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A diagnostic method for gander selection in Zi geese (*Anser cygnoides* L.) and Rhin geese (*Anser anser* L.) using massage reaction and semen quality

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Abstract. The objective of this study was to score reactions to artificial semen collection procedures and to develop an efficient method for gander selection in two original geese breeds. One-hundred and three Zi geese and 110 Rhin geese were used. Gander reaction scores to massage and semen quality parameters were investigated in 10 Zi and 10 Rhin ganders during a complete reproductive season. The relationships between average gander massage reaction score and semen quality were evaluated. After natural mating, egg fertility comparison between Zi ganders selected by massage reaction scores and those not subjected to selection were investigated. Ganders exhibited large variations in artificial semen collection and semen characteristics between breeds. Only 30.1% (31/103) of Zi geese and 46.3% (51/110) of Rhin geese had stable positive massage reactions with semen ejaculation ($P < 0.05$). Average massage reaction score, ejaculate volume, spermatozoa concentration, sperm motility and semen quality factor for single ejaculate were significantly higher in Zi than in Rhin geese. There was a high correlation between average massage reaction score and semen volume, being 0.87 for Zi geese ($P < 0.05$) and 0.82 for Rhin ($P < 0.05$), and this correlation enabled pre-selection of ganders at the beginning of the breeding season. Reproductive efficiency could be improved with massage-selected ganders in natural breeding flocks. Examination of gander massage reaction scores combined with laboratory assessment of semen quality early in the breeding season is a feasible method for predicting fertilising capability of ganders.

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Introduction

China has the largest goose production industry in the world (Wezyk 2005). Goose-meat production in China increased ~35% between 1999 and 2009 (FAOSTAT 2010), but low reproductive efficiency has hindered continued expansion (Liu *et al.* 2008). One reason for this problem is that domestic goose egg fertility is significantly lower than other poultry species. Predominant causes include the high level of gander phallus disease, dysfunction in spermatogenesis and a low percentage of morphologically normal sperm (Svetlik and Weis 2004). It is necessary to select ganders based on reproductive potential to improve reproductive efficiency (Donoghue 1999; Mellor 2001; Cheng *et al.* 2002).

Efficient identification and culling of subfertile ganders has received considerable attention in recent years. On most farms, ganders have been selected based on phallus morphology, including length and circumference, before the breeding season. Ganders with copulatory organ malformation are culled (Liu *et al.* 2002). Svetlik and Weis (2004) stated that unselected ganders in breeding flocks reached 54.7% egg fertility, but when ganders were selected based on ejaculate quality, fertility increased to 87.3% with natural mating. Łukaszewicz and Kruszynski (2003) found that ganders consistently respond differently to semen collection, and massage reaction scores to semen collection are related to the semen characteristics (Lee *et al.* 1999; Svetlik and Weis 2004).

Most domestic geese today are derived from two wild species: the wild Graylag goose (*Anser anser*) and the wild Swan goose (*Anser cygnoides*). Selective breeding from these two ancestors has resulted in breeds that express large variability in multiple phenotypic traits like body size, general behaviour and reproductive characteristics (Łukaszewicz 2010). Reproductive criteria for selection of a gander must be defined and the variation in sperm quality of a population assessed. In China, the Zi goose is famous for its high egg production and is used widely in crossbreeding programs in the north-east of the country. Rhin geese are usually used as a paternal line for their bodyweight and high growth rate (Liu *et al.* 2005). Relatively little research has addressed mean gander massage reaction and semen characteristics in the two breeds.

The objectives of this study were to investigate gander massage reaction and semen characteristics of Zi and Rhin ganders, and to develop a diagnostic tool for gander reproductive selection for these two breeds.

Materials and methods

Animals

The experiment was conducted at the Beifang goose breeding company in Heilongjiang province, China (North latitude: 45°47′). Zi geese (*A. cygnoides* L.), Rhin geese (*Anser anser* L.) and Rhin-Zi crosses were raised under natural lighting and semi-open housing conditions. Birds were fed commercial rations for breeding geese (250–300 g/day) containing 10.80 MJ of metabolisable energy and a crude protein level of 140 g/kg. All ganders were selected based on phallus morphology before the breeding season, which begins in mid February and ends in June. All geese were 8–9 months old.

Experiment I: semen quality and egg fertility during the reproductive cycle

Gander selection by massage reaction

One-hundred and three Zi geese ganders and 110 Rhin ganders were used to investigate reaction to dorso-abdominal massage (Burrows and Quinn 1937; Pawluczuk and Grunder 1989; Grunder and Pawluczuk 1991) twice per week in early March. Particular care was taken to maintain the same staff members, massage method and time of massage. After 1 week of training, gander reactions to the semen collection procedure (two collections from each male) were recorded. Based on their responses to massage, ganders were assigned a score of either 0, 1, 2 or 3: A 0 score meant a lack of reaction or slow reaction (more than 60 s) without ejaculation; 1 meant a quick reaction (less than 60 s) without ejaculation; 2 meant a slow reaction (more than 60 s) ending with ejaculation; 3 meant a quick reaction (less than 60 s) with semen ejaculation (Łukaszewicz and Kruszynski 2003). Only males with a positive reaction with ejaculation in each of the two successive massages were recognised as positive reaction males (massage response score of 2 or 3). As a result, 31 Zi geese and 51 Rhin geese were selected.

Fertility of the two breeds of geese

Throughout the first half of March, 10 of the 31 Zi geese and 10 of the 51 Rhin were randomly chosen. All birds were raised individually. Based on semen quality, pooled semen from each group (separate Zi and Rhin samples) was diluted 1 : 1 with Dulbecco's modified Eagles medium (Liu *et al.* 2008). Sixty 8–9-month-old female Rhin-Zi cross geese were randomly divided into two pens of 30 individuals each. The female geese were inseminated at random with the diluted semen from the male geese within 30 min of semen collection. Each goose was inseminated with 0.1 mL of diluted semen. A second insemination was performed 5 days later. The mean semen quality factor for the pooled semen (SQF-p) was calculated for each group over two inseminations using the calculation: SQF-p = one dose (0.1 mL) of pooled semen $\times$ sperm concentration ($\times 10^6$/mL) $\times$ live and morphologically normal spermatozoa (%) / 100 (Łukaszewicz and Kruszynski 2003; Liu *et al.* 2008). Live and morphologically normal spermatozoa were evaluated with nigrosin-eosin staining (Bakst and Cecil 1997). The volume of semen was measured using a semen collection cup marked to the nearest 10 μL. Sperm concentration was measured with a hemocytometer and motility was estimated using the hanging drop method at 400× magnification (Bakst and Cecil 1997).

Eggs were collected daily from Day 2 after the first insemination to Day 6 after the second insemination. The eggs collected over the 11 days were placed in an automatic incubator. Fertility was determined by candling on Day 10 of incubation.

Semen quality of the two breeds of geese during the reproductive cycle

To estimate the two gander populations' semen characteristics, another test was conducted from the middle of March to the end of June. Semen was collected twice a week from each of the ganders used for the fertility testing, resulting in 32 semen collections from each male during the reproductive cycle. The ganders' massage reaction and semen characteristics were determined once a week.

The mean massage reaction score and semen quality parameters for each gander were calculated for each of the 16 weeks. The correlations between average gander reaction scores and semen volume, sperm concentration, sperm morphology and semen quality factor for single ejaculate (SQF-s) were calculated using the equation: SQF-s = semen volume for individual ganders (mL) $\times$ sperm concentration (10^6/mL) $\times$ live and morphologically normal spermatozoa (%)/ 100 (Łukaszewicz and Kruszynski 2003).

Experiment II: fertility comparison between massage-selected Zi ganders and those not selected by massage procedures

Three-hundred female Zi geese were randomly divided into two groups of 150 individuals each. The 21 selected Zi ganders from Experiment I were mixed with one of the female populations. Thirty additional ganders that did not undergo selection in the early reproductive season were mixed with the other female population. The first group had a low male/female ratio of selected ganders to female geese (1 : 7.14), while the second had a high male/female ratio group of unselected ganders to female geese (1 : 5). Each flock was randomly divided into three replications, each having 50 females and seven males in an experimental group and 50 females and 10 males in a control group. Eggs were collected weekly for 13 weeks and placed in an automatic incubator starting 3 weeks after the males and females were mixed. Fertility was determined every second week from early April to the middle of June by candling on Day 10 of incubation.

Statistical analyses

Data were analysed using the SAS statistical program (version 8.2., SAS Inst. Inc., Cary, NC, USA). Duncan's test was used to determine the differences in average gander reaction scores and semen quality between the Rhin and Zi geese. Results are presented as mean ± s.e.m.

Pearson's Chi-square test was used to analyse differences in fertility between different breeds or gander categories (selected or not selected by massage procedures) as well as reaction differences for massage procedures in the two breeds. Spearman correlations between average massage scores and semen volume, sperm concentration, sperm morphology and SQF were calculated by the CORR procedure using SAS.

Results

Experiment I

Gander reaction to massage procedure

Only 30.1% (31/103) of Zi geese and 46.3% (51/110) of Rhin geese had massage reactions with stable ejaculate volumes. There was a significant difference between the two breeds ($P < 0.05$), with the Rhin breed having more eligible males after the two massage procedures.

Average gander reaction score and semen quality parameters of the two breeds of geese throughout the reproductive season

Average gander reaction scores and semen quality parameters for the two breeds are presented in Table 1. There was a difference ($P < 0.05$) between the Zi geese and the Rhin geese in average gander reaction scores, ejaculate volume, spermatozoa concentration and SQF-s. There were no differences between the two breeds ($P > 0.05$) when comparing the characteristics of live and morphologically normal cells.

Correlations between average gander reaction scores and semen quality parameters in Zi and Rhin geese are presented in Table 2. Correlations between average gander reaction scores and ejaculate volumes through the reproductive season were significant in both breeds ($P < 0.05$).

Fertility test

Fertility test results for the two selected gander populations are presented in Table 3. The fertility was significantly different between the two groups ($P < 0.01$).

Experiment II

Fertility comparison between massage-selected Zi ganders and those not selected by massage procedures

Fertility comparison between Zi ganders based on massage selection and those not selected by massage procedures are presented in Fig. 1. The average fertility rates were 90.5 ± 0.7 and 88.7 ± 0.7%, respectively. There was no difference between the two groups ($P > 0.05$).

Discussion

It was reported that ~60–70% of unselected White Italian (*A. anser*) ganders responded to abdominal massage with a phallus erection, but only 30% routinely produced usable ejaculate (Chelmonska and Łukaszewicz 1995). There was little difference between Zi geese and White Italian ganders in the ratio of positive reactions, but this parameter was higher in Rhin geese at 46.3%. It appears that the response reaction to semen collection stimulation may be breed-dependent.

Unselected ganders include individuals that have good semen quality as well as sterile males and those producing low quality semen (Liu *et al.* 2002). Within a sire family breeding population, the disadvantage of poor reproduction in some unselected ganders clearly appears. In a family selection program for egg production in Zi geese (60 sire families, each with one male and five females), we found that ~10% of the families produced eggs with very low

Table 1. Descriptive statistics of massage reaction and semen quality parameters for Zi and Rhin geese
Means within a row followed by different letters are significantly different ($P < 0.05$)

Item	Average gander reaction scores (0–3)	Volume (mL)	Concentration (1×10^6/mL)	Live, morphologically normal (%)	Motility (%)	SQF-s[B]
Zi geese[A]	2.63 ± 0.08a	0.42 ± 0.04a	617.2 ± 50.1a	40.8 ± 1.6	48.0 ± 3.7a	130.4 ± 29.9a
Range	0–3.0	0.18–1.40	238–2300	28.0–68.0	40.8–78.0	30.8–189.4
Rhin geese[A]	2.26 ± 0.08b	0.31 ± 0.04b	377.0 ± 30.7b	37.4 ± 2.0	43.5 ± 3.1b	51.3 ± 12.80b
Range	0–2.88	0.05–0.58z	203–1490	28.0–76.0	30.8–69.0	13.8–85.3

[A]Number of Zi geese: 10; number of Rhin geese: 10.
[B]SQF-s = semen quality factor for single ejaculate; SQF-s values were calculated as the ejaculated semen volume for individual ganders (mL) × sperm concentration (× 10^6/mL) × live and morphologically normal spermatozoa (%)/100.

Table 2. Correlation coefficients between average massage reaction scores and semen quality parameters in Zi and Rhin geese
*, $P < 0.05$; **, $P < 0.01$

Correlation coefficient		Volume (mL)	Concentration (1×10^6/mL)	Live, morphologically normal (%)	Motility (%)	SQF-s[B]
Massage[A] reaction	Zi geese	0.87*	0.58	–0.18	0.48	0.62
	Rhin geese	0.82*	0.23	0.32	0.44	0.83*

[A]Number of Zi geese: 10; number of Rhin geese: 10.
[B]SQF-s = semen quality factor for ejaculating semen; SQF-s values were calculated as the ejaculated semen volume for individual ganders (mL) × sperm concentration (× 10^6/mL) × live and morphologically normal spermatozoa (%)/100.

Table 3. Effects of semen quality on fertility from different breeds of geese after artificial insemination
Means within a row with no common letter are different ($P < 0.05$)

Group[A]	Mean SQF-p[B]	Eggs set[C]	Fertile eggs	Fertility (%)
Zi geese	34.7	101	84	83.2a
Rhin geese	16.3	89	58	65.1b

[A]Each group's semen was diluted 1 : 1 with Dulbecco's modified Eagle's medium.
[B]SQF-p = semen quality factor for pooled semen, SQF-p = one dose (0.1 mL) of pooled semen × sperm concentration (×10^6/mL) × live and morphologically normal spermatozoa (%)/100.
[C]Data for eggs collected from Day 2 after the first insemination to Day 6 after the last insemination.

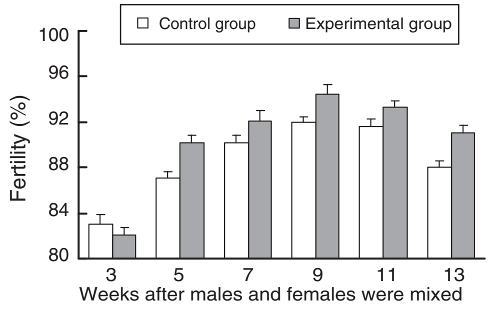

Fig. 1. Flock fertility comparisons between Zi ganders selected by massage reaction and those not selected by massage reaction.

fertility (less than 50%) or infertile eggs over the entire reproductive season (S. J. Liu, unpubl. data), but in most other families, egg fertility varied from 80 to 92%. Within large mating flocks, males of low or questionable fertility may go undetected because in many instances they appear normal and healthy (Wilson *et al.* 1979). In flocks with a higher male/female ratio, because of sperm competition between different males attempting to fertilise the same female (Møller and Ninni 1998), the ganders with higher reproductive capacity could compensate for any production shortage that would have been caused by subfertile or infertile males. This may be why the egg fertility in the control group still reached a satisfactory level despite the presence of some infertile or subfertile ganders.

As a behavioural factor, response to massage may be connected with poultry fertilising potential. Lee *et al*. (1999) divided massage responses into three gradations: response to handling, sensitivity to massage and ease of collection. These three behavioural characters were significantly correlated with semen volume. Furthermore, ease of collection was highly correlated with sperm concentration, sperm motility and testes weight. They also found that males that did not respond positively to handling or were less sensitive to massage appeared to produce less semen. Chelmonska (1972) reported a highly significant correlation (r = 0.8) between the number of positive reactions during the first 3 weeks of the reproductive season and average ejaculate volume during the entire season. In our experiment, there was a high correlation between average gander reaction scores and ejaculate volumes throughout the reproductive season in both breeds. We also found that by categorising Zi geese into low or high massage reaction scores, semen volume differed (0.21 vs 0.49 ml), as did motility (42.2 vs 66%) and semen concentration (380 × 10^6 vs 734 × 10^6/mL) (S. J. Liu, unpubl. data). Despite this, the relationship between response to massage and reproductive efficiency is not simple, as there are many other factors to consider. First, the dorso-abdominal massage is considered a less efficient method for geese compared with other poultry. Second, the frequency of positive reaction of unselected ganders ranged from 70 to 90% (Łukaszewicz 2010), therefore it can be inferred that not all the ganders that lack massage reaction are infertile, after both massage and semen quality selection, we can be assured of each male's status.

Fertility in naturally mating flocks depends on a series of factors, like male dominance status (Jones and Mench 1991), behavioural factors (Burke and Mauldin 1985; Wishart *et al.* 1992), individual semen quality (Perek 1966; Parker and McDaniel 2002; Bilcik and Estevez 2005), and others. The results of a DNA fingerprinting study verified that a large ejaculate volume and sperm mobility may be as important as male mating frequency in high reproductive success in males in a competitive environment (Bilcik and Estevez 2005). A field trial was undertaken to determine if flock reproductive efficiency could be improved by selecting ganders based on massage reaction, and ejaculate quality in Taihu geese gander selection criteria included a massage reaction score of 3, semen volume not less than 0.1 mL, semen concentration not less than 500 × 10^6/mL and motility not less than 60% (Zheng *et al.* 1986). There were different male/female ratios of 1 : 8 and 1 : 10 in the two treated groups. The control group received males selected solely on phallus physical appearance and the male/female ratio was 1 : 5. Compared with the control group, the flock fertility increased to 94.2% in the 1 : 8 sex ratio group (compared with 93.0% in the control group). However, in the 1 : 10 sex ratio group, the fertility dropped to 91.4%. Our results are compatible with these, as there was no difference in fertility in the experimental group, but there was a lower male/female ratio. There may be less frequent mating per goose in a group with a lower male/female ratio. With only fertile males in a flock, even if geese are mated less frequently compared with the control group, it is probable that they will produce a higher percentage of fertilised eggs.

The relationship between massage response of individual ganders and semen quality can help eliminate ganders with poor semen quality. Although this method does not give a complete measurement of fertility, it does offer a quick and reliable method by which the fertility of the whole flock can be improved. It might also lead to the ability to use a lower male to female ratio, allowing for maximised fertilising potential and profitability.

In conclusion, gander reproductive characteristics, including a positive reaction to massage and quality of the first ejaculates, determine the degree of gander fertility in breeding programs. A feasible method for diagnosing the fertility of male geese is the examination of massage reaction and laboratory assessment of semen quality early in the breeding season.

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