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The Effects of Three-way Cross Strategy on the Gander Reproductive Performance

Y.P. Song[1#], Q. Liu[2#], Z.S. Bao[1#], C.G. Xu[1#], F.Z. Wang[3#], Y.X. Zhou[1], Z.Q. Feng[1], Y.F. Sun[1], Y.J. Sui[1], W. Wu[1]

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ABSTRACT

Background: Cross-breeding is the main strategy for improving the economic traits of livestock and poultry within a short time. But there are only a few studies on the comparative assessment of different cross-breeding methods to poultry reproductive performance.
Methods: Here, intersect, back, upgrading and three-way cross-breeding strategies are employed for quantitative comparison of male reproductive performance using Carlos goose, Jilin White goose and Siji goose. The fertility rates and testis weight were analyzed and compared among the five cross-breeding groups. Also, the seminiferous tubule diameter and sperm concentration were measured. The mRNA levels of two reproduction-related genes, GnRH (gonadotropin-releasing hormone) and PRL (prolactin) were quantitatively measured and compared using the qPCR technique.
Result: Finally we found that the three-way cross strategy had the biggest advantage for improving male reproductive performance according to the analysis of fertility rate and testis structure. Also all these data we measured suggested that the three-way cross strategy was beneficial to male reproductive performance and thus we provided a theoretical basis for making appropriate cross-breeding plans.

Key words: Cross-breeding, Carlos goose, Jilin white goose, Siji goose, Three-way cross.

INTRODUCTION

The whole body of the goose is economically valuable including meat, liver, feathers and other byproducts and the goose industry in China is prosperous and profitable. But compared to chickens and ducks, geese exhibit the poorest reproductive performance which severely impedes the development of the industry and limits the income of the farmers. To improve the reproductive performance of the geese, animal breeders have tried several ways from the aspects of the environment (Wang *et al*., 2005; Chang *et al*., 2016), nutrition (Zhang *et al*. 2020) and genetic background (Gao *et al*. 2021). Based on the previous studies, the cross-breeding strategy has become the most promising way for improving reproductive performance to the maximum extent.

Though more attention has been paid to the egg-laying performance of geese (Zhang *et al*., 2020; Gao *et al*., 2021; Guo *et al*., 2019; Zhu *et al*., 2017), the performance of ganders is more crucial such as sperm quality. The factors affecting sperm quality include ejaculate volume, sperm concentration and viability which determine to a great degree the hatchability and fertility (Lukaszewicz *et al*., 2003; Kowalczyk *et al*., 2012). But till now the effects of different cross-breeding strategies on reproductive performance, such as sperm quality, hatchability and fertility are still largely unknown.

In northern China, most goose breeds are smaller though they have better egg-laying performance. On the contrary, in southern China, most goose breeds have better growth performance but poorer egg-laying performance. For example, the Jilin White goose egg production line was bred by Jilin Agricultural University in 2002 and now is famous for its excellent egg-laying performance. They are always used as female parents to improve the egg-laying performance of the offspring with cross-breeding by the farmers.

In recent years, several foreign goose breeds are introduced to improve the performance of domestic breeds. The breeders have been trying to find optimized plans to release the full genetic potential of the parents. Here, using Jilin White goose, Siji goose and Carlos goose, five groups with different compositions of genetic background were compared and analyzed focused on the gander reproductive performance. We found that the three-way cross group showed the best performance with the highest fertility rate ($P< 0.05$), the biggest testis weight ($P< 0.05$), the biggest

[1]College of Animal Science and Technology, Jilin Agricultural University, Changchun 130118, China.
[2]College of Food Engineering, Jilin Engineering Normal University, Changchun, 130052, China.
[3]Qihe Center for Disease Control and Prevention, Dezhou, 251100, China.
[#]These authors contributed equally to this work.

Corresponding Authors: Y.J. Sui, College of Animal Science and Technology, Jilin Agricultural University, Changchun 130118, China. Email: suiyujian2000@163.com

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diameter of seminiferous tubule (P<0.05) and the highest sperm concentration (P<0.05). Our data suggested that the three-way cross was a better strategy to improve the gander reproductive performance though it is more time-consuming.

MATERIALS AND METHODS

Ethics statement

All the animal experiments were approved by the Animal Ethics Committee of Jilin Agriculture University (Changchun, Jilin, China). Except for the feeding and breeding of the geese, all the other experiments were carried out in the College of Animal Science and Technology, Jilin Agricultural University (from 2019 to 2021).

Cross-breeding plans

All the cross-breeding plans were based on the F1 hybrids (Carlos ♂×Jilin White♀). Group I was F1♂×F1♀ (intersect). Group II was Carlos ♂×F1@& (upgrading). Group III was Jilin White♂× F1♀ (back). Group IV was Siji ♂×F1♀ (three-way cross, positive). Group V was F1 ♂× Siji♀ (three-way cross, negative). Each group contained one hundred and twenty-five geese (male:female= 1:4).

Feeding and management

All these five groups were raised separately in the goose house of Jilin Agricultural University. The metabolizable energy of the feed was 11.22 MJ/kg, crude protein was 15.8%, crude fiber was 7%, methionine was 0.38%, lysine was 0.8%, cystine was 0.3%, calcium was 2.3%, phosphorus was 0.3% and salt was 0.4%.

Data and sample collection

The fertility rates (number of fertile eggs/number of total eggs) were calculated and compared in all five groups during different reproductive stages. The testis weight was collected and measured by electronic balance (two ganders each group) at seven different time points. The left side of the testis was placed in liquid nitrogen for RNA extraction and quantification. The right side was fixed in 4% formaldehyde solution for staining of tissue sections.

The diameter of the seminiferous tubule was measured under 100 × magnification and the values from twenty different views were collected and calculated with Scope Photo.

The sperm concentration was measured under 400 ×magnification and the values from twenty different views were collected and calculated with Scope Photo.

Quantitative real-time PCR

Total RNA was extracted using Trizol (Takara, Dalian) and then total RNA was reversely transcripted into cDNA with ReverTra Ace qPCR RT Kit (Toyobo, Shanghai) in the thermocycler (ABI2720, Thermo Fisher Scientific). The cDNA template was used for quantitative PCR with THUNDERBIRD SYBR® qPCR Mix (Toyobo, Shanghai) in the real-time PCR system (ABI StepOnePlus, Thermo Fisher Scientific). β-actin was used as an internal reference gene and the relative expression levels were analyzed by the $2^{-\Delta\Delta CT}$ method. The annealing temperature for GnRH primers was 58° and for PRL was 57°. Cycle2 for qPCR was repeated 40 times (Table 1).

Statistical analysis

The experimental grouping follows the RCBD (randomized complete block design) rule. The data were analyzed by SPSS 23.0 software using the one-way ANOVA procedure and Duncan's multiple range tests. Finally, the data were shown as mean±SD (standard deviation). P>0.05 means that no significant difference and P<0.05 means that the difference is statistically significant.

RESULTS AND DISCUSSION

Fertility rates of different groups with different cross-breeding strategies

The whole experimental period was divided into three stages and the fertility rate of Group d! was the highest (P<0.05) except for the middle stage (P>0.05). The advantage of the three-cross group was more clear at the late stage when the fertility rates of all the other groups decreased significantly. No significant difference in fertility rate was observed at the late stage among the other four groups (P>0.05) (Table 2).

Gander reproductive performance of different groups

For testis weight, no significant difference was observed among all the five groups at pre-stage (P>0.05). And consistent with the fertility rate, Group d! showed a significant advantage at the late stage (P<0.05) but the advantage was not significant at the other stages. The testis weight of the gander increased until the middle stage and then decreased significantly within the next breeding period (Table 3).

For the diameter of the seminiferous tubule, no significant difference was observed until the second half of

Table 1: Primers used for real-time PCR.

Primers	Accession number	Sequence (5′-3′)	Host	Length of product
GnRH-F	DQ023158	GGGACCCTTGCTGTTTTG	Anser anser	182 bp
GnRH-R		GGGCAGGAGCCAGTTGTA		
PRL-F	DQ836024	AGTGAGCACAGCCAACATTA	Anser anser	135 bp
PRL-R		AGAAGGACCACCAGCAACAA		
β–actin-F	M26111.1	GACCACCTTCAACTCCATC	Anser anser	127 bp
β-actin-R		GGCTGTGATCTCCTTCTG		

Table 2: Comparison of fertility rate among different cross-breeding strategies.

Hatching stage	Starting date of hatching	Fertility rate (%)				
		I	II	III	IV	V
Early stage	20th of March	88.89±4.45	88.15±2.56	94.07±1.29	87.41±3.40	97.04±3.39
	20th of April	89.63±3.39	82.96±3.40	90.37±2.57	90.37±3.39	97.78±2.22
	Average	89.26±3.56^{ab}	85.56±3.91^{a}	92.22±2.72^{b}	88.89±3.44^{ab}	97.41±2.02^{c}
Middle stage	4th of May	89.63±1.28	89.63±3.39	88.89±2.22	89.63±3.39	92.59±3.40
	28th of May	88.89±2.22	76.30±4.00	85.92±2.57	86.67±3.85	91.11±2.22
	11th of June	80.00±4.44	62.22±5.88	79.26±2.56	79.26±1.28	89.63±1.28
	Average	85.08±4.18^{a}	82.69±6.46^{a}	84.69±4.77^{a}	83.19±4.27^{a}	86.17±5.30^{a}
Late stage	25th of June	73.33±4.44	56.30±2.57	67.41±3.40	59.26±2.56	85.93±1.29
	9th of July	45.92±4.63	43.70±6.41	45.93±3.39	47.44±3.46	76.30±4.62
	23rd of July	38.52±5.59	36.30±4.63	40.00±4.44	38.52±2.56	65.18±5.59
	Average	52.59±15.44^{a}	45.43±9.69^{a}	51.11±12.91^{a}	48.41±9.35^{a}	75.85±10.38^{b}

Note. All the data in the table were shown as mean±SEM (standard error of the mean). Different superscripts indicated there was a significant difference comparing one to another in the same line (P< 0.05). The same superscript indicated no significant difference (P> 0.05). Group I was F1 ♂ ×F1 ♀ (intersect). Group II was Carlos ♂ ×F1 ♀ (upgrading). Group III was Jilin White ♂ ×F1 ♀ (back). Group IV was Siji ♂× F1 ♀ (three-way cross, positive). Group v was F1 ♂ ×Siji ♀ (three-way cross, negative).

Table 3: Comparison of testis weight among different cross-breeding strategies.

Breeding period	Sampling date	Testis weight (g)				
		I	II	III	IV	V
Pre-stage	15th of February	5.54±0.27^{a}	5.61±0.29^{a}	4.94±0.28^{a}	4.82±0.59^{a}	5.09±0.47^{a}
Early stage	15th of March	7.51±0.49^{a}	7.81±0.67^{a}	6.73±0.40^{a}	6.62±0.26^{a}	7.72±0.33^{a}
	15th of May	12.70±0.42^{bc}	10.86±0.46^{a}	11.72±0.38^{ab}	11.76±0.62^{ab}	13.26±0.40^{c}
Middle stage	1st of June	10.60±0.40^{bc}	10.19±0.29^{ab}	9.67±0.18^{a}	10.58±0.39^{bc}	11.05±0.35^{c}
	15th of June	9.01±0.38^{ab}	8.87±0.64^{ab}	8.62±0.62^{a}	8.75±0.50^{ab}	10.00±0.23^{b}
Late stage	1st of July	6.39±0.40^{a}	6.45±0.37^{a}	6.13±0.70^{a}	5.83±0.52^{a}	7.85±0.47^{b}
	15th of July	5.20±0.23^{a}	5.26±0.34^{a}	4.79±0.30^{a}	5.10±0.19^{a}	6.79±0.32^{b}
Average		8.14±0.37^{ab}	7.86±0.44^{a}	7.51±0.41^{a}	7.64±0.44^{a}	8.82±0.37^{b}

Note. All the data in the table were shown as mean±SEM (standard error of the mean). Different superscripts indicated there was a significant difference comparing one to another in the same line (P< 0.05). The same superscript indicated no significant difference (P> 0.05). Group I was F1♂×F1♀ (intersect). Group II was Carlos♂×F1♀ (upgrading). Group III was Jilin White♂×F1♀ (back). Group IV was Siji ♂×F1@& (three-way cross, positive). Group V was F1♂×Siji♀(three-way cross, negative).

Table 4: Comparison of seminiferous tubule diameter among different cross-breeding strategies.

Breeding period	Sampling date	Diameter of seminiferous tubule (μm)				
		I	II	III	IV	V
Pre-stage	15th of February	239.69±11.52^{a}	241.76±21.92^{a}	230.46±13.50^{a}	227.30±10.86^{a}	234.57±9.41^{a}
Early stage	15th of March	263.41±21.86^{a}	270.19±19.30^{a}	266.61±27.20^{a}	259.27±20.69^{a}	269.16±28.37^{a}
	15th of May	277.49±38.2^{a}	280.46±23.15^{a}	279.36±28.46^{a}	282.45±26.16^{a}	293.32±14.35^{a}
Middle stage	1st of May	264.08±9.21^{a}	254.57±13.67^{a}	274.01±15.94^{a}	263.72±14.88^{a}	287.28±10.87^{a}
	15th of June	215.89±6.4^{a}	219.47±13.24^{a}	225.61±10.70^{a}	216.07±6.27^{a}	264.98±7.42^{b}
Late stage	1st of July	207.01±7.08^{a}	207.47±5.94^{a}	213.04±6.19^{a}	203.33±4.59^{a}	223.81±9.91^{b}
	15th of July	201.88±9.72^{a}	200.46±5.44^{a}	200.81±7.21^{a}	195.24±8.58^{a}	212.26±11.74^{b}
Average		238.49±14.86^{a}	239.20±14.67^{a}	241.41±15.60^{a}	235.34±13.15^{a}	255.05±13.15^{a}

Note. All the data in the table were shown as mean±SEM (standard error of the mean). Different superscripts indicated there was a significant difference comparing one to another in the same line (P< 0.05). The same superscript indicated no significant difference (P> 0.05). Group I was F1♂×F1♀ (intersect). Group II was Carlos ♂×F1♀ (upgrading). Group III was Jilin White ♂×F1♀(back). Group IV was Siji ♂×F1♀ (three-way cross, positive). Group V was F1 ♂×Sij i♀ (three-way cross, negative).

Table 5: Comparison of sperm concentration among different cross-breeding strategies.

Breeding period	Sampling date	Sperm concentration (10^3/mm^2)				
		I	II	III	IV	V
Pre-stage	15th of February	81.52±3.26^a	80.75±3.95^a	91.25±4.04^b	83.06±6.15^a	93.12±3.22^b
Early stage	15th of March	163.18±4.86^b	148.84±3.17^a	171.24±2.40^c	157.79±5.12^b	174.14±5.42^c
	15th of May	259.70±10.72^a	257.60±4.63^a	266.88±7.94^a	264.82±8.45^a	283.92±7.91^b
Middle stage	1st of June	224.04±7.21^b	202.22±9.69^a	229.36±3.92^{bc}	223.90±4.60^b	241.38±6.27^c
	15th of June	142.79±12.71^{abc}	122.73±5.22^a	147.90±8.98^{bc}	139.41±10.41^{ab}	162.33±5.29^c
ate stage	1st of July	80.29±2.72^a	77.16±8.66^a	83.03±8.50^a	78.72±7.25^a	134.60±8.69^b
	15th of July	73.34±9.63^a	69.89±3.42^a	76.83±7.65^a	71.68±5.73^a	105.15±6.15^b
Average		146.41±7.30^a	137.03±5.53^a	149.21±6.20^a	145.63±6.82^a	170.66±6.14^b

Note. All the data in the table were shown as mean±SEM (standard error of the mean). Different superscripts indicated there was a significant difference comparing one to another in the same line (P< 0.05). The same superscript indicated no significant difference (P> 0.05). Group I was F1 ♂ ×F1♀ (intersect). Group II was Carlos ♂ ×F1♀ (upgrading). Group III was Jilin White ♂ ×F1♀ (back). Group IV was Siji ♂ ×F1♀ (three-way cross, positive). Group V was F1 ♂ ×Siji♀ (three-way cross, negative).

Table 6: Comparison of GnRH expression levels among different cross-breeding strategies.

Breeding period	Sampling date	mRNA expression level of GnRH				
		I	II	III	IV	V
Pre-stage	15th of February	1.000±0.088^a	1.000±0.039^a	1.000±0.097^a	1.000±0.066^a	1.000±0.058^a
Early stage	15th of March	1.636±0.042^a	1.734±0.339^a	1.832±0.115^a	1.765±0.166^a	1.736±0.062^a
	15th of May	2.304±0.574^a	2.013±0.213^a	2.732±0.405^a	2.169±0.306^a	3.687±0.094^b
Middle stage	1st of June	1.578±0.076^a	1.762±0.349^{ab}	1.804±0.285^{ab}	1.562±0.217^a	2.145±0.213^b
	15th of June	0.996±0.055^{bc}	0.698±0.027^a	1.161±0.091^{cd}	0.968±0.027^b	1.503±0.205^d
Late stage	1st of July	0.448±0.033^a	0.434±0.033^a	0.929±0.161^c	0.563±0.015^b	1.264±0.185^c
	15th of July	0.442±0.037^a	0.412±0.021^a	0.513±0.017^b	0.488±0.081^{ab}	0.986±0.014^c
Average		1.201±0.129^a	1.150±0.146^a	1.424±0.167^a	1.216±0.125^a	1.760±0.119^b

Note. All the data in the table were shown as mean±SEM (standard error of the mean). Different superscripts indicated there was a significant difference comparing one to another in the same line (P< 0.05). The same superscript indicated no significant difference (P> 0.05). Group I was F1♂ ×F1♀ (intersect). Group II was Carlos♂ ×F1♀ (upgrading). Group III was Jilin White♂ ×F1♀ (back). Group IV was Siji♂ ×F1♀ (three-way cross, positive). Group V was F1♂ ×Siji♀ (three-way cross, negative).

the middle stage. Also, consistent with both the fertility rate and testis weight, the advantage of Group d! was more prominent at the late stage (P< 0.05). No significant difference was observed among the other four groups in the whole period (P> 0.05) (Table 4).

For sperm concentration, the situation was more complicated. At pre-stage and the first half of the early stage, both Group b! and d! had the highest sperm concentrations (P< 0.05). At middle stage, the variation of the sperm concentrations showed no obvious regularity. Until the late stage, Group d! gained the advantage in fertility rate, testis weight and the diameter of the seminiferous tubule mentioned above (P< 0.05) (Table 5).

mRNA expression levels of GnRH and PRL

The mRNA expression levels of two reproduction-related genes, GnRH and PRL, were quantitatively detected in our study. The GnRH expression level of Group d! was the highest on average among the five groups (P< 0.05). The GnRH levels of all the groups increased gradually until the second half of the early stage and then decreased until the end of the experiment. Group b! also showed a significant advantage at the late stage compared with Groups '!, a! and c! (P< 0.05) (Table 6).

Conversely, the PRL expression level of Group d! was the lowest on average among the five groups (P< 0.05). The PRL levels of all the groups decreased gradually until the second half of the early stage and then increased. Group b! also showed a significant advantage consistent with that of GnRH (P< 0.05) (Table 7).

Genetic background is crucial

Several non-genetic factors influence the fertility rate of the geese including nutrition (Chang *et al*., 2016; Zhang *et al*., 2020), environment (Gillette *et al*. 1976) and lighting management (Chang *et al*., 2016; Wang *et al*., 2002; Liu *et al*., 2020), but the genetic background of the geese makes the main contribution (Ottenburghs *et al*. 2016). But till now, how to integrate the genetic advantages of different goose species using optimized cross-breeding plans to improve the fertility rate is still being explored.

Here using three goose species with distinct advantages

Table 7: Comparison of PRL expression levels among different cross-breeding strategies.

Breeding period	Sampling date	mRNA expression level of PRL				
		I	II	III	IV	V
Pre-stage	15th of February	1.000±0.003^{a}	1.000±0.079^{a}	1.000±0.083^{a}	1.000±0.013^{a}	1.000±0.023^{a}
Early stage	15th of March	0.649±0.043^{c}	0.595±0.064^{bc}	0.648±0.033^{c}	0.517±0.031^{ab}	0.468±0.021^{a}
	15th of May	0.404±0.045^{a}	0.412±0.026^{a}	0.306±0.021^{a}	0.402±0.024^{a}	0.332±0.082^{a}
Middle stage	1st of June	0.549±0.061^{b}	0.996±0.031^{d}	0.503±0.047^{ab}	0.735±0.073^{c}	0.419±0.034^{a}
	15th of June	0.989±0.065^{b}	1.204±0.063^{c}	0.780±0.250^{b}	0.909±0.089^{b}	0.667±0.055^{a}
Late stage	1st of July	2.519±0.027^{c}	2.607±0.072^{c}	1.782±0.134^{b}	2.022±0.242^{b}	0.892±0.074^{a}
	15th of July	5.565±0.456^{c}	4.722±0.337^{c}	3.417±0.271^{b}	4.216±0.039^{c}	2.199±0.327^{a}
Average		1.668±0.100^{c}	1.648±0.096^{c}	1.205±0.120^{b}	1.400±0.073^{b}	0.854±0.088^{a}

Note. All the data in the table were shown as mean±SEM (standard error of the mean). Different superscripts indicated there was a significant difference comparing one to another in the same line ($P< 0.05$). The same superscript indicated no significant difference ($P> 0.05$). Group I was F1♂×F1 ♀ (intersect). Group II was Carlos♂×F1♀ (upgrading). Group III was Jilin White ♂×F1 ♀ (back). Group IV was Siji ♂ ×F1 ♀ (three-way cross, positive). Group V was F1♂×Siji ♀ (three-way cross, negative).

of reproductive performance, different cross-breeding methods were employed and compared systematically. We found that the fertility rate of the three-way cross group was the highest ($P< 0.05$) and this advantage was more obvious in the late stage when the fertility rates of the other four groups decreased significantly. Our results suggested that the three-way cross strategy could release the fullest potential of different goose species thus achieving the aim of improving their reproductive performance.

Effects of different cross-breeding strategies on the gander reproductive performance

Given the important influence of the gander reproductive performance on the fertility rate, several studies focused on the correlations between different testis traits and the gander reproductive performance. The development of the testis showed distinct features at different stages and the genitalia grew fast before sexual maturity (Zhang *et al*. 1988). The healthy ganders with good reproductive performance had the bigger testis weight, diameter and epithelial height of the seminiferous tubule compared with the stunted ganders (Liu *et al*. 2002). Our results showed that the optimized three-way cross strategy could improve the gander reproductive performance significantly by increasing the testis weight, the diameter of the seminiferous tubule and the sperm concentration. And the advantage of this strategy was superior to the other cross-breeding strategies.

mRNA expression levels of the genes related to reproductive performance

GnRH is secreted from the hypothalamus and it acts directly on the gonad to promote the synthesis and release of the hormones such as LH (luteinizing hormone) and FSH (follicle-stimulating hormone) (Moore *et al*. 2000). Also, injection of GnRH could potentially increase the level of testosterone (Hirschenhauser *et al*. 2005). Our results showed that GnRH mRNA expression levels of all the five groups displayed the same trends, gradually increasing until the middle stage and decreasing until the end of the late stage. The GnRH mRNA expression trend correlated with that of the testis traits we analyzed including the testis weight, the diameter of the seminiferous tubule and the sperm concentration. On average, the three-way cross group had the highest GnRH level, especially at the reproduction peak.

On the contrary, PRL negatively correlates with reproductive performance (Gumulka and Rozenboim 2015). The PRL mRNA expression level displayed the opposite trend to that of GnRH, gradually decreasing until the middle stage and increasing until the end of the late stage. Our results suggested that PRL inhibited the gander reproductive performance. Also, the three-way cross group had the lowest PRL level.

CONCLUSION

Our data showed that the optimized three-way cross strategy could improve the gander reproductive performance significantly compared with intersect, upgrading and back cross-breeding strategies. The possible underlying mechanism of the improvement may be due to the changes in GnRH and PRL levels.

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Conflict of interest

The authors declare no conflict of interest.

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