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Copper, phosphorus and calcium in bovine blood and seminal plasma in relation to semen quality*

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ABSTRACT

The mean Cu concentration in blood plasma of bulls of 12 Holstein and 13 Czech Spotted breeds was 22.27 μmol.l^{-1}; P, 1.78 mmol.l^{-1}; Ca, 3.04 mmol.l^{-1}; P:Ca ratio, 0.64. The mean Cu concentration in seminal plasma of bulls was 38.17 μmol.l^{-1}; P, 12.07 mmol.l^{-1}; and Ca, 3.52 mmol.l^{-1} and the P:Ca ratio was 3.31. The relationship between Cu, P and Ca concentration in blood and seminal plasma is expressed by calculated phenotypic coefficients of correlation. Highly significant positive coefficients of correlation ($P<0.01$) were found between the Cu, P and P:Ca ratio in blood and seminal plasma (Cu - r_p=0.36, P - r_p=0.32 and P:Ca ratio r_p=0.28). In contrast, no positive relationship between the Ca concentration in blood plasma and seminal plasma was found; the calculated coefficient of correlation was negative (r_p=-0.16). This was probably caused by the higher Ca concentration in blood plasma of bulls.

Statistically significant ($P<0.05$) positive coefficients of correlation were found between the Cu concentration in blood plasma and the total number of sperm in ejaculate (r_p= 0.33), between the Cu concentration in blood plasma and the total number of sperm with progressive motility (r_p=0.35), and between the Ca concentration and monthly value of sperm motility (r_p=0.26).

Positive coefficients of correlation were found between the Cu concentration in seminal plasma and the mean monthly volume of ejaculate (r_p=0.36, $P<0.01$), between the Cu concentration in seminal plasma and sperm motility (r_p=0.33, $P<0.05$), and Cu concentration in seminal plasma and total number of sperm with progressive motility in ejaculate (r_p=0.28, $P<0.05$).

The P concentration in seminal plasma of bulls was positively correlated with the sperm concentration in ejaculate collected on test days (r_p=0.36, $P<0.01$). The correlation between the P:Ca ratio in seminal plasma and the sperm concentration in ejaculate collected on test days was positive (r_p=0.39, $P<0.01$) and so was the correlation between the P:Ca ratio and the mean monthly sperm concentration (r_p=0.28, $P<0.05$). The calculated coefficient of correlation between the Ca concentration in seminal plasma and the mean monthly sperm motility was negative (r_p=-0.38, $P<0.01$).

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It can be concluded that there is a positive relationship between the Cu and P concentrations and P:Ca ratio in seminal plasma of bulls and the quality and quantity parameters of ejaculate of active stud bulls. A higher concentration of Ca in seminal plasma negatively affected mainly sperm motility in this study.

KEY WORDS: bulls, copper, phosphorus, calcium, blood plasma, seminal plasma, sperm cells, ejaculate

INTRODUCTION

The observation of ejaculate quality and quantity of stud bulls kept in an AI station revealed that the quality of ejaculate of the same bull changes depending on internal and external conditions.

A number of authors have pointed to the role of minerals in sperm. Wong et al. (2001) showed the essential role of minerals in spermatogenesis and fertility. Sorensen et al. (1999) highlighted the effect of calcium in seminal plasma. They found a statistically significant difference in sperm motility in ejaculates with high and low calcium contents. Also Dragileva et al. (1999) stressed the role of calcium in the process of capacitation.

The copper content of the semen also plays a meaningful role. Jockenhovel et al. (1990) found a statistically significant positive correlation between copper concentration in semen and progressive motility of sperm cells (r=0.23). Dhami et al. (1994) reported lower copper concentrations in ejaculates without motility than in motile ejaculates. Leonhard-Marek (2000) and Dhami et al. (1994) stressed the impact of copper on sperm motility. Gamčík et al. (1992) determined the concentration of calcium, 8.48 $\mu mol.l^{-1}$ and phosphorus, 26.48 $mmol.l^{-1}$, in whole semen.

The level of minerals is different in semen plasma and blood plasma. Dhami et al. (2001) pointed out the negative associations of zinc and copper with blood and semen. On the other hand, Wong et al. (2001) found a positive correlation (r=0.25) between the copper concentration in blood plasma and sperm motility.

Plasma copper levels of cattle were reported by Vrzgula et al., 1990 (12.6-18.9 $\mu mol.l^{-1}$). The calcium concentration ranged from 2.25 to 3.00 $mmol.l^{-1}$. Reece (1998) presented values in the range of 4.50-6.00 $mmol.l^{-1}$ for dairy cows. The mean phosphorus concentration reported by Vrzgula et al. (1990) ranged from 1.60 to 2.26 $mmol.l^{-1}$, and by Reece (1998) between 1.00 and 3.5 $mmol.l^{-1}$.

This study deals with the relationship between calcium, copper and phosphorus concentrations in blood and seminal plasma and concentration, sperm motility and volume of ejaculate in breeding bulls of Holstein and Czech Spotted breeds.

MATERIAL AND METHODS

Observations of the relationship between concentration of calcium, copper and phosphorus in blood and seminal plasma and concentration, sperm motility and volume of ejaculate were carried out in an experimental group of 25 stud bulls (12 Holstein and 13 Czech Spotted breeds) aged from 4 to 6 years. The bulls were actively used for collection of ejaculate in AI stations. All of the bulls were housed in a tethered housing system where the same daily regime was applied and their ejaculate was collected regularly (twice a week) for production of insemination doses. They were fed identical rations based on grass hay, silage, straw and mixed concentrate feed - the ration was calculated according to recommendations of Sommer et al. (1994). The bulls were fed two times daily.

The observation proceeded for three months, from December 2000 to February 2001. The ejaculate was periodically collected (twice a week) and the following parameters were determined: sperm motility and concentration, volume of ejaculate, total number of sperm in ejaculate (TNS) and total number of motile sperm in ejaculate (TNMS). After dilution of the native ejaculate with physiological solution (1:1) at a temperature of 37±1°C the percentage of motile sperm cells (progressive movement forward) was assessed by a subjective method. The sperm concentration was assessed haemocytometrically in Bürker's cellules.

In addition, blood samples were taken monthly and were analysed for Cu, P and Ca concentration in blood plasma. Blood was always taken from the median caudal vein (*vena caudalis mediana*) between 8 and 11 a.m. into test tubes coated with heparin at a ratio of 0.05 ml heparin to 5.00 ml blood. The concentration of some minerals (Cu, P and Ca) was determined in blood plasma colorimetrically by means of Bio-LA kits (Lachema, Czech Republic) within 24 h. In December and February the ejaculates were taken shortly before blood samples in order to determine actual sperm motility, concentration, and volume of ejaculate. Also, the ejaculates were centrifuged (2,500 rpm) in order to separate seminal plasma. Cu, P and Ca concentrations were determined in seminal plasma using Bio-LA sets (Pliva, Lachema, Czech Republic) procedures.

The results were worked up using mathematical and statistical methods according to Venčikov and Venčikov (1977). All of the presented results in tables are mean values (with standard deviation). The results obtained were subjected to statistical analysis using Student's t–test; the relationships between concentration of minerals in blood plasma and concentration of minerals in seminal plasma and their correlation with concentration, sperm motility and volume of ejaculate were assessed by calculated phenotypic coefficients of correlation.

RESULTS AND DISCUSSION

The mean Cu concentration in blood plasma of bulls during the whole experimental period was 22.27 μmol.l^{-1} and ranged from 15.99 to 28.04 μmol.l^{-1} in individual months (Table 1). The mean P concentration in blood plasma was 1.78 mmol.l^{-1} and ranged from 1.42 to 2.02 mmol.l^{-1} in individual months. The mean Ca concentration in blood plasma of bulls was 3.04 mmol.l^{-1} during the whole experimental period and ranged from 2.94 to 3.09 mmol.l^{-1} in individual months. The P:Ca ratio was 0.64. The Cu concentration value was higher than the generally acknowledged range of values for bulls, 12.6 μmol.l^{-1} to 18.9 μmol.l^{-1} given by Vrzgula et al. (1990). Similarly, the Ca concentration was also higher than the values presented by Vrzgula et al. (1990), 2.25 mmol.l^{-1} to 3.00 mmol.l^{-1}. Reece (1998) reported a range of 4.5 to 6.00 mmol.l^{-1} for dairy cows. The mean P concentration is in keeping with the values presented by Vrzgula et al. (1990) and Reece (1998). The P:Ca ratio was 0.64.

TABLE I

The average concentrations of the copper, phosphorus and calcium of the blood and seminal plasma on test day (t-test)

Month	n		Blood plasma				Seminal plasma			
			Cu μmol.l^{-1}	P mmol.l^{-1}	Ca mmol.l^{-1}	P:Ca	CU μmol.l^{-1}	P mmol.l^{-1}	Ca mmol.l^{-1}	P:Ca
XII.2000	25	x	28.04	1.89	3.09	0.69	41.93	11.49	3.52	3.27
		s_x	7.34	1.32	0.72	0.52	15.24	3.07	0.36	0.86
I.2001	25	x	21.80	2.02	3.08	0.68	–	–	–	–
		s_x	7.57	0.90	0.45	0.35	–	–	–	–
II.2001	25	x	15.99	1.42	2.94	0.45	33.92	12.89	3.52	3.38
		s_x	6.91	0.71	0.53	0.23	9.51	2.70	0.49	1.06
TOTAL	75	x	22.27	1.78	3.04	0.64	38.17	12.07	3.52	3.31
		s_x	8.77	1.04	0.58	0.52	13.72	3.00	0.42	0.92

The mean Cu concentration in seminal plasma of bulls over the whole observation period was 38.17 μmol.l^{-1}, P 12,07 mmol.l^{-1} and Ca 3.52 mmol.l^{-1}. The P:Ca ratio in seminal plasma was 3.31. Ca and P concentrations in seminal plasma were lower than the concentration in whole ejaculate (Ca - 8.48 μmol.l^{-1}, P -26.48 mmol.l^{-1}) reported by Gamčík et al. (1992); the P:Ca ratio corresponds with values given by Gamčík et al. (1992).

While the Cu and Ca concentrations in blood plasma correspond with their concentrations in seminal plasma, the P concentration in seminal plasma was 6.8 ti-

TABLE 2

The average values of the quality and quantity parameters of ejaculate of the Holstein and Czech Spotted bulls on test days and average monthly values (t - test)

Month		Average semen guality in day of blood collection							Average monthly semen quality					
			Sperm						Sperm					
		n	ejaculate volume cm^3	concen-tration $.10^9 . cm^{-3}$	sperm motility %	TNS $. 10^9$	TNMS $. 10^9$	n	ejaculate volume cm^3	concen-tration $.10^9 . cm^{-3}$	sperm motility %	TNS $. 10^9$	TNMS $. 10^9$
XII.2000	x	25	5.47	1.16	67.1	6.32	4.23	225	10.00	1.25	70.2	12.74	8.98
	s_x		2.39	0.41	5.7	3.39	2.28		4.19	0.23	2.9	5.85	4.10
I.2001	x		–	–	–	–	–	225	8.16	1.22	68.5	10.27	7.10
	s_x		–	–	–	–	–		4.06	0.22	5.4	5.67	4.03
II.2001	x	25	6.00	1.30	67.5	7.65	5.24	200	6.87	1.64	68.0	10.51	7.27
	s_x		3.34	0.52	8.3	5.28	3.78		2.75	2.75	1.2	6.21	5.57
TOTAL	x	50	5.71	1.22	67.3	6.96	4.65	650	8.31	1.37	68.9	11.13	7.76
	s_x		2.81	0.46	33.3	4.32	3.04		3.92	0.73	5.2	5.80	4.14

mes higher than that in blood plasma. This indicates a higher requirement of P in ejaculate.

The mean quality and quantity parameters of ejaculate collected on a test day (when blood samples were taken) and mean monthly values are shown in Table 2. The mean sperm concentration on test day ($1.22 \cdot 10^9 \cdot cm^{-3}$) was lower than the sperm concentration during the whole observation period ($1.37 \cdot 10^9 \cdot cm^{-3}$). The mean motility on test days was 67.3 %, while the monthly motility value was 68.9%. Similarly the mean volume of ejaculate collected on test days (when blood samples were taken) was lower (5.71 cm^3) than the mean monthly value (8.31 cm^3). This was due to the subsequently repeated collection of sperm (so-called "double mount") that is performed in order to obtain a larger volume of sperm. This factor also affected the difference between the total number of sperm cells on test days ($6.96 \cdot 10^9$) and the mean monthly value ($11.13 \cdot 10^9$) and also the total number of sperm cells with progressive motility in ejaculate ($4.65 \cdot 10^9$ vs $7.76 \cdot 10^9$). The inquest values are similar to those found in bulls before, i.e. sperm concentration ($1.13 \cdot 10^9 \cdot cm^{-3}$), motility 69.3%, volume of ejaculate (6.89 cm^3), respectively (Máchal, 2001).

The relationship between the Cu, P and Ca concentration in blood and seminal plasma is expressed by calculated phenotypic coefficients of correlation (Table 3). Statistically highly significant positive coefficients of correlation ($P<0.01$) were found between the Cu, P and P:Ca ratio in blood and seminal plasma (Cu, $r_p=0.36$, P, $r_p=0.32$ and P:Ca ratio, $r_p=0.28$). In contrast, no positive relationship between the Ca concentration in blood plasma and seminal plasma was found; the calculated coefficient of correlation was negative ($r_p=-0.16$). This was probably caused by the higher Ca concentration in blood plasma of bulls.

TABLE 3

Phenotypic correlations between copper, phosphorus, calcium in blood plasma and seminal plasma (50 bulls)

In blood	Seminal plasma			P:Ca
	Cu	P	Ca	
Cu	0.36++			
P		0.32++		
Ca			-0.16	
P:Ca				0.28++

++ $P<0.01$

The calculated phenotypic coefficients of the correlation between Cu, P and Ca concentration, P:Ca ratio in blood and seminal plasma and parameters of ejaculate quality on test days (when samples of blood and ejaculate were taken) are shown in Table 4. Phenotypic correlations between Cu, P, Ca, P:Ca ratio in blood and se-

TABLE 4

Calculated phenotypic coefficients of correlation of copper, phospohorus, calcium concentrations in blood plasma and seminal plasma to values of the ejaculate quality in the bulls on test days

Month	Indices	n	Blood plasma				Seminal plasma			
			Cu	P	Ca	P:Ca	Cu	P	Ca	P:Ca
XII.2000	Ejaculate volume	25	0.00	-0.10	0.08	-0.06	-0.24	-0.07	-0.10	-0.04
	Sperm concentration	25	0.51++	0.46+	-0.05	0.43+	0.11	0.40+	0.02	0.43+
	Sperm motility	25	0.14	0.30	-0.02	0.31	-0.22	0.02	-0.04	0.04
	TNS	25	0.37	0.12	0.03	0.16	-0.11	0.09	-0.16	0.15
	TNMS	25	0.38	0.18	0.01	0.22	-0.17	0.10	-0.16	0.16
II.2001	Ejaculate volume	25	0.31	-0.28	-0.08	-0.21	0.47+	-0.31	-0.22	-0.21
	Sperm concentration	25	0.19	-0.30	0.30	-0.42+	0.13	0.30	-0.12	0.37
	Sperm motility	25	0.10	-0.06	0.28	-0.19	0.35	0.21	-0.51++	0.34
	TNS	25	0.42+	-0.43+	0.12	-0.46+	0.51++	-0.01	-0.31	0.13
	TNMS	25	0.42+	-0.43+	0.15	-0.47+	0.53++	0.02	-0.36	0.18
TOTAL	Ejaculate volume	50	0.10	-0.14	0.01	-0.10	0.08	-0.14	-0.13	-0.16
	Sperm concentration	50	0.24	0.16	0.13	0.09	0.08	0.36++	-0.02	0.39++
	Sperm motility	50	0.15	0.09	-0.15	-0.08	0.15	0.11	-0.22	0.13
	TNS	50	0.33+	-0.06	0.08	-0.06	0.05	0.06	-0.19	0.16
	TNMS	50	0.35+	-0.04	0.08	-0.04	0.03	0.07	-0.22	0.16

+ $P<0.05$; ++ $P<0.01$

minal plasma and mean values of parameters of ejaculate quality during the whole month are presented in Table 5.

Analyses of the relationship between the Cu, P and Ca concentration in blood plasma of bulls and the actual values of ejaculate quality (collected on the same day as blood samples) revealed statistically significant ($P<0.05$) positive coefficients of correlation only between the Cu concentration and the total number of sperm cells in ejaculate ($r_p=0.33$), between the Cu concentration and the total number of sperm cells with progressive motility ($r_p=0.35$) and between the Ca concentration and the monthly value of sperm motility ($r_p=0.26$). These findings are in agreement with results of Jockenhovel et al. (1990) and Wong et al. (2001).

More significant relationships were found between the Cu, P and Ca concentration in seminal plasma and parameters of quality and volume of ejaculate. A statistically highly significant ($P<0.01$) positive coefficient of correlation was found between the Cu concentration in seminal plasma and the mean monthly volume of ejaculate ($r_p=0.36$) and statistically significant ($P<0.05$) positive coefficients of correlation between the Cu concentration in seminal plasma and sperm motility ($r_p=0.33$) and the Cu concentration in seminal plasma and the total number of sperm cells with progressive motility in ejaculate ($r_p=0.28$). These results correspond with the studies of Dhami et al. (1994) and Leonhard-Marek (2000).

Similarly, a highly significant ($P<0.01$) positive coefficient of correlation ($r_p= 0.36$) was found between the P concentration in seminal plasma of bulls and the sperm concentration in ejaculate collected on test days. The coefficient of correlation between the P concentration in seminal plasma of bulls and the mean monthly sperm concentration was positive ($r_p=0.22$), however, this relationship was not statistically significant. A highly significant ($P<0.01$) positive coefficient of correlation was found between the P:Ca ratio in seminal plasma and the sperm concentration in ejaculate collected on test days ($r_p=0.39$); the P:Ca ratio was significantly ($P<0.05$) positively correlated to the mean monthly sperm concentration ($r_p=0.28$).

A statistically highly significant ($P<0.01$) negative coefficient of correlation was found between the Ca concentration in seminal plasma and mean monthly sperm motility ($r_p=-0.38$). A negative coefficient of correlation was calculated between the Ca concentration in seminal plasma and sperm motility in ejaculate collected on test days ($r_p=-0.22$), however, this coefficient was not statistically significant.

It can be concluded that there is a positive relationship the between Cu and P concentration and P:Ca ratio in seminal plasma of bulls and the quality and quantity parameters of ejaculate of active stud bulls. In this study, a higher concentration of Ca in seminal plasma negatively affected mainly sperm cell motility.

TABLE 5

Calculated phenotypical correlations of copper, phospohorus, calcium concentrations in blood and seminal plasma to average monthly ejaculate parameters in the bulls

Month	Indices	n	Blood plasma				Seminal plasma				
			Cu	P	Ca	P:Ca		Cu	P	Ca	P:Ca
	Ejaculate volume	25	-0.16	0.29	-0.29	0.44+	25	0.14	0.04	0.12	-0.03
XII.2000	Sperm concentration	25	0.51++	0.16	0.32	0.09	25	0.00	0.40+	0.42+	0.23
	Sperm motility	25	0.17	0.08	0.10	0.06	25	0.06	0.09	0.25	-0.01
	TNS	25	0.05	0.25	-0.09	0.41+	25	0.10	0.13	0.20	0.03
	TNMS	25	0.05	0.25	-0.08	0.41+	25	0.09	0.12	0.21	0.02
	Ejaculate volume	25	0.20	0.13	0.00	0.08		–	–	–	–
I.2001	Sperm concentration	25	0.16	-0.08	0.22	-0.17		–	–	–	–
	Sperm motility	25	0.19	0.15	0.18	0.05		–	–	–	–
	TNS	25	0.23	0.15	0.03	0.09		–	–	–	–
	TNMS	25	0.23	0.17	0.05	0.10		–	–	–	–
	Ejaculate volume	25	0.36	-0.26	0.14	-0.26	25	0.48+	-0.32	-0.28	-0.20
II.2001	Sperm concentration	25	-0.26	-0.18	-0.10	-0.14	25	0.11	0.07	-0.18	0.16
	Sperm motility	25	0.11	-0.11	0.34	-0.25	25	0.44+	0.06	-0.51++	0.23
	TNS	25	0.03	-0.37	0.03	-0.33	25	0.38	-0.05	-0.36	0.11
	TNMS	25	0.03	-0.37	0.05	-0.35	25	0.42+	-0.04	-0.41+	0.13
	Ejaculate volume	75	0.21	0.19	-0.01	0.24	50	0.36++	-0.24	-0.18	-0.21
TOTAL	Sperm concentration	75	-0.16	-0.10	-0.04	-0.10	50	-0.05	0.22	0.05	0.28+
	Sperm motility	75	0.16	0.06	0.26+	-0.01	50	0.33+	-0.02	-0.38++	0.13
	TNS	75	0.13	0.09	0.01	0.13	50	0.25	0.01	-0.10	0.07
	TNMS	75	0.14	0.10	0.03	0.12	50	0.28+	0.00	-0.13	0.07

+ P<0.05; ++ P<0.01

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STRESZCZENIE

Koncentracja miedzi, fosforu oraz wapnia w plazmie krwi i nasieniu buhajów; stosunek do jakości

Przeciętna koncentracja miedzi w plazmie krwi buhajów wynosiła 22,27 μmol.l^{-1}, fosforu 1,75 mmol.l^{-1}, wapnia 3,04 mmol.l^{-1}, a stosunek fosforu do wapnia wynosił 0,64. Stwierdzona przeciętna koncentracja miedzi w nasieniu wynosiła 38,17 μmol.l^{-1}, wapnia 3,52 mmol.l^{-1}, fosforu 12,07 mmol.l^{-1}, a stosunek fosforu i wapnia 3,31. Stosunek koncentracji miedzi, fosforu i wapnia w plazmie krwi oraz w nasieniu został określony poprzez obliczone korelacje fenotypowe. Między koncentracją miedzi, fosforu i stosunkiem fosforu i wapnia w plazmie krwi i w nasieniu otrzymano statystycznie istotne ($P<0,01$) dodatnie korelacje (miedź $r_p=0,36$, fosfor $r_p=0,32$; stosunek fosforu do wapnia $r_p=0,28$). Stwierdzono statystycznie nieistotną ujemną korelację ($r_p=-0,16$) pomiędzy koncentracją wapnia w plazmie krwi i w nasieniu.

Otrzymano statystycznie istotne dodatnie korelacje ($P<0,05$) między koncentracją miedzi w plazmie krwi i ogólną liczbą plemników w nasieniu, $r_p=0,33$, między koncentracją miedzi w plazmie krwi i ogólną liczbą plemników z progresywną ruchliwością, $r_p= 0,35$ oraz między koncentracją wapnia w plazmie krwi i miesięczną ruchliwością plemników, $r_p= 0,26$.

Stwierdzono dodatnią korelację między koncentracją miedzi w nasieniu i przeciętną miesięczną objętością ejakulatu -$r_p=0,36$ ($P<0,01$), między koncentracją miedzi w nasieniu i ruchliwością plemników, $r_p=0,33$ ($P<0,05$), między koncentracją miedzi w nasieniu i ogólną liczbą plemników z progresywnym ruchem w nasieniu, $r_p=0,28$

Koncentracja fosforu w nasieniu buhajów była dodatnio skorelowana z koncentracją plemników w nasieniu pobranym w dniu pobierania krwi, $r_p=0.36$ ($P<0,01$). Stosunek fosforu do wapnia w nasieniu do koncentracji plemników w nasieniu pobranym w dniu pobierania krwi był dodatni, $r_p=0,39$ ($P<0,01$), podobnie jak stosunek fosforu do wapnia do przeciętnej miesięcznej koncentracji plemników, $r_p=0,28$ ($P<0,05$). Między koncentracją wapnia w nasieniu i przeciętną miesięczną ruchliwością plemników otrzymano ujemną korelację, $r_p=-0,38$ ($P<0,01$).

W podsumowaniu stwierdzono, że istnieje dodatnia korelacja pomiędzy koncentracją miedzi i fosforu a stosunkiem fosforu do wapnia w nasieniu buhajów oraz jakościowymi i ilościowymi cechami nasienia u buhajów hodowlanych. Większa koncentracja wapnia w nasieniu miała negatywny wpływ na ruchliwość plemników.