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Performance differences of Rhode Island Red, Bashang Long-tail Chicken, and their reciprocal crossbreds under natural cold stress

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Objective: The Bashang Long-tail chicken (BS), an indigenous Chinese breed, is considered cold tolerant. We selected BS, the Rhode Island Red (RIR), and their reciprocal crossbreds for the present study. The objectives were: i) to validate whether BS is cold tolerant and whether egg production and cold tolerance of crossbreds could be improved; and ii) to determine the physiological characteristics that underlie cold tolerance and favorable egg production performance in cold environments.
Methods: A total of 916 chickens were reared in warm and natural cold environments (daily mean ambient temperature varied from 7.4°C to 26.5°C in the warm environment and from –17.5°C to 27.0°C in the cold environment). To investigate their adaptability to the cold environment, the egg production performance and body weight were monitored and compared between breeds and environments. The cloacal temperature and serum biochemical parameters were monitored to reveal the physiological characteristics underlie cold tolerance and favorable egg production performance in the cold environment.
Results: The warm environment experiment showed that RIR had the highest egg production performance, and that the reciprocal crossbreds had a higher egg production performance than BS. While in the cold environment RIR had the lowest egg production performance, and the reciprocal crossbreds had a higher egg production performance than BS. In the cold environment BS and reciprocal crossbreds had higher triiodothyronine, tetraiodothyronine levels than RIR. At 35 and 39 wk of age, when the ambient temperature was extremely low (varied from –20°C to 0°C), serum glucose, follicle-stimulating hormone, luteinizing hormone, estradiol of BS and crossbreds were higher than RIR.
Conclusion: Bashang Long-tail chicken has a favorable cold tolerance ability. Crossbreeding with RIR and BS is an effective way to develop cold tolerant chickens with improved egg production performance.

Keywords: Cold Stress; Serum Biochemical Parameters; Cloacal Temperature; Egg Production Performance; Bashang Long-tail Chicken; Rhode Island Red

INTRODUCTION

Birds have a higher body temperature compared to mammals, which makes them more vulnerable to cold environments [1,2]. Constantly increasing requirements for animal welfare and for the production of high quality animal food have resulted in free-range chicken production systems becoming more widespread [3]. However, modern high performance commercial strains, which have been selected under specific conditions, do not adapt well to realistic environments [4,5]. In natural environment, cold stress is one of the prominent challenges, especially in cold regions. Previous studies have shown that cold stress affects the development, health and welfare of chickens [6-8]. Frequently, climate events, like extreme low temperature weather conditions, always cause great economic losses [6,9]. Thus, there is a need to develop cold tolerant chickens for regions that experience extreme cold.

The Bashang Long-tail chicken (BS), found in northern China, is considered to be a cold tolerant breed [10], as it is adapted to the climate and geography of the Bashang region (41°14′-41°56′N, 114°50′-116°04′E; average altitude of 1,418 m, mean annual temperature of 1.9°C). Another remarkable feature of BS is favorable egg-laying performance under the natural environmental conditions of that region (about 150 eggs/hen per year according to local records). These are the main reasons that BS was selected for the present study.

Previous studies have reported that White Leghorns crossed with Bedouin fowl (a breed that is adapted to desert conditions and can withstand both hot summers and cold winters) can produce chickens that are similarly well adapted [6]. Other studies report that Fayoumi, a heat tolerant breed, can be crossed with other breeds to produce crossbreds with improved adaptation and fitness traits [11]. Thus, crossing BS with a commercial line to produce cold tolerant high performance laying chickens might be feasible. The objectives of the present study were to: i) validate whether BS is cold tolerant and whether egg production performance and cold tolerance of the crossbreds could be improved; and ii) determine the physiological differences that underlie cold tolerance and favorable egg production performance in cold environments.

MATERIALS AND METHODS

Animal care

The animal care protocol used in the present study was approved by The Animal Welfare Committee of China Agricultural University (permit number: DK996).

Animals

We first obtained 120 BS chickens (20 males+100 females; 14 wk of age) from a poultry conservation farm in the Bashang region, northern China. Another group of 110 RIR chickens (20 males+90 females; 14 wk of age) were obtained from a local layer breeding corporation in Beijing, China. To obtain the sample population of BS, RIR, and their reciprocal crosses (RMBF, RIR males cross BS females; RFBM, RIR females cross BS males), the females of each breed were evenly and randomly assigned to two groups (2 groups/breed×2 breeds = 4 groups), and reared in individually housed stair-step cages at the Zhuozhou Farm facility of China Agricultural University. When the laying rate of BS and RIR reached 60%, the two groups of females of each breed were artificially inseminated with the semen of either BS or RIR (once every four days). Eggs were collected for 14 consecutive days and stored at 20°C. These eggs were then hatched at the farm. We obtained more than 450 birds/group on May 1, 2015. After being hatched, the chicks were artificially sexed and individually marked with numbered wing-bands. Birds were kept together during brooding and in rearing cages from the time of hatching to 14 wk of age. The lighting and feeding programs were followed the farm management guide for RIR. Briefly, nutrient requirements were followed recommendations of the NRC [12].Start pullets with 20 to 22 hours of continuous and 30 lux light during the first week of age, after which it was reduced to 10 lux and was reduced weekly to reach 10 hours at 10 weeks of age. From 17 weeks of age, the light was gradually increased in increments of 5 lux and 1 hour per week to 20 weeks of age.

For the adult birds of the four groups (BS, RIR, RMBF, and RFBM) used in the present study, the basic morphological information was provided here. Most of the BS birds have a yellow body with a black tail; the other colors found in BS include brown, black, white and speckled. The BS cock has a long tail feather which length is about 40 to 50 cm. The eggshell color of BS is brown and egg weight is about 54.2 g. Adult BS female weight is 1,645.6 g (22 wk of age). For RIR, the bird's feathers are rust-colored. The eggshell color of RIR is brown and egg weight is about 60.5 g. Adult RIR female weight is 1,699.3 g (22 wk of age). For the two crossbreds, most of the birds have yellow and brown feathers, the other colors found include black, white and speckled. The eggshell color of the crossbreds is brown and egg weight is about 57.5 g. Adult female weight of the crossbreds is 1,686.3 g for RMBF and 1,673.3 g for RFBM (22 wk of age).

Experimental design and treatment

We designed the study as two parts, a warm environment experiment (used as a control) and a cold environment experiment. The purpose of the warm environment experiment is to investigate egg production performance of the four groups (BS, RIR, and their reciprocal crossbreds) under optimal conditions. While the cold environment experiment was set to test the egg production performance changes of the four groups compared to their counterparts under the warm environment, which can indicate the cold tolerance of the four groups. Moreover, for the four groups under the cold environment, their serum biochemical parameters, cloacal temperature (Tc) and body weight (BW) were monitored to find the underlying physiological characteristics of the four groups.

The cold environment experiment was conducted under conditions of a natural cold environment, from September 1, 2015 to January 29, 2016, during the autumn and winter seasons in Beijing, China. Four groups of chickens (BS, RIR, RMBF, RFBM) were reared from 18 to 39 weeks of age for this study. On September 1, 2015, 102 healthy 18-wk-old females of each group were randomly selected and transferred to 12 identical half-open sheds (0.17 m2/bird, 34 chickens/shed, 3 replicates/group; natural cold environment) at an ecological ranch in the Yanqing County of Beijing (40°26′N, 116°05′E, average altitude of 520 m) with *ad libitum* access to water and feed. All sheds (2×3 m) included a patch of sandy ground (2×1 m) and were individually enclosed by woven wire fencing and equipped with perches (18 cm/bird), nests (1 per 6 hens), nipple drinkers, a feeder, a feed silo, and slatted floors (2×2 m, suspended 0.5 m above the ground). A

conveyor belt running along the back of the nests collected the eggs. Another four groups of birds (n = 102, 18-wk-old) were reared in individually housed stair-step cages (688 cm^2/bird), under standard housing conditions (warm environment), at the Zhuozhou Farm facility of China Agricultural University. Adjacent hens (34 birds) were set as a replicate, so there were three replicates per group in the warm environment. Under both environments, all birds received 16 h of light per day. The daily ambient temperature (Ta) range was recorded with maximum minimum centigrade thermometers (TFA Dostmann, Reicholzheim, Germany; ±1°C), and no differences were found between sheds at any given time. The daily mean Ta was calculated as the mean of the daily maximum and minimum temperatures (daily mean Ta varied from approximately 7.4°C to 26.5°C in the warm environment and from –17.5°C to 27.0°C in the natural cold environment). Relative humidity was recorded with digital hygrometers (TFA Dostmann, Germany), and no differences existed between sheds at any given time (in the warm environment: approximately 28% to 85%; and in the natural cold environment: approximately 19% to 92%).

Egg production performance

The daily egg-laying performance was recorded. The weekly laying rate was calculated as follows:

$$\text{Weekly laying rate} = \frac{\text{Total number of eggs produced during 7 days}}{\text{Total number of hen - days in the same period}} \quad (1)$$

Sampling and parameters measurement

The BW, Tc, and serum biological parameters (glucose [GLU], triglycerides [TRI], triiodothyronine [T3], tetraiodothyronine [T4], follicle-stimulating hormone [FSH], luteinizing hormone [LH], and estradiol [E2]) of the birds reared under the natural cold environment were monitored. The BW was monitored throughout the study with 20 birds weighted every time. Tc was measured at 22, 27, 31, 34, 36, and 39 wk of age with 20 birds measured every time. At 20, 27, and 31 wk of age, serum biological parameters were measured with 12 birds were measured every time; while at 35 and 39 wk of age, 25 birds were measured to increase statistical power. To minimize disturbances, all sampling and measurements were conducted at 19:00 h and were completed within 2 h. Birds were randomly selected from sheds for sampling and measurements. The Tc was measured with a digital thermometer (Citizen CTE502; ±0.1°C). Blood samples (3 mL) were collected from the brachial vein into plastic vacuum tubes, which were kept in an incubator over night at 37°C for clotting. Then the clot was removed by centrifugation (Eppendorf 5804R, Hamburg, Germany) and the resulting supernatant serum was carefully collected using a pipette. Serum samples (1 mL) were stored at 4°C until further analysis. The concentrations of GLU and TRI were analyzed by an automatic biochemical analyzer (RA-1000, Bayer Corp., Tarrytown, NY, USA) using colorimetric methods, following the instructions of the corresponding reagent kits (Zhongsheng Biochemical Co., Ltd., Beijing, China). Serum T3, T4, E2, FSH, and LH were tested using commercially available ^{125}I-labeled radioimmunoassay (RIA) kits (Beijing Sino-uk institute of Biological Technology, Beijing, China). Of these parameters, T3, T4, and E2 RIA kits were validated for measurements on chicken samples [13,14]; however, FSH and LH RIA kits were validated on human samples. All serum samples were analyzed within 48 h.

Statistical analysis

Data was analyzed using SPSS (SPSS for Windows Release 20.0, SPSS Inc.; Armonk, NY, USA). The egg production performance data was analyzed with two-way analysis of variance using the general linear models procedure. The main effects of rearing environment (temperature), genotype and the interaction between them were tested. The simple main effects analysis was performed to determine the mean difference in egg production performance between groups at each rearing environment, as well as between rearing environments for each group. The BW, Tc and serum biochemical parameters were analyzed using the mixed model, with a completely randomized, repeated measure design. Significant differences were determined using the least squares differences test with 5% probability.

RESULTS

Egg production performance

The weekly laying rate of the four groups reared in the warm environment is presented in Figure 1A. The peak laying rate of RIR, BS, RMBF, and RFBM were 94.8%, 76.8%, 94.5%, and 93.1% respectively. The average number of eggs produced per hen at 39 wk of age was 130.7, 91.1, 117.6, and 121.1 for RIR, BS, RMBF, and RFBM, respectively. The number of RIR eggs was higher than that of RMBF and RFBM ($p = 5.0\times10^{-4}$ and $p = 6.0\times10^{-3}$ respectively), which in turn were higher than that of BS (both $p<1.0\times10^{-4}$; Table 1).

The weekly laying rate of the four groups reared in the natural cold environment is presented in Figure 1B. The peak laying rate of RIR, BS, RMBF, and RFBM were 67.1%, 73.8%, 87.5%, and 80.4%, respectively. The average number of eggs produced per hen at 39 wk of age was 57.7, 70.5, 84.16, and 78.62 for RIR, BS, RMBF, and RFBM, respectively. The egg number of RMBF and RFBM was higher than that of BS ($p = 3.0\times10^{-4}$ and $p = 1.6\times10^{-2}$ respectively), which in turn were higher than that of RIR ($p = 0.0011.0\times10^{-3}$, Table 1).

As shown in Figure 1, it is evident that the cold environment adversely affected egg production performance. During the whole experimental period, the average number of eggs produced by each group reared in the natural cold environment was lower than that reared in the warm environment ($p<1.0\times10^{-4}$ for all groups). The interactions of environment by genotype have a

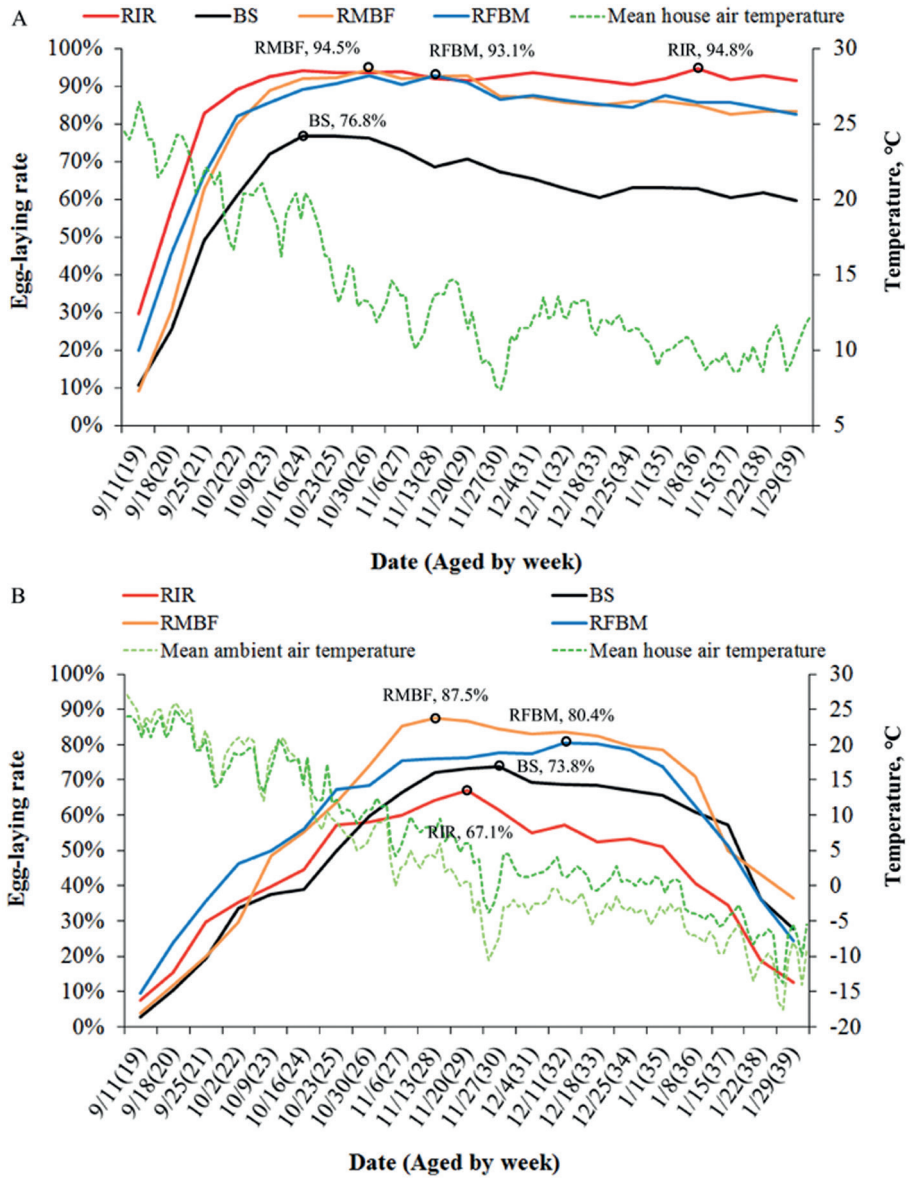

Figure 1. Weekly laying-rate of the four groups reared in the warm and natural cold environments. (A) Weekly laying-rate in the warm environment. (B) Weekly laying-rate in the natural cold environment. Circled points represent the peak laying-rate of each group. X-axis: date (aged by week). RIR, Rhode Island Red; BS, Bashang Long-tail chicken; RMBF, RIR males cross BS females; RFBM, RIR females cross BS males.

statistically significant effect on the egg production performance ($p<1.0\times10^{-4}$, Table 1). The results also showed that the egg production performance of the crossbreds was improved under conditions of natural cold environment.

Body weight

The BW changes between groups reared in the natural cold environment are presented in Figure 2. Before 34 wk of age, BW of each group increased gradually and no significant differences

Table 1. Egg production performance and number of dead birds of the four groups during the whole experimental period under both cold and warm environments

	Cold				Warm				p-value		
	BS	RIR	RMBF	RFBM	BS	RIR	RMBF	RFBM	E	G	E×G
Egg No.	70.5^{B}	57.7^{C}	84.16^{A}	78.62^{A}	91.1^{C}	130.7^{A}	117.6^{B}	121.1^{B}	<0.01	<0.01	<0.01
Egg mass/hen (g)	3,823.9^{B}	3,488.0^{C}	4,839.2^{A}	4,523.8^{A}	4,941.3^{C}	7,900.8^{A}	6,762.0^{B}	6,968.1^{B}	<0.01	<0.01	<0.01
Dead birds No.	3	3	2	4	0	1	0	0	Na	Na	Na

BS, Bashang Long-tail chicken; RIR, Rhode Island Red; RMBF, RIR males cross BS females; RFBM, RIR females cross BS males; E, environmental effects; G, genotype effects; E × G, interactions of environment by genotype; Na, not available.

$^{A-C}$ Means within each parameter at the same time with different superscript letters are significantly different ($p<0.01$).

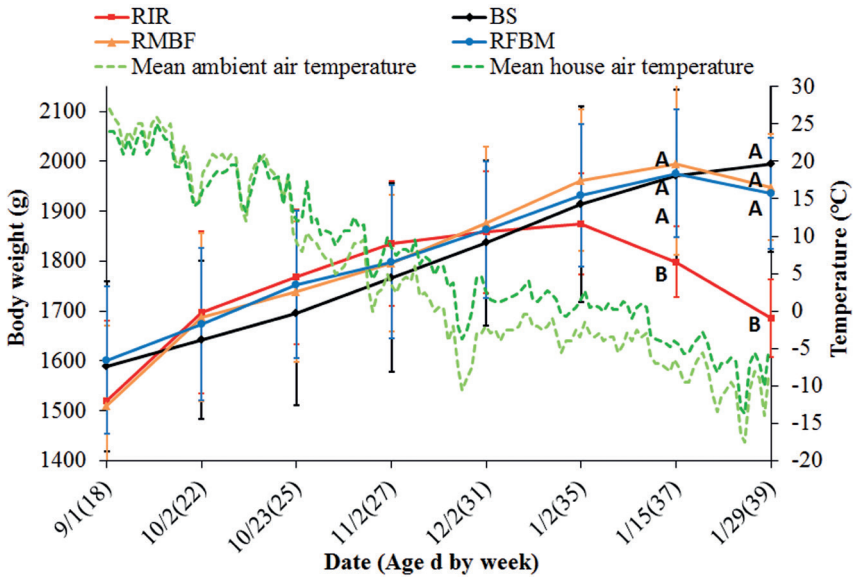

Figure 2. Body weight (mean±standard deviation) of the four groups reared in the natural cold environment. A, B at the same time point, different letters indicate significant difference ($p<0.01$). X-axis: date (aged by week). RIR, Rhode Island Red; BS, Bashang Long-tail chicken; RMBF, RIR males cross BS females; RFBM, RIR females cross BS males.

were observed between groups. However, at 37 and 39 wk of age, when the Ta was below –5°C (varied from –17.5°C to –5°C), the BW of RIR declined and fell to a value that was lower than that of the other three groups ($p<1.0\times10^{-4}$).

The mean BW of RIR at 37 wk of age (1,798.2 g) was lower than that at 34 wk of age (1,875.7 g, $p = 1.0\times10^{-3}$), and that at 39 wk of age (1,685.2 g) was lower than that at 37 wk of age ($p = 1.0\times10^{-4}$). The BW of crossbreds was also reduced at 39 wk of age, but was not significantly lower than that at 37 wk of age ($p = 0.15$ for RMBF and 0.13 for RFBM). BS was the only breed to show no reduction in BW and kept steadily increasing throughout the entire experimental period.

Cloacal temperature

Variations in Tc between groups reared in the natural cold environment are presented in Figure 3. As the Ta decreased, the Tc of BS showed a reduction from 22 to 34 wk of age ($p<1.0\times10^{-4}$). The Tc of RMBF and RFBM showed a slight reduction and that of RIR showed a slight increase throughout the experimental period.

Serum biochemical parameters

Table 2 and Table 3 show the effects of group, time and the interactions between them on serum biochemical parameters in the natural cold environment. T3 and T4 levels of BS and the cross-

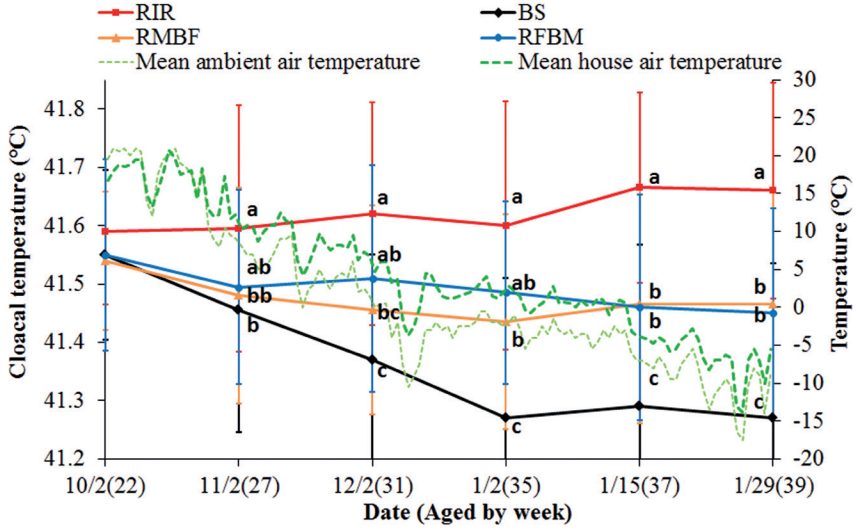

Figure 3. Cloacal temperature (mean±standard deviation) of the four groups reared in the natural cold environment. [a-c] The same time point, different letters indicate significant differences between groups ($p<0.05$). X-axis: date (aged by week). RIR, Rhode Island Red; BS, Bashang Long-tail chicken; RMBF, RIR males cross BS females; RFBM, RIR females cross BS males.

Table 2. p-Value for the influence of group, time and their interactions on biochemical parameters in the natural cold environment

Item	GLU	TRI	T3	T4	FSH	LH	E2
Group[1)]	0.14	0.61	0.00	0.00	0.002	0.001	0.09
Time[2)]	0.00	0.00	0.997	0.00	0.00	0.003	0.00
Group × time	0.002	0.03	0.52	0.002	0.00	0.004	0.03

GLU, glucose; TRI, triglycerides; T3, triiodothyronine; T4, tetraiodothyronine; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, estradiol; RIR, Rhode Island Red; BS, Bashang Long-tail chicken; RMBF, RIR males cross BS females; RFBM, RIR females cross BS males.
[1)] Groups: RIR, BS, RMBF, and RFBM.
[2)] Time: 20, 27, 31, 35 and 39 wk of age.

breds were higher than those of RIR at 27, 31, 35, and 39 wk of age (Table 3, $p<0.05$). GLU, FSH, LH, and E2 levels of BS and the crossbreds were higher than those of RIR at 35 and 39 wk of age (Table 3, $p<0.05$). Levels of TRI were comparable between groups throughout the study.

During the whole experimental period, the total number of dead birds of each group under both environments are shown in Table 1.

DISCUSSION

Cold environments can dramatically affect health, performance and welfare of chickens. Over the past few decades, most breeding programs have only considered the economically relevant traits and have generally ignored the fitness related traits [4]. On the other hand, there is a drastic difference between the environment of selection and that of production. In reality, animals face acute heat or cold exposure, gradual seasonal changes in temperature, and changes in diurnal temperature cycles. Thus, their ability to adapt to such environmental conditions has become an important issue. As results, many problems about production performance and welfare arose in the actual production environment [15]. In the present study, we examined the egg production performance of RIR, BS, and their reciprocal crossbreds. The results show that cold stress negatively affects egg production performance, a finding that is consistent with previous studies [6]. Under the natural cold environment, BS was the only breed to show no reduction in BW and kept steadily increasing throughout the entire experimental period, which suggests RIR is more vulnerable to cold environments. It also shows that the egg production performance of the crossbreds was higher in the natural cold environment. This suggests that crossing RIR with cold tolerant BS is an effective way to improve egg production performance in natural cold environments. Similarly, previous studies on Bedouin and Fayoumi strains have demonstrated that their crosses with commercial lines effectively improved performance of the crossbreds in suboptimal environmental conditions [6].

Homeothermic animals develop adaptive modifications to cope with Ta fluctuations that include adjustments in metabolism, insulation, and behavior [16]. Previous studies in birds have demonstrated that lower critical temperatures are reduced and metabolism is increased, as Ta decreases. The present results showed that when Ta fell below a threshold of –5°C, the BW of RIR was significantly reduced, and that of the crossbreds was just

Table 3. Serum biochemical parameters of the four groups reared in the natural cold environment (mean±SD)

Age (date)	Group	GLU (mmol/L)	TRI (mmol/L)	T3 (ng/mL)	T4 (ng/mL)	FSH (mIU/mL)	LH (mIU/mL)	E2 (pg/mL)
20 wk (1 Oct)	RIR	11.42±1.40	9.44±0.94	0.54±0.11	23.45±7.29^{b}	6.66±1.02^{ab}	11.24±1.53	233.08±30.04
	BS	10.94±1.07	9.49±0.52	0.54±0.11	25.52±5.90^{ab}	7.39±1.45^{a}	11.60±1.34	228.89±27.67
	RMBF	10.65±1.17	9.19±1.22	0.59±0.12	27.80±6.74^{a}	6.02±0.90^{b}	10.78±1.76	208.51±16.74
	RFBM	10.76±1.21	9.27±1.21	0.53±0.07	24.03±5.20^{b}	6.84±1.28^{ab}	10.23±1.32	196.93±21.58
27 wk (6 Nov)	RIR	12.30±1.25	6.48±0.15	0.45±0.07^{b}	11.69±2.53^{c}	6.35±0.64	12.63±1.429	419.20±113.21
	BS	11.84±1.26	6.62±0.09	0.59±0.11^{a}	24.68±5.30^{a}	6.43±1.03	13.75±1.459	397.22±85.08
	RMBF	12.02±0.74	6.55±0.11	0.58±0.11^{a}	20.98±5.45^{ab}	6.06±0.98	13.31±2.157	408.79±93.07
	RFBM	11.76±1.14	6.05±0.93	0.58±0.11^{a}	19.76±6.86^{b}	6.44±1.52	12.96±2.896	400.33±109.68
31 wk (4 Dec)	RIR	10.96±1.64	5.17±0.19	0.47±0.19^{b}	14.69±3.30^{c}	5.65±0.69	11.41±1.57	349.85±45.98
	BS	12.07±1.40	5.22±0.14	0.60±0.20^{a}	23.92±4.54^{a}	5.73±0.81	12.43±2.50	338.74±89.02
	RMBF	11.00±1.85	5.10±0.18	0.58±0.22^{a}	19.52±4.73^{b}	6.50±0.97	12.25±1.80	325.91±85.45
	RFBM	11.55±2.07	5.02±0.23	0.58±0.16^{a}	18.68±3.21^{b}	5.80±1.27	12.19±1.64	341.23±70.35
35 wk (1 Jan)	RIR	10.86±1.21^{c}	5.28±0.84	0.41±0.15^{b}	20.33±5.34^{c}	5.00±1.51^{b}	8.08±2.27^{b}	261.01±41.55^{b}
	BS	13.05±1.79^{ab}	5.43±0.56	0.65±0.21^{a}	30.88±7.68^{a}	6.52±2.56^{a}	15.14±11.41^{a}	266.47±81.27^{ab}
	RMBF	12.52±1.75^{b}	5.64±0.19	0.63±0.19^{a}	25.21±6.53^{b}	8.17±3.42^{a}	15.03±9.95^{a}	305.11±82.12^{a}
	RFBM	13.76±1.56^{a}	5.62±0.26	0.55±0.15^{a}	22.49±5.66^{bc}	6.14±2.52^{ab}	11.37±5.82^{ab}	314.72±69.18^{a}
39 wk (29 Jan)	RIR	11.83±1.02^{b}	8.43±0.192	0.46±0.07^{c}	19.79±3.32^{c}	6.19±0.98^{b}	9.00±1.85^{c}	204.62±48.60^{b}
	BS	12.95±1.09^{a}	8.45±0.55	0.62±0.08^{a}	29.05±5.31^{a}	7.02±1.08^{a}	11.08±1.76^{b}	226.73±81.40^{b}
	RMBF	12.63±1.53^{a}	8.45±0.24	0.58±0.09^{ab}	28.06±5.79^{a}	7.09±1.37^{a}	12.44±2.27^{a}	299.32±81.65^{a}
	RFBM	12.92±1.27^{a}	8.46±0.22	0.55±0.06^{b}	24.25±5.82^{b}	7.15±1.20^{a}	11.69±2.39^{ab}	282.50±77.18^{a}

SD, standard deviation; GLU, glucose; TRI, triglycerides; T3, triiodothyronine; T4, tetraiodothyronine; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, estradiol; RIR, Rhode Island Red; BS, Bashang Long-tail chicken; RMBF, RIR males cross BS females; RFBM, RIR females cross BS males.
[a-c] Means within each parameter at the same time with different superscript letters are significantly different ($p<0.05$).

slightly reduced at the lowest Ta encountered in the study, whereas the BW of BS showed a steady increase. Similarly, Spinu and Degen [6] showed that cold environments significantly reduced BW in White Leghorns, but not in Bedouin fowl, a breed adapted to desert conditions. Other studies [8,17] have demonstrated that abdominal fat content is negatively affected by low environmental temperatures. In the cold environment, heat loss must be compensated with comparable levels of heat production to maintain a constant body temperature range. Therefore, reductions in BW might be due to enhanced catabolism to generate more heat. Furthermore, the feed intake of RIR was also reduced at a later stage (data not shown), and this was directly associated with the reductions in BW. Therefore, extreme cold could have an influence on the nervous system and thereby affect feed intake. In this study, the Tc of RIR was kept stable, however, it of other three groups was reduced (the Tc of BS was reduced earlier and to a much greater extent than that of RMBF and RFBM). In cold environments, a lower body temperature facilitates the minimization of heat dissipation. These results, together with egg production performance, suggest that BS was better adapted to cold environments and that the crossbreds had improved cold tolerance. During the whole experimental period, the dead birds of the four groups under both environments varied from 0 to 4, we presume that these deaths were due to poor cold tolerance or bad health status.

Birds must increase heat production as Ta decreases, a process that entails hormonal regulation [18,19]. The success with which chickens cope with cold depends on their physiological ability to respond appropriately. Thyroid hormones, including T3 and T4, reportedly regulate thermogenesis, particularly the basal metabolic rate and cold-induced thermogenesis [20,21]. Previous studies [8,22] have reported that chicken T3 and T4 levels are elevated under cold stress. Serum levels of thyroid hormones were considered to be associated with energy production [23,24]. Guo et al [25] assumed that thyroid stimulating hormone and T4 secretion are enhanced to increase the metabolic rate, as the bird adapted to colder environments. In the present study, we found that T3 and T4 levels were significantly higher in BS than in RIR, and those of crossbreds showed improvement to a different extent. This indicates that BS could generate more heat to keep body temperature stable in cold environments, and that the heat generating capacity of the crossbreds was also enhanced. At later stages, GLU levels of BS, RMBF, and RFBM were also significantly higher than that of RIR. Puvadolpirod and Thaxton [26] demonstrated that serum GLU is important for homeostasis.

The female reproductive system is comprised of the ovary and oviduct, and is regulated by the hypothalamus-pituitary-gonadal axis. The hypothalamus produces gonadotropin-releasing hormone (GnRH) that stimulates the pituitary gland to produce LH and FSH, two hormones that in turn regulate ovarian follicular growth and ovulation. The ovary produces gonadal steroids, primarily estradiol and progesterone that return to the central nervous system through the bloodstream, and provide feedback regulation of hypothalamic GnRH production and release [27]. Differences in egg-laying performance have been associated with differences in plasma levels of reproductive hormones, such as LH, FSH, and inhibins [28]. During the later stages of the present study, LH, FSH, and E2 of BS, RMBF and RFBM were significantly higher than that of RIR. The different concentrations of these hormones might represent indicators or factors responsible for the differences in egg-laying performance; nevertheless, the timing of the differences observed in the levels of these hormones was not synchronized with the changes observed in the egg-laying rate. Reproduction is a complicated process that involves many hormones, and is affected by physiological conditions and by the external environment [29]. It should be noted that the present study examined only three reproductive hormones (LH, FSH, and E2), and other hormones such as inhibin, progesterone, and GnRH were not analyzed. Some studies have reported that the effects of gonadotropins on follicular development and ovulation rate are mediated by intraovarian growth factors such as the insulin-like growth factor, bone morphogenetic proteins, and epidermal growth factor [30]. Thus, it may be possible to determine the mechanisms that favor egg-laying performance in cold environments by analyzing the levels of a greater number of reproductive hormones and indices in future studies.

In individual growth and development, the levels of biochemical parameters are in a dynamic state. These levels can be affected by age, environment changes, and physiological conditions [27]. Thus, levels of the serum biochemical parameters vary at different times. Despite the pulsatility of hormone secretion, we also observed notable variations in serum T3 and T4 levels in the present study. T3 and T4 concentrations in RIR were relatively higher at 20 wk of age, but became lower from 27 to 39 wk of age. In contrast, T3 and T4 concentrations of BS were increased to different extents from 27 to 39 wk of age. These findings might be due to breed differences, and the levels of T3 and T4 could be potential biomarkers for future selection of cold tolerant breeds.

In summary, the present study shows that BS is more cold tolerant, and could be a potential candidate for future selection and crossbreeding of cold tolerant breeds. Crossbreds of RIR and BS exhibit both favorable cold tolerance and favorable egg production performance.

IMPLICATIONS

The present study demonstrated that crossbreeding with RIR and BS is an effective way to develop cold tolerant chickens with improved egg production performance. In this study, the birds with favorable cold tolerance and egg production performance under the cold environment showed higher serum T3 and T4 levels, as well as GLU, FSH, LH, and E2 levels. By analyzing these biochemical parameters, we can investigate the cold tolerance and egg production performance of chickens under cold environments.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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