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RESEARCH ARTICLE

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Effect of mating combination and environmental factors on hatchability of chicken eggs in Tibet

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Abstract To improve the hatchability of introduced high-yield chickens in Tibet, and ameliorate the egg yield and reproduction traits of the local Tibetan chickens, four mating combinations between the Recessive White and Tibetan chicken were employed to investigate the effect of the mating combination and environmental factors on hatchability in this study. The results showed that the fertility of the eggs with Tibetan chicken as female parent was significantly lower than that with Recessive White as female parent ($P < 0.05$), while hatchability was the reverse ($P < 0.05$). The embryonic mortality of Recessive White was significantly higher by 7.95% than the Tibetan chickens, particularly during the last stage of incubation. The hatchability of combination III, whose female parent was Tibetan chicken, was higher by 20.44% than combination II, whose female parent was Recessive White. This provided a theoretical basis for a cross using the Tibetan chicken as female parent. Binary logistic regression analysis results also showed that the mating combination and environmental temperature were of importance to egg hatchability.

Keywords Tibetan chicken, Recessive White, mating combination, embryo mortality, fertility, hatchability

1 Introduction

Known as “the Ridge of the World”, Tibet, at an average altitude of 4 500 m, is marked by a distinct plateau climate with variform physiognomy (Zhong et al., 2004). There are plenty of distinct species resources in Tibet because of such an ecological environment. Tibetan chicken, as the characteristic poultry in Qinghai-Tibet Plateau, is welcomed by Tibetans for its tender, delicate and nutritious meat (Du et al., 2004). This is why intensified production of Tibetan chickens has become a preferred industry there. But Tibetan chickens cannot meet the demand of the local market because of their low egg yield and low fertility rate. Therefore, it is necessary to improve their production performance and reproductive traits (Li et al., 2005). The very low hatchability at high altitude conditions, low oxygen partial pressure (11.9 kPa), cold and dry climate in Tibet restricts the introduction and popularization of other chicken breeds. Improving the egg hatchability in Tibet is one of the critical problems in developing its poultry industry. Investigations on the effect of different mating combinations and environment temperature on egg hatchability was performed in Linzhi of Tibet, and the results are important for the development of Tibetan poultry industry in the aspects of economy and practice. It may also provide some references for analyzing cross mating improvement and environment adaptability among other chicken breeds.

2 Materials and methods

2.1 Local climate

This research was carried out at the College of Agricultural & Animal Husbandry of Tibet University, located in the southeast of Tibet. The Hengduan Mountains, Himalayas and Nianqingtanggula Mountains are located in the east, west and north of Linzhi Prefecture, respectively. This causes the special tropical and subtropical moist climate in the area. Linzhi district has an annual average rainfall range of 400 mm to 2 200 mm, annual average sunlight of 2 022.2 h, and a yearly average temperature of 8.7°C.

2.2 Collecting pedigree eggs

Four mating combinations are listed in Table 1. The Tibetan chickens used were offsprings of Tibetan chickens collected

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Table 1 Eggshell thickness, fertility and hatchability of four mating combinations

Combination code	I	II	III	IV
Mating combination	Tibetan chicken ♂× Tibetan chicken ♀	Tibetan chicken ♂× Recessive White ♀	Recessive White ♂× Tibetan chicken ♀	Recessive White ♂× Recessive White ♀
Egg thickness / mm	0.268 5a	0.287 7b	0.268 9a	0.288 1b
Fertility / %	52.61a	69.87b	49.45a	81.13b
Hatchability / %	65.56a	25.15c	45.59b	23.26c

Note: The letters in the same row denote the significant level at 0.05.

from peasants in the Linzhi district. The Recessive White chickens were purchased from Jiangsu Poultry Institute. All of the provided chickens were grown in the hatchery, and the hens which were housed in individual cages were fed under the same breeding conditions. Artificial insemination was conducted every four days, and the total of normal hatched eggs within 24 days were weighed and numbered. According to incubation stage, the eggs were sorted into two batches of 431 eggs and 429 eggs, respectively.

2.3 Incubator and environmental conditions

The incubator brand Yiai EIF/CDM8400 used was made in Qingdao, China. The temperature was maintained between 36.5°C and 37.8°C, and the relative humidity ranged from 50.5% to 61.3% in the period of incubation and from 60% to 64% in the out-hatching period. House temperature was maintained between 3.6°C and 8.7°C, and relative humidity ranged from 48% to 51%. Eggs were turned over every two hours.

2.4 Data test and statistical analysis

Eggs were weighed on the third, sixth, 12th, and 18th day after incubation and checked with egg candlers every 6 days from 15:00 to 17:00. SAS 6.12 software was employed to carry out the analysis of variance, Chi-Square test and regression analysis.

3 Results

3.1 Effect of different mating combinations on eggshell thickness, fertility and hatchability

χ^2 tests showed no significant differences in eggshell thickness, fertility and hatchability between the two batches of hatching eggs. So the two batches of the same mating combinations were integrated (Table 1).

The difference in eggshell thickness and fertility rate between different mating combinations, for the same female parent, was not significant ($P>0.05$), but for the different female parent, it was significant ($P<0.05$). The difference was higher with the Tibetan chicken as female parent than that with Recessive White.

The hatchability of the fertile eggs of combination I (65.56%) was the highest and was significantly higher than that of combination III, whose female parents were all Tibetan chicken. The hatchability of combinations II and IV, whose female parents were Recessive White, was the lowest but there was no significant difference between them.

As seen from Fig. 1, the embryonic mortality on the 3rd day was higher than on the 5th day in combinations I and III, whose female parents were Tibetan chicken, while in combinations II and IV, whose female parents were Recessive White, it was the reverse. It was a surprise that the embryonic mortality of combination IV was 10.2% higher than that of I during the later stages of incubation.

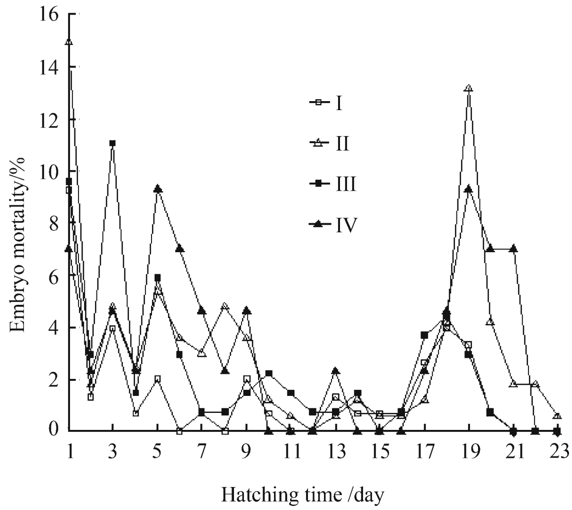

Fig. 1 Embryo mortality of four mating combinations

3.2 Effect of egg preservation time on hatchability

To study the effective mechanism of egg preservation time on hatchability clearly, the survival and hatchability of the embryos which had been incubated for six days were plotted on a graph as shown in Fig. 2.

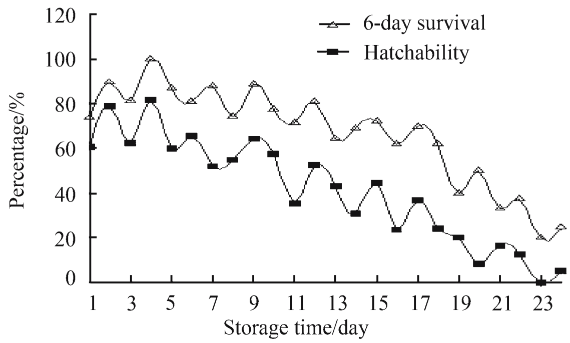

Fig. 2 Embryo survival rate and hatchability after six days of storage

Along with the preservation time being prolonged, it can be seen that the survival and hatchability of the embryos incubated for six days did not decrease in a straight line but in a curve. In addition, for the fresh eggs stored within two days, their survival and hatchability were not yet the highest on the 6th day. Data analysis demonstrated that the hatchability of fertile eggs reserved for 2–6 days reached 67.96%. This was 7.1% higher than that of the eggs reserved for 0–1 day and 9.2% higher than that of the eggs reserved for 7–9 days, indicating that it was better to reserve eggs for 2–6 days before incubation. The hatchability of eggs reserved for 13–18 days still reached 32.8%, and even if reserved for 19–24 days, the fertile eggs still had the ability to incubate, but with only 8.9% of hatchability.

3.3 Weight loss of eggs during incubation

Weight loss of eggs directly influenced the hatchability. The analysis of weight loss in two batches of eggs with different female parents and different hatching results was done in this study. The results can be seen in Table 2, Fig. 3 and Fig. 4.

Table 2 Weight loss of incubated eggs

Batches	Female parents breed	Type	Hatching result	Absolute weight loss/g	Absolute weight loss rate/%
The first batch	Tibetan chicken	1	hatched	1.114 1e	2.338 6e
		2	non-hatched	1.289 8d	2.7017bc
	Recessive White	3	hatched	1.535 8c	2.641 0cd
		4	non-hatched	1.676 6b	2.877 2ab
The second batch	Tibetan chicken	1	hatched	1.187 7dc	2.446 3de
		2	non-hatched	1.262 2d	2.629 8cd
	Recessive White	3	hatched	1.559 0bc	2.707 4bc
		4	non-hatched	1.811 4a	3.063 9a

Note: Different letters within the same column denote the significant level at 0.05. Type1: Hatched eggs from Tibetan chicken as female parent; Type 2: Non-hatched eggs from Tibetan chicken as female parent; Type 3: Hatched eggs from Recessive White as female parent; Type 4: Non-hatched eggs from Recessive White as female parents.

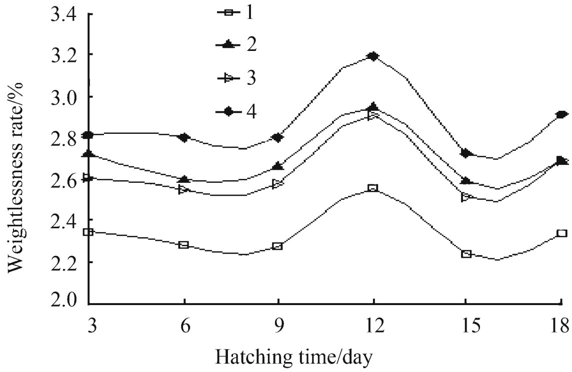

Fig. 3 The weight loss rate of the first batch of eggs

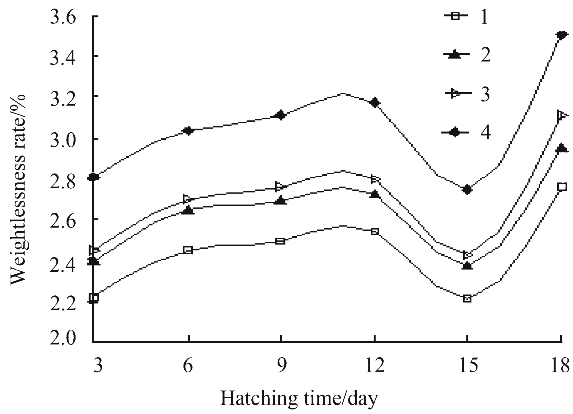

Fig. 4 The weight loss rate of the second batch of eggs

As seen from Table 2, the difference in the absolute rate of weight loss between the two batches of eggs with the same female parents and hatch result was not significant ($P > 0.05$). The rate of egg weight loss was lower than that of non-hatched eggs, although their female parents were of the same breed. The absolute weight loss rate of Recessive White eggs was higher than Tibetan chicken eggs with similar hatching result.

By comparing Figs. 3 and 4, the following similarities were found: (1) The weight loss rate of type 4 was the highest while type 1 was the lowest. (2) The lowest moisture loss of hatching eggs appeared on the 15th, l6th day while the peak appeared on the 11th, 12th, and 18th day. The following differences were also observed: (a) The weight loss rate curve of the first batch of eggs was similar to a “W” shape and the second batch was similar to “the Big Dipper” shape; (b) The weight loss rate of type 2 was higher than type 3 in the first batch of eggs, but it was contrary to the second. (3) On the third day, the differences of weight loss rate were significant in all types, except for type 1. (4) For the first batch of eggs, the rate of weight loss decreased slowly during the first 8 days, and it was lower than that on the 12th day, but this phenomenon was contrary to the second batch.

3.4 Logistic Regression analysis of the factors affecting incubation

As the dependent variables is binary (i.e., it takes on only two possible values), Logistic Regression (LR) was employed to analyze the five variables which may affect the hatching results.

Firstly, an observation which is far from the mean of the x variables is said to have high leverage. Extreme observation can, but do not always, have high influence on regression estimation. So the standardized residual was employed to find the outlier (Kaps and Lamberson, 2004).

Secondly, Table 3 listed the LR results which were carried out using LOGISTIC procedure in the SAS software. As seen from the Table 3, the variables, batch, surface area and preservation time, should be removed because they were not significant in the Full model.

Table 3 LR statistics for full model

variables	*DF*	Chi-Square	*P*-value
Mating combination	2	6.78	0.0337
Batch	1	3.39	0.0654
Surface area	1	2.16	0.1413
Preservation time	1	3.48	0.0621
Relative weight loss rate	1	13.23	0.0003

Note: Mating combination 4 was not considered because of many missing values.

Thirdly, LR analysis was performed after variable selection. In the reduced model, variable selection was carried out in the SAS LOGISTIC procedure using the backward elimination procedure (Johnson, 1998). As a result, surface area, preservation time and batch were removed from the set of predictor variables one after another. The final model includes the variables mating combination and relative weight loss rate as shown in Table 4. From Table 4, we can see that the variables were all very significant.

Table 4 LR statistics for reduced model

Parameters	*DF*	Estimate	Standard Error	Wald Chi-Square	*P*-value
Intercept	1	5.6804	1.1554	24.1703	0.0001
Mating combination I (x_1)	1	2.0577	0.6865	8.9837	0.0027
Mating combination II (x_1)	1	–1.6052	0.3881	17.1102	0.0001
Relative weight loss rate (x_2)	1	–0.2509	0.0696	12.9808	0.0003

Note: The estimates of mating combination 3 = 0.

Finally, using SAS procedure, if PHAT >0.5 then predict = "hatched"; else predict = "non-hatched". Table 5 summarized classifications obtained from the full model and reduced model, respectively.

We can see that the predict results of full model and reduced model were similar although the reduced model comprises of only two variables. It implies that the two variables (mating combination and weight loss rate) contribute to hatchability almost entirely. As a result, the fitted equations for hatchability in Tibet are listed as follows:

$$g(x) = 5.6804 + 2.0577\,x_1 - 0.2509\,x_2$$
when mating combination is I,

$$g(x) = 5.6804 - 1.6052\,x_1 - 0.2509\,x_2$$
when mating combination is II,

$$g(x) = 5.6804 - 0.2509\,x_2$$
when mating combination is III.

4 Discussion

4.1 Mating combination influencing the hatchability

Four mating combinations between Tibetan chicken and Recessive White were employed in this study. The results showed that the fertility of the hatching eggs using Tibetan chicken as female parent was significantly lower than that of Recessive White as female parent ($P<0.05$). This indicated that Recessive White was more adaptable to artificial insemination than Tibetan chicken that had been raised dispersedly for a long time and had gotten used to wild pairing. But for hatchability, the fertile eggs of Tibetan chicken were significantly higher than those of Recessive White ($P<0.05$), and the fertile eggs using Tibetan chicken as male and female parents were the highest, followed by combination III. These results confirmed that the female parent had an important effect on nutrition of fertile eggs, quality of eggshell and development of early embryo (Christensen et al., 2001; Li et al., 2002), and it was mainly apparent in the eggshell thickness ($P<0.05$) and the high correlation with hatchability (correlation coefficient was 0.608 ± 0.090) (Hulet et al., 1985; Ken and De, 1987). The local Tibetan chicken eggs with thinner eggshells could not only be stored for long time under local physical environment full of fresh air, but also accommodated itself to the anoxic hatching circumstance during the last stage of incubation. On the other hand, the embryos of combination II and IV whose female parents were Recessive White died in great numbers during the last stage of incubation as the bad permeability of the thicker eggshell resulted in fulminating anoxia. Of course, the hatchability was also influenced by the male parent to some degree (Landauer, 1967). The hatchability of combination III with Tibetan chicken as female parent was significantly higher than combination II with Recessive White as female parent by 20.44% ($P<0.05$). This indicated that Recessive White had not accommodated itself to the anoxic circumstance of cold nights and hot days during storage and incubation even though it was grown in Linzhi, Tibet. The results further provided a theoretical basis for crossing using the Tibetan chicken as female parent to improve the hatchability of introduced chicken eggs in Tibet.

Table 5 Predict results of full model and reduced model

	Full model with all variables		Reduced model with only Mating combination and Relative weight loss rate	
	Non-hatched (observed)	Hatched (observed)	Non-hatched (observed)	Hatched (observed)
Non-hatched (predict)	31(50%)	20(12.58%)	29(46.77%)	20(12.58%)
Hatched (predict)	31(50%)	139(87.42%)	33(53.23%)	139(87.42%)

In addition, the results of Logistic Regression analysis further confirmed that mating combination (genetic factor) was of importance to hatchability ($P < 0.01$).

4.2 Weight loss rate influencing the hatchability

It is cold and dry with a thin and fresh air in the Tibet Plateau, and this is suitable for storing eggs naturally. Although the cold nights and hot days make the temperature fluctuate greatly (Benton et al., 1996; Brake and Walsh, 1993), the hatchability was not affected significantly by preservation time when the eggs were stored less than 24 days as seen from the results of LR analysis ($P > 0.05$).

Hatchability was influenced by environmental temperature and humidity to some extent, and this influence was mainly reflected in weight loss rate (Landauer, 1967). Especially in the cold and dry winter, the simple incubator produced could hardly meet the need for predetermined humidity within the acceptable temperature range in the Plateau. This greatly contributed to moisture loss and as a result, it negatively influenced normal embryonic development and changed the curve of absolute weight loss rate from the normal "W" shape (Geng and Wang, 1990; Duan, 1995) to "the Big Dipper" shape. In this case, the thicker eggshell of Recessive White could have reduced moisture evaporation, but the larger eggshell surface area deprived it of such an advantageous factor. Therefore, the hatchability was not affected significantly by surface area of eggshell in this assay ($P > 0.05$). In addition, Table 2, Fig. 3 and Fig. 4 indicated that the rate of weight loss of hatched eggs was lower than non-hatched eggs although their parents were the same. The result was in accordance with the findings of others (Geng and Wang, 1990; Duan, 1995) and further demonstrated that it was disadvantageous for the normal embryonic development when moisture loss was too fast.

As seen from Fig. 3 and Fig. 4, the curves of absolute weight loss rate were not similar between the two batches. Does this phenomenon imply the batch significantly affected the egg hatchability as the weight loss rate? The results of LR analysis tell us that it is not the case. Batch is just a time concept of hatching eggs, and couldn't influence the weight loss rate. In fact, the reason for the different curves of absolute weight loss rate between two batches is the external environmental temperature and humidity (Wilson et al., 1984). Therefore, it is necessary to increase the heat and moisture appropriately during incubation at high altitudes. For example, the number of heating tubes and the area of wet film can be increased when the incubators are used at Tibetan altitudes.

It is necessary for embryo development to exchange air with its surroundings by absorbing oxygen and eliminating carbon dioxide and moisture during incubation. But in the Qinghai-Tibet Plateau, the air is thin with low amounts of oxygen. The chicken embryos would die in great numbers because of oxygen deficiency especially after 18 days when chicken embryos increase their aerobic capacity due to the transition from allantois chorion respiration to pulmonary respiration. Under the condition of oxygen deficiency within the incubator, normal metabolism will be restrained, while speeding up metabolism of the compensation mechanism of chick embryo (Visschedijk and Rahn, 1981; Bagley et al., 1990). As a result, to retain normal embryonic development under the anoxic condition of the Plateau, it is necessary to take some oxygenation measures besides ventilation.

In this study, we analyzed the effect of mating combination and environmental factors on hatchability. Although the intrinsic qualities of eggs (egg weight, egg-shape index, eggshell air pore density and surface area, etc.) have some significant effects on hatchability (Brake and Walsh, 1993; Visschedijk and Rahn, 1981; Kirk et al., 1987), according to the results of this study, it is also important to further analyze the effect of atmospheric pressure and the amount of oxygen in the atmosphere on hatchability.

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