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ORIGINAL ARTICLE

Determination of calcium, magnesium, phosphorus, iron, and copper contents in rooster seminal plasma and their effects on semen quality

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Abstract
It has been documented that minerals affect male fertility and subsequent reproduction in human and animals. The aim of this investigation was to assess calcium (Ca), magnesium (Mg), phosphorus (P), iron (Fe), and copper (Cu) contents of rooster seminal plasma following normal feeding of diet and investigate their correlation with spermatozoa characteristics. Fifty-one semen samples were collected from ten roosters. Semen samples were evaluated concerning sperm motility and viability. Ca, P, Mg, Fe, and Cu contents of seminal plasma were evaluated by commercial kits. The mean concentrations of Ca, P, Mg, Fe, and Cu were 6.52 ± 0.4 mg/dl, 3.89 ± 0.18 mg/dl, 3.89 ± 0.2 mg/dl, 231.58 ± 6.23 μg/dl, and 425.40 ± 17.18 μg/dl, respectively. Forward progressive motility of spermatozoa was significantly associated with Ca ($r = 0.57$), Mg ($r = 0.42$), Fe ($r = 0.87$), and Cu ($r = 0.66$) concentrations of seminal plasma ($P < 0.05$). Also, there was a significant association between viability of spermatozoa and Ca ($r = 0.58$), Mg ($r = 0.41$), Fe ($r = 0.87$), and Cu ($r = 0.67$) contents of seminal plasma ($P < 0.05$). In conclusion, Ca, Mg, Fe, and Cu concentrations of seminal plasma may differ between roosters following feeding with the same diet and correlated with spermatozoa characteristics.

Keywords Minerals · Rooster · Semen parameters

Introduction

Semen evaluation plays an important role in poultry breeding for selecting males and monitoring their reproductive performance (Cheng et al. 2002). Minerals significantly modify the male fertility and subsequent reproduction (Smith and Akinbamijo 2000). Some of them are important for proper spermatozoa cell activities (e.g., sodium, potassium, calcium, magnesium); others are needed in relatively narrow limits (e.g., zinc, copper, manganese, cobalt, selenium, iron) (Massanyi et al. 2004). The impact of major biologically active minerals on spermatozoa viability parameters has been studied in animals and humans (Massanyi et al. 2008; Sorensen et al. 1999).

Ca stimulates motility activity of spermatozoa (Ashizawa and Wishart 1987). Ca is involved in chemotaxis, hyperactivation, capacitation, and the acrosome reaction of spermatozoa which are essential in fertilization process (Rahman et al. 2014). Ca is important for thermo-tolerance and cryosurvivability of spermatozoa (Kanyinji and Maeda 2010). P has multiple roles including reproduction. Decreased feed intake, fertility rate, irregular estrous cycles, decreased ovarian activity, increased occurrence of cystic ovaries, low conception rates, and delayed sexual maturity have been reported when P deficiency is seen (Bindari et al. 2013). Mg as a macromineral is necessary for biological procedures of body. Mg exerts positive effect on testosterone (Maggio et al. 2014).

Fe and Cu are important trace minerals playing important roles in fertility and productivity of flocks. Cu roles in fetal development (Hurley and Keen 1979), the cytochrome C oxidase (Mills and Williams 1962), superoxide dismutase (Prohaska and Wells 1975), oxidative stress (Zidenberg-Cherr and Keen 1991), and other cuproenzymes such as peptidyl glycine monooxygenases (Prohaska and Bailey 1995) have been identified. Cu is a cofactor for many enzymes including cytochrome c oxidase, superoxide dismutase, diamine oxidase, and tyrosinase (Uriu-Adams and Keen

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2005). Akinloye et al. (2011) showed that Cu had significant positive effects on semen volume. Iron as an essential micro mineral is involved in many physiologic functions of cells (Lieu et al. 2001), which are linked to spermatogenesis and spermatozoa metabolism (Wise et al. 2003). In addition, Nikolaev et al. (1998) stated that Fe has an important role in normal sperm production and semen characteristics. Deficiency or excess of either mineral causes adverse effects on genital system that eventually leading to reduced fertility (Tvrda et al. 2015).

Information related to mineral contents of seminal plasma in roosters is scant. This study was conducted to evaluate Ca, Mg, P, Fe, and Cu values of the seminal plasma in Ross 308 roosters and test if there is any relationship between these minerals and semen quality.

Material and methods

Birds and semen collection

Ten adult males (Ross 308) of 40-week-old were raised under standard conditions (14L: 10D; 18–22 °C) and fed with standard commercial diet for breeders (2750 Kcal ME/Kg, CP 12%, Met 0.32%, Lys 0.50%; Ca 0.7%, available P 0.35%, Na 0.16%, Cu 10 mg/Kg, Fe 50 mg/Kg; 142 g/d) according to the instructions of Ross broiler breeder nutrition guide 2013. Water prepared ad-libitum to the birds. The roosters were trained to give semen 3 weeks before the collection began. The semen was obtained using graduated collection tube via the abdominal massage method (Lake 1957) twice a week. The samples were taken carefully to avoid any contamination with cloacal contents and blood. Volume of each sample was measured in graduated collecting tubes and recorded on individual case report forms.

Semen analysis and measurements

The determination of percentage of spermatozoa with forward progressive motility and viable spermatozoa was conducted after collection. Forward progressive motility of spermatozoa was assessed by placing a portion of ejaculate diluted with 2.9% sodium citrate solution (1:200) on a slide with a coverslip being placed over the sample, with using an Olympus (BX41, Japan) compound light microscope (×400 magnification), equipped with a warm stage being used for spermatozoa assessments. For forward progressive motility percentage, 200 spermatozoa were evaluated. The percentage of sperms which show moderate to rapid forward movement indicate as motility (Ommati et al. 2013).

Staining of a part of semen samples with eosin-nigrosin solution was used to evaluate viability of the spermatozoa (Bakst and Cecil 1997). The stained seminal preparation was ready in duplicate, and 200 spermatozoa per slide were checked. The slides were evaluated for cell viability, where live sperms stain nothing. Concentration of spermatozoa was figured out by Neubauer hemocytometer.

The spermatozoa and the seminal plasma were separated by centrifugation. Semen was centrifuged for 10 min at 550*g*. The supernatant was centrifuged two more times, first for 10 min at 550 *g*, and then for 30 min at 3000*g*. The supernatant was considered to be the seminal plasma (Blesbois et al. 1993) and kept frozen (− 20 °C) until further analyses. Ca, Mg, P, Cu, and Fe concentrations of seminal plasma were measured by commercial kits (Zeist Chem Diagnostics, Iran) according to the manufacture instruction.

All equipment used for semen collection and measurement of minerals was first soaked in a hydrochloric acid solution for 24 h, rinsed thoroughly with double distilled water, allowed to air-dry, and then used for the experiment.

Statistical analysis

All percentage data were subjected to arcsine transformation. To assess the association between parameters of the semen with mineral contents of the seminal plasma, the Pearson correlation coefficient test was used. The data were categorized in three groups of excellent (with greater than 90% forward progressive motility), good (with forward progressive motility of 70–90%), and fair (with lesser than 70% forward progressive motility) according to their forward progressive motility of spermatozoa. The comparison of the semen parameters and mineral contents of the seminal plasma in excellent, good, and fair groups was carried out by one-way ANOVA. The data analyzed using SigmaStat software (version 3.5; Chicago, IL). Results are presented as the mean ± standard error. For all statistical analyses, differences with $P < 0.05$ were considered significant.

Results

The results of the semen characteristics as well as Ca, P, Mg, Fe, and Cu contents of seminal plasma of 51 samples are summarized in Table 1.

Forward progressive motility of spermatozoa was significantly associated with Ca ($r = 0.57$), Mg ($r = 0.42$), Fe ($r = 0.87$), and Cu ($r = 0.66$) concentrations of seminal plasma ($P < 0.05$). Also, there was a significant association between viability of spermatozoa and Ca ($r = 0.58$), Mg ($r = 0.41$), Fe ($r = 0.87$), and Cu ($r = 0.67$) contents of seminal plasma ($P < 0.05$). However, it showed a positive non-significant association between P contents and progressive motility ($r = 0.32$) and viability ($r = 0.34$) of spermatozoa.

In order to have a better judgment about the results, data were categorized in three groups of excellent ($n = 31$), good ($n = 9$), and fair ($n = 11$) quality according to their forward progressive motility of spermatozoa. The mean values for

Table 1 Characteristics of the rooster semen (Mean ± SEM); $n = 51$

Ejaculate volume (ml)	0.29 ± 0.02
Spermatozoa concentration ($\times 10^6$ cells/ml)	2.39 ± 0.2
Forward progressive motility (%)	81.49 ± 2.6
Viability (%)	86.74 ± 2.17
Seminal plasma calcium (mg/dl)	6.52 ± 0.4
Seminal plasma magnesium (mg/dl)	3.89 ± 0.2
Seminal plasma phosphorus (mg/dl)	3.89 ± 0.18
Seminal plasma iron (μg/dl)	231.58 ± 6.23
Seminal plasma copper (μg/dl)	425.40 ± 17.18

progressive motility were recorded as 93.28 ± 0.43% in excellent, 79.55 ± 1.76% in good, and 49.9 ± 3.78% in fair groups, which were significantly different ($P < 0.001$). The comparison of the data of the excellent, good, and fair groups is presented in Table 2.

In excellent group, correlation between progressive motility and Ca, P, Mg, Fe, and Cu was 0.13, 0.10, 0.08, 0.07, and 0.03, respectively ($P > 0.05$). Also, there were non-significant association between progressive motility and spermatozoa concentrations and semen volume ($P > 0.05$), but the association with viability was significant ($r = 0.91$; $P < 0.001$).

In good group, there was non-significant association between progressive motility and Ca ($r = 0.51$), P ($r = 0.11$), Mg ($r = 0.60$) Fe ($r = 0.51$), and Cu ($r = 0.60$) contents of seminal plasma ($P > 0.05$). Furthermore, correlation between progressive motility and semen volume and spermatozoa concentrations was not significant ($P > 0.05$).

In fair group, there was non-significant association between progressive motility and Ca ($r = -0.18$), P ($r = 0.11$), Mg ($r = 0.28$), Fe ($r = 0.21$), and Cu ($r = 0.27$) contents of seminal plasma ($P > 0.05$). The progressive motility showed positive association with viability ($r = 0.91$; $P < 0.001$), while no significant association was observed between spermatozoa concentrations and semen volume ($P > 0.05$).

Discussion

The present study was designated to evaluate the relationship between seminal plasma minerals and semen characteristics in roosters. The findings of this study showed that the concentrations of Ca, Mg, Fe, and Cu in seminal plasma were correlated positively with forward progressive motility and viability of spermatozoa.

Male fertility is one of the important factors affecting productivity of breeder flocks. Minerals are crucially important in the fertility of males. There are a few similar reports in the literature about mineral contents of rooster semen and their relationship with semen characteristics. Studies focused on the addition of minerals to ration to describe their effects on semen characteristics.

Our results indicate a positive association between Ca and progressive motility ($r = 0.57$) and viability ($r = 0.58$) of spermatozoa. Aghaei et al. (2010) reported that seminal calcium contents of indigenous roosters with low, medium, and high progressive motility groups were 5.21 ± 1.04, 5.47 ± 0.92, and 10.11 ± 2.36 mg/dl, respectively. And positive correlation was observed between Ca contents of seminal plasma and progressive motility. The positive effects of Ca and Mg on the motility and morphology of spermatozoa were reported (Eghbali et al. 2010a). In the present experiment, calcium contents (6.52 ± 0.40 mg/dl) of seminal plasma within the range of values were published by Aghaei et al. (2010). Kanyinji and Maeda (2010) observed that addition of 2% Ca to rooster ration causes higher spermatozoa motility. They noted that increase in spermatozoa motility in males fed 2% added Ca may be linked to a reduction in blood cholesterol that optimized the functions of the Leydig and Sertoli cells, leading to production of spermatozoa with high membrane

Table 2 Comparison of the semen parameters (Mean ± SEM) of roosters allocated in excellent, good, and fair groups according to the forward progressive motility of spermatozoa

Variable	Groups			
	Excellent ($n = 31$)	Good ($n = 9$)	Fair ($n = 11$)	P value
Forward progressive motility (%)	93.28 ± 0.43^a	79.55 ± 1.76^b	49.9 ± 3.78^c	< 0.001
Viability (%)	96.67 ± 0.39^a	84.66 ± 1.82^b	60.45 ± 2.94^c	< 0.001
Concentration ($\times 10^9$ cells/ml)	2.89 ± 0.26^a	2.05 ± 0.43^{ab}	1.23 ± 0.43^b	0.004
Volume (ml)	0.35 ± 0.2^a	0.20 ± 0.03^b	0.22 ± 0.05^b	0.011
Calcium contents (mg/dl)	7.63 ± 0.45^a	6.56 ± 0.78^a	3.36 ± 0.57^b	< 0.001
Phosphorus contents (mg/dl)	3.96 ± 0.18^a	4.17 ± 0.61^a	3.47 ± 0.45^a	0.43
Magnesium contents (mg/dl)	4.26 ± 0.24^a	4.11 ± 0.59^a	2.68 ± 0.16^b	0.005
Iron contents (μg/dl)	258.92 ± 3.78^a	227.04 ± 4.58^b	158.26 ± 4.15^c	< 0.001
Copper contents (μg/dl)	488.41 ± 17.57^a	386.54 ± 31.67^b	279.64 ± 14.39^c	< 0.001

Different superscript letters (a, b, and c) denote a significant difference within a row ($P < 0.05$)

integrity. They also cited that adding 4% Ca to the rooster diet suppressed spermatozoa motility, which may be due to effects of glutamate which induces spermatozoa mitochondrial degeneration. Increased cAMP concentrations that are linked with spermatozoa motility following administration of Ca to the diet provide another explanation for spermatozoa motility (Kopf et al. 1983; Wishart and Ashizawa 1987). Stegmayr and Ronquist (1982) evaluated effects of Mg, Ca, and Zn ions on sperm motility. They stated that Mg and Zn have greatest and lowest stimulatory effects, respectively. Jarinkovičova et al. (2012) observed negative correlations between concentration of Mg in blood plasma and frequency of morphologically defective spermatozoa of cocks and a positive relationship between Mg concentrations and spermatozoa motility. Calcium and P concentrations in blood plasma showed any significant correlation with ejaculate parameters. They concluded that Mg has key role in quality of ejaculate in layers.

It has been demonstrated that iron positively affects the motility and morphology of spermatozoa (Eghbali et al. 2010b). Our study showed that concentrations of Fe in plasma of semen were positively correlated with forward progressive motility of spermatozoa. Under ROS over-production or in a state of Fe deficiency, the motility of spermatozoa will be lost (Loganathasamy 2012). Some pathways related to Fe metabolism in cells are linked to spermatogenesis (Hales 2010; Metzendorf and Lind 2010). Tvrda et al. (2012) indicated that Fe contents of bovine seminal plasma and sperm motility were positively associated, as seen in our result. An in vitro study showed that Fe (≤ 250 μM/L FeSO4.7H2O) sustained sperm motility and energy metabolism. Inversely, low doses of Fe increased sperm motility (≤ 62.50 μM/L FeSO4.7H2O) (Knazicka et al. 2012).

It has been demonstrated that concentrations of Cu in plasma of semen in low, medium, and high progressive motility were 3.18 ± 0.67, 7.24 ± 1.24, and 12.25 ± 2.83 μg/ml, respectively, and positive correlation was reported among seminal plasma concentrations (Aghaei et al. 2010). These findings were in agreement with the present study. In another study, copper contents of seminal plasma were 6.79 ± 6.42 μg/ml in roosters (Massanyi et al. 2008). In our findings, concentrations of copper (425.40 ± 17.18 μg/dl) in seminal plasma were consistent with those of others (Aghaei et al. 2010). Saleh et al. (2008) indicated that concentration of Cu in the seminal plasma in azoospermic males was lower than healthy controls. Wong et al. (2001) demonstrated a positive correlation between blood Cu levels and sperm motility. Furthermore, Machal et al. (2002) reported that Cu concentration in bovine blood plasma has a positive effect on the spermatozoa count and progressive motility. Decreased spermatozoa count and motility have also been reported in Cu deficient rats (Lyubimov et al. 2004).

It could be concluded that concentrations of Ca, Mg, and Cu in seminal plasma after normal feeding of broiler breeders may affect the semen characteristics in roosters. Additional research should be conducted to determine benefits of these minerals on fertility of males, hatchability of eggs, and productivity of breeder flocks.

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Compliance with ethical standard

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed in this study.

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