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The effect of zinc supplementation on zinc content in blood serum and seminal plasma and on the quality of stallion semen

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Summary

For 90 days (30+30+30) 26 Primitive Polish Horse stallions (12+5+9) underwent 3 experiments. In the first one, the stallion diet containing 30 mg Zn/kg d.m. (dry matter) was enriched with $ZnSO_4 \cdot 7H_2O$ to 45 mg (n=4) and 100 mg Zn/kg d.m. in fodder (n=4), respectively. In the second experiment, 3 stallions fed on 22 mg Zn daily were added $ZnSO_4 \cdot 7H_2O$ at the same time up to 100 mg Zn/kg d.m. in fodder. In the third experiment, the diet of 3 stallions, containing initially 26 mg Zn/kg and 1.25% Ca in d.m. (after adding $CaCO_3$), was enriched with $ZnSO_4 \cdot 7H_2O$ up to 161 mg Zn/kg of d.m., and 3 other stallions were added only $CaCO_3$ up to 1.25% Ca in d.m. The remaining 9 stallions (4+2+3) were a control group, without any mineral supplements. The experiments examined the influence of 30 day zinc supplemenation on the content of this element in blood serum and seminal plasma as well as on the quality of stallion semen. The experiments revealed that increased supply of zinc in the quantity of 100 mg/kg of d.m. influenced the change of Zn concentration in seminal plasma. Changes in some indexes of the semen quality were related to it, especially a statisticant increase in spermatozoa concentration in the stallion semen was reported. Enrichment of the diet containing excessive amount of calcium with zinc, in turn, only influenced the zinc concentration in the seminal plasma of the stallions positively. The applied zinc supplement caused the semen to deteriorate as much as it was the case in the group of stallions fed with the diet containing 1.25% Ca.

keywords: stallion, Zn supplement, Zn blood serum, Zn seminal plasma, sperm-quality

Der Einfluß des Supplements von Zink auf den Zinkgehalt im Blutserum und Samenplasma und auf die Sameneigenschaften von Hengsten

Innerhalb von 90 Tagen (30+30+30) wurden drei Versuche an 26 Hengsten der Rasse Primitives Polnisches Pferd (12+5+9) durchgeführt. Im ersten Versuch wurde die 30 mg Zn/kg Trockenmasse enthaltende Diät der Hengste mit $ZnSO_4 \cdot 7H_2O$ entsprechend auf 45 mg (n=4) und 100 mg Zn/kg Futtertrockenmasse (n=4) angereichert. Im zweiten Versuch wurde zugleich $ZnSO_4 \cdot 7H_2O$ bis zu 100 mg Zn/kg Trockenmasse zum Futter der drei mit 22 mg Zn täglich gefütterten Hengsten zugesetzt. Im dritten Versuch wurde die Diät der drei Hengste, die zuerst 26 mg Zn/kg und 1,25 % Ca in der Trockenmasse (nach Zugabe von $CaCO_3$) enthielt, mit $ZnSO_4 \cdot 7H_2O$ auf 161 mg Zn/kg Trockenmasse angereichert. Dem Futter der drei anderen Hengste wurde nur $CaCO_3$ – 1,25 % Ca in der Trockenmasse – zugesetzt. Die übrigen neun Hengste (4+2+3) waren eine Kontrollgruppe, die keine Mineralzusätze bekam. Im Rahmen dieser Versuche wurde der Einfluß der 30tägigen zusätzlichen Gaben von Zink auf den Gehalt dieses Elements im Blutserum, Samenplasma sowie auf die Sameneigenschaften der Hengste untersucht. Die Untersuchungen haben ergeben, daß die erhöhte Zugabe von Zink in 100 mg/kg Trockenmasse die Änderung der Zinkkonzentration im Samenplasma beeinflußt hat. Damit waren auch Änderungen mancher Indikatoren der Sameneigenschaften verbunden, besonders wurde ein wesentlicher Anstieg der Spermienkonzentration im Samen der Hengste notiert. Die Anreicherung der Diät, die Kalzium im Überfluß enthielt, mit Zink, beeinflußte dagegen nur das Zn im Samenplasma der Hengste positiv. Der eingesetzte Zusatz von Zink hat bewirkt, daß die Sameneigenschaften sich in gleichem Maße verschlechtert haben, wie bei der Hengstgruppe, deren Diät 1,25 % Ca enthielt.

Schlüsselwörter: Hengst, Zn-Zugabe, Zn-Blutserum, Zn-Samenplasma, Spermaqualität

Introduction

The research by *Harrington et al. (1973)* indicates that during zinc deficiency, the foals show similar symptoms to growing as individuals of other species of animals. Foals fed diets containing 5 mg of zinc per kg of diet develop skin lesions (parakeratosis) similar to those seen in pigs fed zinc-deficient diets. The zinc deficiency in foals also resulted in reduction of growth, serum alkaline phosphatase and tissue zinc. *Anke (1977)* adds that zinc deficiency in horses may cause deviation of reproduction functions of both sexes, reflected in delayed ovulation in mare, low libido and poor semen quality in stallion.

Sex organs of males were among the first organs which underwent detailed examination of zinc changes on the basis of report of its high concentration in semen and after the relation between the metal and degenerative changes in the teste of animals affected by zinc deficiency. *(Apgar, 1985; Hidiroglou and Knipfel, 1984; Martin et al., 1994; Mason et al., 1982).* Enrichment of a diet with zinc can level the testicular lesions and positively influence male spermatogenesis *(Abbasi et al., 1980; Diamond et al., 1971; Underwood and Somers, 1969).* Personal research conducted so far *(Danek and Wisniewski, 1992a; Danek and Wisniewski, 1992b)* indicates that stallions

fed on low-zinc fodder show a decrease of Zn concentration in hair, blood serum and seminal plasma. It is accompanied by a substantial decrease of quantity of leukocytes in peripheral blood and by a decrease of activity of alkaline phosphatase in blood serum and by decrease of spermatozoa concentration in stallion semen. Later studies *(Danek et al., 1995; Danek et al., 1996)*, in turn, described deficiency of zinc caused by excess of calcium in fodder and proved negative influence of that state on the quality of stallion semen.
Currently the influence of supplemental zinc in fodder on zinc content in blood serum and seminal plasma and on the quality of stallion semen is being examined.

Material and Methods

The research was carried out on a total number of 26 clinically healthy Primitive Polish Horse stallions kept in a stud breeding. In the first examination, 12 stallions aged 4–9 years took part, in the second one 5 stallions aged 5–9 years, and in the third one 9 stallions aged 4–8 years. All the horses were fed individually on fodder consisting of 2 kg of rolled oat and 5 kg hay per day and watered with permanent mineral content water. The research was conducted for 3 months (April–June) of the copulative season 1989/1990. As far as the stallions from experiments 1 and 3 are concerned, the research was preceded by one-month preparation period for a breeding season, in which the stallions each gave four full ejaculates. The stallions from experiment 2 continued the season in a national studs with the average number of 4 matings per month before the experimental start.
The experimental design is given in Table 1. In the first experiment, four stallions received a supplement of 0.42 g $ZnSO_4 \cdot 7H_2O$ to 45 mg for 30 days (May), and the other 4 stallions 2.0 g $ZnSO_4 \cdot 7H_2O$ to 100 mg Zn/ kg d.m. The other stallions were a control group fed on fodder containing 30 mg Zn/ kg d.m. In the second experiment, 3 stallions had their diet enriched (supplement of 2,15 g $ZnSO_4 \cdot 7H_2O$/ day) up to 100 mg Zn/ kg d.m. of fodder, and 2 control stallions received 22 mg Zn/ kg d.m. to fodder per day. In the third experiment, 3 stallions also for 30 days (May) received 3.8 g $ZnSO_4 \cdot 7H_2O$ to fodder up to 161 mg Zn/kg d.m. and 148 g $CaCO_3$ to 1.25% Ca in d.m.. Next 3 stallions received only a supplement of calcium to 1.25% Ca in d.m. The other stallions were a control group that were dayly fed with 26 mg Zn/kg d.m. and 0.32% Ca in d.m. in fodder for the whole period of the experiment. The amount of Zn supplement to fodder with excess of calcium was established on the basis of recommendations given by *Haaranen (1963)* for cattle.
Once a month during the research period, a sample from fodder was taken to chemical analysis for the content of zinc. Full blood was taken just before getting semen from external jugular vein, ejaculate with the help of an artificial vagina at one week intervals. The received serum was put into ampoules and frozen at a temperature of –20°C. The ejaculate underwent quantitive and qualitive analysis *(Bielański, 1979)* taking into account initial evaluation of semen, spermatozoa concentration (with the cytometric method) and their total number in semen and percentage of live spermatozoa together with their morphological examination (dyeing with eosin and nigrosines). Seminal plasma was received after centrifugation of fresh samples of gel-free ejaculate (g=1000, for 15 minutes). The samples were frozen at a temperature of –20°C and gradually underwent examination determining total protein concentration with refractometric method and activity of alkaline phosphatase (on the basis of *Bessey et al.* method, *1946*) using Bio-Test Lachema set. The samples of blood serum and seminal plasma were deproteinized with 10% TCA and the content of zinc was determined with the absorption spectrophotometric method *(Prasad et al., 1965)* using a AAS III VEB Carl Zeiss Jena instrument.
The results of conducted research were statistically analysed with the t-Student test.

Results

Experiment 1
According to Table 2 only in the 2nd group of stallions (fed on 100 mg Zn/kg d.m. of fodder for 30 days) the concentration of zinc in the seminal plasma changed statistics significantly ($P<0.001$). In comparison with initial period (1.40 mg/l), increase in the zinc concentration took place at the time of the experiment to 1.70 mg/l, and after the end its decrease, to 1.19 mg/l. Significant differences were also found between the period of giving zinc sulfate and a month when the experiment ended.
In this group some indexes of semen quality changed (Table 3). A statistically significant increase was found in: spermatozoa motility, from the initial value of 65.5% to 72.2% during the experiment ($P<0.001$) and to 68.8% after the experiment ended ($P<0.01$) as well as a significant ($P<0.001$) increase in spermatozoa concentration in semen, from 186.6 x 10^6 to 282.4 x 10^6 /ml, respectively. Significant differences referred also to the period of $ZnSO_4$ administration and the experiment termination. In the other groups of stallions, i.e. in the group receiving 45 mg Zn/kg d.m. with a diet and the control one fed 30 mg Zn/kg, there were no statistically significant changes in the value of the parameters mentioned above. From among biochemical indexes of semen quality, the concentration of total protein in seminal plasma changed significantly ($P<0.02$) from the initial value 19.5 g/l to 23.4 g/l during the experiment.
A significant difference concerned also the supplementation period and a month after it. The alkaline phosphatase activity in stallion seminal plasma, in turn, did not change statistics significantly.

Experiment 2
The enrichment of diet with zinc caused a statistically significant ($P<0.001$) increase of Zn in the seminal plasma of the stallions from group II, from 1.49 mg/l to 1.99 mg/l during

the experiment, and its drop to 1.24 mg/l after the end of the experiment. Significant differences also concerned the period of Zn supplementation and a month after the end of the experiment. In this experiment no significant changes in values of the indexes above were found in the stallions of control group III (Table 4).

total ejaculate volume (P<0.001) took place, from 25.7 ml before the beginning of the experiment to 72.5 ml during the experiment and of gel-free volume, from 21.5 ml to 49.2 ml, respectively. Significant changes referred also to the periods during the experiment and after it. The stallions were also found to have a significant increase in spermato-

Tab. 1: Experimental design

Schema der Untersuchung

	Group stallions								
	I			II			III		
	A	B	C	A	B	C	A	B	C
	Experiment 1								
Zn (mg/kg d.m.)	30	(n=4) 45	30	30	(n=4) 100	30	30	(n=4) 30	30
	Experiment 2								
Zn (mg/kg d.m.)				22	(n=3) 100	22	22	(n=2) 22	22
	Experiment 3								
Zn (mg/kg d.m.)	26	(n=3) 161	26	26	(n=3) 26	26	26	(n=3) 26	26
Ca (%)	0.32	1.25	0.32	0.32	1.25	0.32	0.32	0.32	0.32

A 30 days before the beginning of the experiment (April) ,
B 30 days during of the experiment (May)
group I, II in the experiment 1 and group II in the experiment 2 with supplement, +Zn ,
group I in the experiment 3 with supplement +Zn+Ca,
group II in the experiment 3 with supplement +Ca,
group III in the experiment 1, 2, 3 without any mineral supplements,
C 30 days after the end of the experiment (June), n= number of stallions

Tab. 2: Content of zinc in blood serum and in seminal plasma of stallions in the experiment 1 (mean±SD).

Zinkgehalt im Blutserum und Samenplasma von Hengsten in Versuch 1 (Mittelwert ± Standardabweichung).

Zn	Group stallions								
	I			II			III		
	A	B	C	A	B	C	A	B	C
Serum(mg/l)	0.88±0.14	0.92±0.12	0.96±0.16	0.89±0.16	1.01±0.18	0.99±0.18	0.86±0.10	0.85±0.07	0.86±0.09
Seminal plasma(mg/l)	1.69±0.35	1.86±0.23	1.59±0.28	1.40±0.16[a]	1.70±0.26[b]	1.19±0.15[c]	1.68±0.25	1.61±0.22	1.55±0.19

A 30 days before the beginning of the experiment,
B 30 days during of the experiment, group I, II with supplement +Zn, group III without mineral supplement,
C 30 days after the end of the experiment,
Significantly different: a:b, a:c, b:c (P<0.001)

Some changes in the semen quality were found in stallions receiving 100 mg Zn in kg of d.m. of fodder daily (Table 5). Thus it is clear that a statistically significant increase in the

zoa concentration in semen, from 213.7 x 10^6 /ml before the beginning of the experiment to 358.7 x 10^6 /ml during the experiment (P<0.001), and to 245.7 x 10^6 after its end

(P<0.05), and in the total number of spermatozoa in semen, from 4.6 x 10^9 to 16.0 x 10^9, respectively (P<0.001).

es of the parameters above were not statistically significant in the control horses (fed on 22 mg Zn/kg d.m. of fodder).

Tab. 3: Semen quality of stallions in the experiment 1 (mean ±SD).
Samenqualität der Hengste des 1. Versuchs (Mittelwert ± Standardabweichung).

Examined parameter	Group stallions I A	I B	I C	II A	II B	II C	III A	III B	III C
Semen									
Total volume(ml)	71.6±53.0	82.9±29.9	69.6±29.6	85.0±34.2	72.1±39.4	92.2±41.1	53.4±10.4	59.2±17.9	58.6±14.6
Gel-free volume(ml)	57.0±38.2	60.1±21.6	46.0±26.3	62.5±27.4	48.4±23.7	66.2±28.4	39.3±13.4	43.5±13.7	43.1±11.7
pH	6.87±0.13	6.96±0.09	6.88±0.03	6.98±0.09	7.01±0.19	6.93±0.07	6.94±0.10	6.88±0.08	6.85±0.10
Spermatozoa									
Motility(%)	70.0±5.0	67.5±5.2	70.2±5.6	65.5±5.2^a	72.2±3.4^b	68.8±3.4^c	72.2±6.6	74.4±5.2	73.3±5.0
Live(%)	73.6±6.0	70.1±3.7	71.3±5.0	69.9±5.4	73.1±3.9	72.7±4.2	76.1±5.0	77.5±5.9	79.5±3.8
Normal(%)	79.5±2.6	78.4±1.9	80.6±3.0	78.3±4.9	79.3±2.1	81.2±2.7	82.3±3.5	84.3±3.4	84.5±3.2
Concentration(10^6/ml)	176.1±73.6	172.0±81.8	216.4±99.6	186.6±17.1^a	282.4±79.9^b	186.6±39.2^a	232.2±85.6	242.2±61.4	244.2±60.3
Total number(10^9)	10.7±3.2	10.4±3.0	11.9±3.5	11.7±2.3	12.5±3.3	14.2±5.4	10.0±7.0	10.0±3.0	10.1±4.6
Seminal plasma									
Total protein(g/l)	21.2±3.4	20.6±5.0	22.4±4.8	19.5±4.2^{a*}	23.4±3.9^{b*}	19.7±2.6^c	21.7±5.1	20.7±5.1	19.7±3.0
Alkaline phosphatase(U/lx10^3)	12.4±6.3	12.8±5.8	11.3±2.9	12.3±6.1	11.3±4.4	14.4±4.8	12.1±4.1	12.8±6.2	13.10±4.8

Significantly different: a:b (P<0.001), a:c (P<0.05), b:c, b*:c (P<0.01), a*:b* (P<0.02)

A significant difference was also found in spermatozoa concentration and in total number of these cells in semen between the beginning and end of the experiment. In the first group of stallions, one more significant change took place (P<0.01), namely increase in the total protein concentration was found, from initial value 19.2 g/l to 25.5 g/l within the

Experiment 3

In the I group of stallions receiving supplementary zinc to fodder with excessive calcium, a statistically significant drop in the Zn concentration in blood serum was found, from 1.03 mg/l before the beginning of experiment, to 0.86 mg/l during the experiment (P<0.01), and to 0.77 mg/l after

Tab. 4: Content of zinc in blood serum and in seminal plasma of stallions in the experiment 2 (mean ± SD)
Gehalt von Zink im Blutserum und im Samenplasma der Hengste während des 2. Versuchs.

Zn	Group stallions II A	II B	II C	III A	III B	III C
Serum(mg/l)	0.74±0.20	0.89±0.18	0.90±0.16	0.74±0.09	0.72±0.08	0.73±0.08
Seminal plasma(mg/l)	1.49±0.18^a	1.99±0.11^b	1.24±0.08^c	1.36±0.11	1.30±0.15	1.29±0.08

A 30 days before the beginning of the experiment,
B 30 days during of the experiment, group II with supplement +Zn, group III without mineral supplement,
C 30 days after the end of the experiment.
Significantly different: a:b, a:c, b:c (P<0.001)

month of giving $ZnSO_4$. A significant difference concerned also the period during and after the end of experiment. The changits end (P<0.001). In the II group of stallions, the zinc concentration in blood serum statistics significantly dropped,

from 0.98 mg/l to 0.62 mg/l (P<0.001) and to 0.76 mg/l (P<0.01), respectively. A significant difference concerned of semen quality were found (Table 7). On the other hand, in the group II of stallions a significant increase of semen

Tab. 5: Semen quality of stallions in the experiment 2 (mean ± SD)
Samenqualität derHengste in Versuch 2 (Mittelwert ± Standardabweichung).

Examined parameter	Group stallions					
	II			III		
	A	B	C	A	B	C
Semen						
Total volume(ml)	25.7±15.5[a]	72.5±13.1[b]	32.5±13.7[a]	64.0±15.6	51.0±11.4	74.0±15.6
Gel-free volume(ml)	21.5±13.5[a]	49.2±12.2[b]	25.7±11.9[a]	51.0±11.4	36.0±10.6	49.0±11.4
pH	6.95±0.13	6.94±0.05	7.01±0.12	6.85±0.07	6.90±0.08	6.85±0.07
Spermatozoa						
Motility(%)	72.5±5.0	75.5±5.7	70.5±5.0	78.0±4.1	77.0±7.0	76.0±7.0
Live(%)	74.0±4.5	76.7±4.7	71.7±5.5	80.5±2.1	79.0±5.6	78.0±1.4
Normal(%)	75.3±6.2	78.5±3,5	79.0±4.8	82.0±1.4	82.2±2.3	83.0±1.3
Concentration(10^6/ml)	213.7±31.1[a]	358.7±41.4[b]	245.7±34.9[c]	201.5±19.2	180.0±14.1	187.5±17.6
Total number(10^9)	4.6±2.8[a]	16.3±4.0[b]	6.8±2.4[a]	10.4±1.9	8.0±1.1	9.1±1.4
Seminal plasma						
Total protein(g/l)	19.2±4.0[a]	25.5±4.3[b*]	18.5±2.3[a*]	21.7±2.4	20.0±2.4	19.5±2.9
Alkaline phosphatase(U/l x 10^3)	13.3±5.0	11.3±3.4	13.5±3.1	12.8±3.1	13.6±3.4	3.8±3.5

Significantly different: a:b, b:c, b*:a* (P<0.001), a:b* (P<0.01); a:c (P<0.05)

also the month of the experiment and the period after its end. In the seminal plasma a similarly significant drop in the zinc concentration was found (P<0.001), from 2.41 mg/l to 1.28 mg/l and to 1.10 mg/l, respectively. In the control group III, fed on 26 mg Zn/kg d.m. daily, the above indexes did not change statistics significantly during the research (Table 6).

In the groups I and III of stallions, no statistically significant changes in the values of macro- and microscopic indexes pH (in comparison with the initial period 7.01) to 7.11 during the period of giving $CaCO_3$ (P<0.05), and to 7.16 during the period after the end of the experiment (P<0.001). In that period, the motility of spermatozoa in semen decreased significantly (P<0.01), from 70.0% to 62.5%, respectively. Similarly, the concentration of spermatozoa decreased statistics significantly (P<0.02), from 288.7 x 10^6 /ml to 196.6 x 10^6 /ml and to 189.6 10^6 /ml, and also the total number of these cells in semen significantly decreased (P<0.05),

Tab. 6: Content of zinc in blood serum and in seminal plasma of stallions in the experiment 3 (mean ± SD)
Gehalt von Zink im Blutserum und im Samenplasma der Hengste während des 3. Versuchs.

Zn	Group stallions								
	I			II			III		
	A	B	C	A	B	C	A	B	C
Serum(mg/l)	1.03±0.13[a]	0.86±0.12[b]	0.77±0.15[b*]	0.98±0.18[a]	0.62±0.12[b*]	0.76±0.18[b]	0.90±0.13	0.81±0.14	0.80±0.16
Seminal plasma(mg/l)	2.15±0.56	1.69±0.58	1.54±0.72	2.41±0.62[a]	1.28±0.35[b*]	1.10±0.33[b*]	1.18±0.64	1.23±0.26	1.26±0.52

A 30 days before the beginning of the experiment,
B 30 days during of the experiment, group I with supplement +Ca + Zn, group II with suplement +Ca, group III without any mineral supplements,
C 30 days after the end of the experiment.
Significantly different; a:b* (P<0.001), a:b (P<0.01), b*:b (P<0.05)

respectively from 12.8 x 10^9 , to 7.4 x 10^9 and to 8.0 x 10^9, respectively. The other examined indexes did not change during the experiment and after its end.

From among biochemical indexes of semen quality, stallions receiving zinc supplemented to fodder were found to have a statistically significant drop ($P<0.05$) in the total protein concentration, from initial 23.7 g/l to 20.7 g/l during the experiment and a significant ($P<0.02$) drop in the alkaline phosphatase activity in seminal plasma from 15.7 U/l x 10^3 during the experiment, to 11.4 U/l x 10^3 after its end. In the other groups of stallions the concentration of total protein and the activity of alkaline phosphatase in seminal plasma did not change significantly.

Discussion

Both in the first (30 mg Zn/kg d.m. of fodder), and especially in the second (22 mg Zn/kg d.m. of fodder) and the third (26 mg Zn/kg d.m. of fodder) experiment, the basic diet did not cover full demand of zinc accepted for mature horses. A horse diet, according to NRC 1989 recommendations, should contain at least 40 ppm Zn in the ration. The supplement of $CaCO_3$ to fodder caused increase of calcium to 1.25% in d.m. Thus the diet of the experimental stallions included 5 times as much calcium as the accepted minimum demand (0.25%) for this element for horses *(Ralston, 1992).*

In the current research, a short-time supply of 100 mg Zn positively influenced the content of zinc in blood serum but here there were insignificant increases. On the other hand, significant changes of zinc concentration in seminal plasma appeared in both groups of stallions fed 100 mg Zn/kg of d.m.. In experiment 1, in the month of treatment with $ZnSO_4$, an almost 21% increase of zinc concentration in seminal plasma took place up to 1.70 mg/l, in the 2nd experiment a 32% increase in the zinc concentration up to 1.99 mg/l, and in the month after the experiment termination, a drop to 1.19 mg/l and 1.24 mg/l, respectively.

By comparison, the personal research *(Danek and Wisniewski, 1992b)* shows that stallions fed for 7 months of the season on 17.5 mg Zn/kg d.m. of fodder have the zinc concentration in seminal plasma at the level of 1.13 mg/l on the average.

Pallauf and Kirchgeßner (1972) proved that the zinc level in the serum growing rats dropped within two days on the deficient diet by more than 30% and in four days by more than 50% of the original value. Addition of zinc restored the serum zinc to normal.

Miller et al., (1970) established for cattle that when dietary zinc was increased from 33 to 233 ppm (+200 ppm Zn as ZnO or $ZnSO_4$), there were small but insignificant increases in the zinc content of the whole blood and serum. Further raising of dietary zinc from 233 to 633 ppm usually resulted in more rapid and significant increase in serum, whole blood zinc (8 and 15 days), and testicles of calves (21 days after initiations of dietary treatments).

The research conducted by *Besson et al., (1977)* shows that the effect of supplemental dietary zinc on blood zinc

Tab. 7: Semen quality of stallions in the experiment 3 (mean ± SD).
Samenqualität der Hengste im Versuch 3.

Examined parameter	Group stallions I A	I B	I C	II A	II B	II C	III A	III B	III C
Semen									
Total volume(ml)	68.1±52.9	82.2±50.8	58.3±31.3	58.1±36.9	68.6±24.8	87.4±68.7	101.6±80.1	113.3±84.4	97.5±63.2
Gel-free volume(ml)	50.0±37.2	49.7±23.0	42.6±20.2	45.6±17.1	41.6±20.1	43.3±17.1	63.0±40.2	65.0±33.7	64.1±37.1
pH	7.00±0.15	7.06±0.23	7.05±0.17	7.01±0.05^a	7.11±0.15^b	7.16±0.07^{b*}	7.10±0.16	7.10±0.09	7.00±0.10
Spermatozoa									
Motility(%)	70.0±6.5	72.5±8.6	74.1±5.1	70.0±6.0^{a*}	66.6±4.9	62.5±4.5^{b*}	68.6±6.1	69.1±5.1	67.5±5.9
Live(%)	73.8±5.7	75.5±9.3	75.8±5.7	73.2±6.2	72.2±6.0	70.1±4.2	70.8±8.9	71.2±6.1	73.4±5.2
Normal(%)	83.6±5.5	83.7±4.6	85.1±2.7	83.0±3.01	81.8±2.3	81.1±2.4	77.7±0.9	80.8±2.4	80.3±2.6
Concentration(10^6/ml)	259.1±80.6	256.6±61.5	294.5±99.2	288.7±94.3^{a*}	196.8±87.2^b	189.6±86.3^b	150.2±30.7	157.1±30.5	160.4±52.8
Total number(10^9)	12.1±6.4	10.7±5.9	10.7±5.4	12.8±8.1^a	7.4±2.5^b	8.0±3.7^b	10.1±0.9	10.1±3.2	10.2±5.8
Seminal plasma									
Total protein(g/l)	23.7±3.2^a	20.7±3.1^b	21.3±3.7	22.6±6.6	20.1±6.9	22.3±5.3	19.0±3.2	19.5±3.2	20.8±2.6
Alkaline phosphatase(U/lx10^3)	13.7±5.3	15.7±3.7^{a*}	11.4±3.8^b	13.6±2.4	12.3±1.4	12.9±2.0	15.5±7.1	13.5±3.1	14.7±2.3

Significantly different: a:b* ($P<0.001$), a*:b* ($P<0.01$), a*:b ($P<0.02$), a:b ($P<0.05$)

levels in beef cattle are inconsistent, and supplemental zinc had virtually no effect on blood serum levels of zinc except when the dietary level was extremely high (300 or 620 mg/kg Zn as ZnO). While *Schryver et al., (1980)* showed that the ponies absorbed and retained more stable Zn when fed 250 mg of supplemental zinc that when fed the basal diet (35 mg Zn/kg) alone or the basal diet plus 520 mg of supplemental Zn as ZnO. The plasma Zn concentration was significantly higher when the ponies were given a daily supplement of 520 mg of zinc that when they received unsupplemented diets or 250 mg of supplemental Zn/day.
Neathery et al., (1972) on the other hand, found for goats fed on fodder supplemented with 36 ppm Zn significant differences in zinc content in blood plasma in the 12th week of the experiment, in comparison with the group fed on 4 ppm of zinc.
It seems that similar mechanisms could have operated with stallions described in experiments, especially fed a lower amount of zinc in diet [22 mg Zn/kg d.m. of fodder].
The supplement of Zn (up to 161 mg/kg d.m.) applied in experiment 3 to fodder in a way undermined the negative influence of Ca excess on Zn status for stallions. An established 16% drop in the zinc concentration in stallion serum of group I (to 0.86 mg/l) was smaller than it was noted in the same period in the group of stallions receiving diet with $CaCO_3$ supplement [0.62 mg/l (a drop by 36%)]. For comparison, the previous research *(Danek et al., 1995)* found a drop in the zinc content in stallion serum during feeding fodder containing 1.25% Ca in d.m. to 0.54 mg/l on the average. The concentration was also significantly lower than determined for stallions fed for 7 months of the season on fodder including 17.5 mg Zn/kg d.m. [0.75 mg/l] *(Danek and Wisniewski, 1992a)*.
Many authors point the negative influence of calcium excess on zinc absorption and distribution in animal body. *Berry et al. (1961)* found for swine that calcium (0.6%) added to a low zinc ration (29 ppm), decreased blood, plasma and total tissue Zn^{65} levels. However, calcium added to a zinc supplemented ration (100 ppm) increased blood and total tissue Zn^{65} activity. Inadequate proportions of the examined elements, Ca, Zn, Cu, were found in the fodder, and caused the increase of zinc level in plasma and hair as well as disappearance of parakeratosis and mycosis symptoms *(Wisniewski, 1984)*.
Pond (1983) proved for lambs fed two levels of Ca (0.5 and 0.8%) and Zn (20 and 100 ppm), that plasma Zn tended to decline during the first 28 days in the lambs fed diets unsupplemented with Zn, but plasma Zn concentration of lambs fed all diets increased between day 28 and 56 and then declined at day 84 to a level lower than that on day 0. Raising the level of dietary Ca does not induce clinical signs of zinc deficiency in the growing lamb, in contrast to the general observations of this relationship in growing swine. *Roth and Kirchgeßner (1989)*, on the other hand, showed that after 28 days of experiment the serum zinc concentration of the zinc – deficient rats (–Zn+Ca) – was reduced by 63% as compared to control animals (+Zn+Ca), but zinc deficiency did not influence the calcium concentration.
In contrast to blood serum there was no statistically significant drop in the zinc concentration in seminal plasma of stallions of group I. It also differed from what was established for the group of stallions receiving excessive calcium. In this case the concentration of this element in comparison with the initial period in the month of giving $CaCO_3$ decreased by 46% to 1.28 mg/l. In the previous research *(Danek et al., 1996)* a 41% fall of zinc concentration was found in seminal plasma of stallions fed 1.25% in d.m. of fodder.
The enrichment of stallion diet with zinc in experiment 1 and 2 affected some indexes of semen quality. Total volume of ejaculate and gel-free volume increased significantly in the stallions in experiment 2, and spermatozoa motility increased after zinc was added (up to 100 mg/ kg of d.m.) only in the stallions from experiment 1. The supplement of zinc did not have a significant effect on indexes of stallion semen quality analysed next, namely on the percentage of live and morphologically normal spermatozoa. Earlier research *(Danek and Wisniewski, 1992b)* did not show significant correlation between low content of zinc in fodder, and semen volume, spermatozoa motility, percentage of live and morphologically normal spermatozoa (except for negative correlation with % of spermatozoa changed secondarily) in stallion semen. The negative correlation was found only between zinc content in coat and % of live spermatozoa. Whereas *Hunt et al., (1992)* showed that seminal volume and total seminal zinc loss per ejaculate are sensitive to short-term zinc depletion in young men.
Kynaston et al,. (1988) found in subfertile men with idiopathic asthenozoospermia and/or oligospermia after oral administration of zinc sulphate (220 mgs., twice daily for a period of three months) significantly increased zinc concentrations in seminal fluid, a progressive percentage of total sperm motility and no significant changes in percentage of dead or abnormal forms. While *Danscher et al., (1978)* noted a drop in spermatozoa motility with an increase of zinc concentration in semen.
In stallions receiving a supplement of zinc to 100 mg/kg of fodder, the spermatozoa concentration (experiment 1 and 2) and total number of spermatozoa in semen (experiment 2) increased. The personal research *(Danek and Wisniewski, 1992b)* showed negative influence of a low-zinc diet on concentration of spermatozoa in stallion semen. *Saaranen et al., (1987)* and *Stanwell-Smith et al,. (1983)* also showed a positive correlation between zinc content in seminal plasma and semen density. *Underwood and Somers (1969)*, on the other hand, proved that lambs fed on diet supplemented with zinc sulphate to provide total zinc level of 17.4 ppm and 32.4 ppm showed no signs of zinc deficiency reported for sheep fed on a diet with 2.4 ppm of zinc. Testicular growth and sperm production were markedly improved by both the zinc supplements. Similarly *Abbasi et al. (1980)* established that spermatozoa concentration and their total number in semen decrease significantly in people fed on low-zinc diet (24–40 weeks). While *Hartoma et al., (1977)* found an increase of sperm-count in infertile men after 4–8 weeks of oral administration of zinc sulphate together with an increase of zinc concentration in serum.

The last of parameters changing during the experiments was total protein concentration in seminal plasma of stallions. Its statistically significant change was found in stallions in experiment 1 and 2 fed on fodder with 100 mg Zn/kg of d.m. For comparison, earlier research *(Danek and Wisniewski 1992b)* showed in stallions fed in the season on a zinc-reduced diet a decrease in the total protein concentration in seminal plasma. In these examinations, just like in the current ones, there was no change of alkaline phosphatase activity in seminal plasma of stallions. The results show that zinc, just like it was found in semen of boars *(Luberda et al. 1988)* and bulls *(Strzezek and Glogowski, 1979)* does not play such a role as it was proven in the case of other molecular forms of this enzyme. For example in blood serum of zinc-depletion stallions lower activity of alkaline phosphatase was found *(Danek and Wisniewski 1992b)*.

Although in stallions (in experiment 3) fed on zinc-supplemented fodder with high content of calcium some changes in values of some macro- and microscopic indexes of semen quality were reported, in comparison with initial period the differences were statistically insignificant. Similar tendencies were observed in the control group of stallions fed on mineral- unsupplemented diet. Significant changes concerned only biochemical indexes of seminal quality. Namely a decrease in total protein concentration during the experiment and lowering of alkaline phosphatase in the month after the experiment ended were found. In the group receiving only elevated amount of calcium (also earlier research *Danek et al., 1996*), no statistically significant changes in values of these biochemical indexes were reported. However, in previous experiments (1 and 2), after the enrichment of a diet with zinc an increase in total protein concentration in seminal plasma was found, but no significant changes of alkaline phosphatase activity.

In the group of stallions fed 1.25% Ca/kg d.m. semen pH increased, spermatozoa motility lowered and the concentration and the total number of these cells in semen decreased. These results prove some earlier personal observations *(Danek et al., 1996)* and show the negative influence of excessive calcium supply on some characteristics of semen quality of stallions.

To sum up it must be noted that short-term supply of 100 mg Zn/kg of d.m. affected zinc content only in seminal plasma of stallions (experiment 1, 2). Changes in some semen characteristics were connected to it, especially the increase of spermatozoa concentration in stallion semen was found. The supplement of zinc sulphate applied in experiment 3 in a way levelled the negative influence of calcium excess on the zinc in seminal plasma and the semen quality.

It seems however that the extent of changes depended on the zinc supply degree in an organism and on initial quality of stallion semen.

References

Abbasi A.A., Prasad A.S., Rabbani P. and Dumouchelle E. (1980): Experimental zinc deficiency in man. J.Lab.Clin.Med. 96, 544–550.

Anke M. (1977): Die Bedeutung der Mengen- und Spurenelemente in der Fütterung von Zucht -und Sportpferden. Tierzucht 7, 310–312.

Apgar J. (1985): Zinc and Reproduction. Ann.Rev.Nutr. 5, 43–68.

Berry R.K., Bell M.C. Grainger R.B. and Buescher R.G. (1961): Influence of dietary calcium and zinc on calcium-45, phosphorus-32 and zinc-65 metabolism in swine. J.Anim.Sci. 20, 433–439.

Bessey R.K., Lowry O.H. and Brock M.J. (1946): A method for the rapid determination of alkaline phosphatase with five cubic milimeters of serum. J. Biol. Chem. 164,321–323.

Besson W.M., Perry T.W. and T.D. Zurcher T.D. (1977): Effect of supplemental zinc on growth and on hair and blood serum levels of beef cattle. J.Anim.Sci. 45, 160–165.

Bielanski W. (1979): Rozród zwierzat. PWRiL, Warszawa, p. 437.

Danek J. and Wisniewski E. (1992a): Wplyw niedoboru cynku w diecie na wskazniki hematologiczne, aktywnosc fosfatazy zasadowej i stezenia bialka calkowitego w surowicy oraz na zawartosc cynku, miedzi i wapnia w surowicy i siersci ogierow. Med. Wet. 48, 521–523.

Danek J. and Wisniewski E. (1992b): Zmiany jakosci nasienia w niedoborze cynku u ogierów. Med. Wet. 48, 566–568.

Danek J., Wisniewski E., Krumrych W. and Dabrowska J. (1995): Wplyw nadmiaru wapnia w paszy na wskazniki hematologiczne oraz biochemiczne w surowicy krwi i siersci ogierow. Med. Wet. 51, 544–546.

Danek J., Wisniewski E. and Krumrych W. (1996): Wplyw nadmiaru wapnia w paszy na jakosc nasienia ogierow. Med. Wet. 52, 459–561.

Danscher G., Hammer R., Fjerdingstand E. and H. Robbe H. (1978): Zinc content of human ejaculate and the motility of sperm cells. Int.J.Androl. 1, 576–581.

Diamond I., Swenerton H. and Hurley L.S. (1971): Testicular and esophageal lasions in zinc- deficient rats and their reversibility. J. Nutr. 101, 77–84.

Haaranen S. (1963): Zinc requirement of dairy cattle. Feedstuffs, March, 9.

Harrington D.D., Walsh J. and White V. (1973): Clinical and pathological findings in the horses fed zinc-deficient diets. Proc. 3rd Equine Nutr. and Physiol. Symp.Gainesville. F l. p. 51–54.

Hartoma R.T., Nahoul K. and Netter A. (1977): Zinc, plasma androgens and male sterility. Lancet 26, 1125–1126.

Hidiroglou M. and Knipfel J.E. (1984): Zinc in Mammalian Sperm: A review. J.Dairy Sci. 67, 1147–1156.

Hunt C.D., Johnson P.E., Herbel J. and Mullen L.K. (1992): Effects of dietary zinc depletion on seminal volume and zinc loss, serum testosterone concentrations, and sperm morphology in young men. Am. J.Clin.Nutr. 56, 148–157.

Kynaston H.G., Lewis-Jones D.I., Lynch R.V. and Desmond A.D. (1988): Changes in Seminal Quality Following Oral zinc Therapy. Andrologia 20 (1), 21–22.

Luberda Z., Strzezek J., Bielski A. (1988): Wplyw zawartosci cynku w plazmie nasienia knura na aktywnosc Dnaz i fosfataz oraz wlasciwosci precypitujace tego plynu wobec zoltka jaja kurzego. Med. Wet. 44, 298–301.

Martin G.B., White C.L., Markey C.M. and Blackberry M.A. (1994): Effects of dietary zinc deficiency on the reproductive system of young male sheep; testicular growth and the secretion of inhibin and testosterone. J.Reprod. Fertil. 101, 87–86.

Mason K.E., Burns W.A. and J.C.Jr Smith J.C. Jr (1982): Testicular Damage Associated with Zinc Deficiency in Pre- and Postpubertal Rats: Response to Zinc Repletion. J.Nutr. 112, 1019– 1028.

Miller W.J., Blackmon D.M., Gentry R.P. and Pate E.M. (1970): Effects of high but nontoxic levels of zinc in practical diets on ^{65}Zn and zinc metabolism in Holstein calves. J.Nutr.100, 893–902.

Neathery M.W., Miller W.J., Blackmon D.M., Pate F.M. and Gentry R.P. (1972): Effects of Long term Zinc Deficiency on Feed Utilization, Reproductive Characteristics, and Hair Growth in the Sexually Mature Male Goat. J.Dairy Sci. 56, 98–105.

Pallauf J. and Kirchgeßner M. (1972): Zinkkonzentration in Blut und Serum wachsender Ratten bei Zinkmangel. Zbl.Vet.Med. A 19, 594–604.

Pond W.G. (1983): Effect of dietary calcium and zinc levels on weight gain and blood and tissue mineral concentrations of growing Columbia – and Suffolk-Sired lambs. J.Anim.Sci. 56, 952–959.

Prasad A.S., Oberleas D., Halsted J.H., Collins R.S. (1965): Determination of zinc in biological fluids by atomic absorpion spectrofotometry in normal and cirrhotic subject. J.Lab.Clin.Med.66, 508–516.

Ralston S.J. (1992): Diagnosis of Common Mineral Imbalances. *in:* Current Therapy in Equine Medycyne (3), edit. *E.Robinson*, Philadelphia WB Saunders Company. p. 717.

Roth H.P. and Kirchgeßner M. (1989): Influence of zinc and/or calcium deficiency on prolactin concentration in rat serum. Trace Elem. Med. 6, 139–141.

Saaranen M., Suistoma U., Kantola M., Saarikoski S. and Vanha Perttula T. (1987): Lead, magnesium, selenium and zinc in human seminal fluid: comparison with semen parameters and fertility. Hum. Reprod. 2, 475–479.

Schryver H.F., Hintz H.F. and Lowe J.E. (1980): Absorption, extrection and tissue distribution of stable zinc and ^{65}zinc in ponies. J. Anim. Sci. 51, 896–902.

Stanwell-Smith R., Thompson S.G. Haenes A.P., Ward R.J., Cashmore G., Stendronska J. and Hendry W.F. (1983): A comparative study of zinc, copper, cadmium, and lead levels in fertile and infertile men. Fertil.Steril. 40, 670–677.

Strzezek J. and Glogowski J. (1979): Molecular forms of alkaline phosphatase in bull seminal plasma. Int. J. Biochem. 10, 135–146.

Underwood E.J. and Somers M. (1969): Studies of zinc in sheep. In the relation of zinc to growth, testicular development, and spermatogenesis in young rams. Aust. J.agric.Res. 20, 889–897.

Wisniewski E. (1984): Preventive and therapeutic applications of zinc in bovine dermatomy coses. Bull.vet.Inst. Pulawy 27, 22–35.

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