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Impact of Calcium, Zinc and Copper Concentrations in Seminal Plasma on Semen Parameters in Zie Geese

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Abstract: The relationship between Zinc (Zn), Calcium (Ca) and Copper (Cu) concentrations in seminal plasma and semen quality parameters was investigated in geese. Eighty, 1 year old Zie ganders (*Anser cygnoides* L.) were used. The ganders were ranked by their average Semen Quality Factor (SQF) value. Scores less than or greater than one standard deviation from the population mean were categorized as a low or high SQF phenotype (n = 10 males per phenotype), respectively. Seminal plasma was obtained by centrifuging semen samples. Zn, Ca and Cu concentrations in seminal plasma were determined using an atomic absorption spectrophotometer. Concentrations in seminal plasma were in the order Ca>Zn>Cu. There were positive correlations between the concentration of Ca with semen motility (0.47, $p<0.05$) and live morphologically undamaged sperm number (0.32, $p<0.05$). Cu and Zn concentrations were not correlated with any semen analysis parameters.

Key words: Zie goose, semen analysis parameters, seminal plasma, semen quality factor, China

INTRODUCTION

Domestic geese are seasonal breeders and amongst all the poultry species have the lowest semen quality, egg production, fertility and hatchability rates. Small ejaculate volume, low sperm concentration and a high percentage of malformed sperm cells characterize ganders (Lukaszewicz, 2010; Svetlik and Weis, 2004). On account of the extremely low percentage of morphologically normal sperm, the Semen Quality Factor (SQF) has been used as a predictor of gander fertilizing ability (Lukaszewicz and Kruszynski, 2003; Liu *et al*., 2008).

The quality of semen is affected by numerous factors. When gander semen is collected artificially, the ejaculate blends with lymph-like fluid on the surface of the everted cloaca. Geese lack accessory sex glands and deferent duct fluid and transparent fluid are chemically distinct, therefore an effective method for inferring extra-gonadal duct function is from analyzing seminal plasma composition (Al-Aghbari *et al*., 1992).

Fowl semen contains high concentrations of Zinc (Zn), Calcium (Ca) and Copper (Cu) in ionic and bound forms. Seminal plasma is vital because it provides a nutritive and protective environment for spermatozoa to survive (Cheah and Yang, 2011). Contact with toxic mineral elements in the seminal plasma can affect developing spermatozoa. Abnormal levels of these elements may affect spermatogenesis with respect to sperm production, maturation, motility and fertilizing potential (Wong *et al*., 2001).

Sperm motility, sperm concentration, morphology and SQF are semen quality parameters used to evaluate gander fertility. To date, there are no similar reports available in geese. Here, researchers investigated the relationship between three important mineral elements (Zn, Ca and Cu) and semen quality parameters by quantifying the concentrations in the seminal plasma of high and low SQF geese.

MATERIALS AND METHODS

Eighty, 1 year old Zie geese ganders of similar weight (4.0 kg) were used. All geese were raised under natural lighting and semi-open housing conditions. Birds were fed commercial rations for breeding geese (280-350 g day^{-1}), containing 10.80 MJ metabolizable energy and 140 g crude protein/kg. The ganders were trained to give semen 7 days before the collection began. Semen was collected by the abdominal massage method (Lake, 1957).

Semen was collected from all geese to determine semen parameters. The volume of semen was measured by

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reading the semen collection cup to the nearest 10 μL. Sperm concentration was measured by hemocytometer and motility was estimated using the Hang Drop Method at 400x magnification (Bakst and Cecil, 1997). Morphology of spermatozoa was evaluated after nigrosin-eosin staining (Bakst and Cecil, 1997; Siudzinska and Lukaszewicz, 2008). SQF values were calculated as the ejaculatedsemen volume for individual ganders (mL) x sperm concentration ($\times 10^6$ mL^{-1}) x live morphologically normal spermatozoa (%) (Lukaszewicz and Kruszynski, 2003).

Males were ranked by average SQF values in two successive trials any males with a score less than or greater than one standard deviation from the population mean was categorized as a low or high SQF phenotype (n = 10 males per phenotype), respectively.

Ejaculates were pipetted into 1.5 mL microcentrifuge tubes and centrifuged to procure seminal plasma. Seminal plasma samples in each group were frozen at -20°C for further analysis. Zn, Ca and Cu concentrations in seminal plasma samples were determined using an atomic absorption spectrophotometer. Data were analyzed using the statistical program (SPSS, Version 16). The results were given as Mean±SEM. The Mann-Whitney U-test was used to evaluate the significance of differences in characteristics of seminal plasma concentrations of trace elements between high and low SQF groups. Spearman's rank correlation coefficients were calculated to determine associations between Zn, Ca and Cu concentrations and semen parameters. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Semen parameters and the mineral concentrations (Zn, Ca and Cu) of seminal plasma from geese with different SQF are shown in Table 1. The sperm concentration, motility and the percentage of live morphologically undamaged sperm were significantly ($p<0.05$) higher in the high SQF males than that in the low SQF males. The concentration of Ca was significantly higher in the high SQF males than that in low the SQF males (24.8±0.17 vs. 7.8±0.08 ug kg^{-1}).

Table 1: Mineral concentrations of seminal plasma in geese of high or low Semen Quality Factor (SQF)

	Semen Quality Factor (SQF)	
Parameters	High (n = 10)	Low (n = 10)
Semen volume (mL)	0.31±0.04	0.27±0.04
Concentration (1×10^6 mL^{-1})	620.00±9.08^a	37.20±6.83^b
Live morphologically undamaged sperm (%)	40.80±1.60^a	17.40±1.20^b
Motility (%)	47.00±1.70^a	21.50±1.90^b
SQF	135.40±9.90^a	19.30±2.80^b
Seminal plasma concentration		
Zinc (ug kg^{-1})	6.40±0.20^a	6.40±0.19^a
Calcium (ug kg^{-1})	24.80±0.17^a	7.80±0.08^b
Copper (ug kg^{-1})	1.80±0.28^a	1.70±0.28^a

SQF = Sperm Quality Factor; SQF values were calculated as the ejaculated semen volume for individual ganders (mL) x sperm concentration ($\times10^6$ mL^{-1}) x live morphologically normal spermatozoa (%); ^{a,b}Means within a row with different superscripts differed significantly ($p<0.05$)

When studying the correlation between the elements Zn, Ca and Cu and the semen analysis parameters there were positive correlations between concentration of Ca with semen motility and the percentage of live morphologically undamaged sperm, being 0.47 ($p<0.05$) and 0.32 ($p<0.05$), respectively. Cu and Zn concentrations were not correlated with any semen analysis parameters.

Studies on the relationship between Ca and the semen analysis parameters report inconsistent results. Wong *et al.* (2001) showed neither beneficial nor adverse associations between Ca concentration and sperm parameters in fertile and subfertile males. Others have found a significant difference between Ca concentration in high and low sperm motility groups which are consistent with the results. Here, the higher the Ca concentration in seminal plasma, the higher the percentage of motile spermatozoa in gander semen. The increase in sperm motility is probably due to a rise in intracellular Ca in spermatozoa induced by increases in seminal Ca which raises cAMP concentrations that are associated with sperm motility (Wishart and Ashizawa, 1987). Kanyinji and Maeda (2010) demonstrated that additional dietary Ca elevated seminal Ca and lowered blood Ca which subsequently enhanced sperm motility.

Ca is important for sperm motility (Lindemann *et al.*, 1987), metabolism (Peterson and Freund, 1976) and acrosome reaction and fertilization (Yanagimachi and Usui, 1974). Meseguer *et al.* (2004) found intracellular concentrations of Ca were connected with sperm morphology. Semen samples with <5% normal sperm compared with samples with >11% normal sperm had a higher concentration of seminal Ca. This is in agreement with the results where there was a positive correlation between the percentage of live morphologically undamaged sperm and seminal Ca concentrations in gander semen samples.

Total content of Zn in fowl semen is high and has been found to be critical for spermatogenesis but there have been conflicting reports on the effect of seminal Zn on sperm quality. Amen and Al-Daraji (2011) found supplementation of broiler breeder males with Zn caused significant improvement in seminal plasma traits. Aghaei *et al.* (2010) concluded that Zn concentration of semen decreased with decreasing number and motility of spermatozoa. Xu *et al.* (1993) found that there was a significant positive correlation between sperm density

and the concentration of Zn in seminal plasma in normospermic subjects. There are however, some reports in which no significant correlations between Zn concentration in seminal fluid and sperm parameters were found (Wong *et al*., 2001; Lewis-Jones *et al*., 1996; Lin *et al*., 2000). Here, there was no difference in Zn concentrations of different SQF geese.

CONCLUSION

Semen quality parameters are affected by variations of seminal plasma Ca in ganders. Concentration of seminal plasma Ca is an important marker of geese sperm quality.

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