loci detected may explain the low number of heterozygotes. Values of H_O was higher than in other studies; however, the values of H_O were similar to the findings of Zanetti *et al*. (2010). These findings indicated loss of genetic diversity.

Table 3 – Expected (H_E) and observed (H_O) heterozygosity, within-breed molecular coancestry (f_{IJ}) for each type analyzed and polymorphic information content (PIC).

Types	Treatment groups											
	Control				Heat stress				Light			
	H_o	H_E	PIC	f_{ij}	H_o	H_E	PIC	fij	H_o	H_E	PIC	f_{ij}
Hilly RB	0.75	0.50[b]	0.63[a]	0.15	0.81	0.43	0.46[a]	0.12[a]	0.67[a]	0.57[b]	0.75	0.19
Hilly SBW	0.88	0.33[a]	0.86[b]	0.22	0.75	0.50	0.86[b]	0.22[b]	0.87[b]	0.34[a]	0.63	0.15

Legend: RB= Reddish Brown; and SBW= Spotted Black and White

The superscript a and b indicate significant differences between genotypes at 5% level of significance.

The within-breed molecular coancestry (fij) values for hilly reddish brown type chicken varied from 0.12 to 0.19 and for hilly spotted white chicken varied from 0.15 to 0.22. This value indicated that these two types of hilly chickens have a minimum genetic differentiation. A similar finding was reported by Tadano *et al.* (2007) for native Japanese poultry breeds and in contrast with lower genetic differentiation values found in 8 Finnish chicken breeds by Vanhala *et al.* (1998) and Monira *et al.* (2011) of a Bangladesh study. The genetic diversity of the two hilly types was also assessed by calculating the polymorphic information content (PIC) values. PIC values vary from 0.46 to 0.75 for reddish brown type chicken and 0.63 to 0.86 for spotted white type chicken, respectively. These PIC values indicated the moderate diversity.

Among the two types of hilly chickens, the reddish brown type produced more eggs than spotted black and white. In addition to egg production, other performance of reddish brown chicken was better than spotted black and white chicken and their genetic diversity was low to moderate. Therefore, the selection of reddish brown type hilly chickens will be the better option for the incorporation of further genetic improvement programs.

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