THE INFLUENCE OF THE ORGANIC AND INORGANIC FORM OF ZINC ON VOLUME EJACULATE, SPERM – CONCENTRATION AND PERCENTAGE OF PATHOLOGIC SPERMS

Horký P., Jančíková P., Zeman L.

Mendel University in Brno, Czech Republic
pavel.horky@mendelu.cz

Abstract

The experiment was established to study the feeding of organic and inorganic forms of zinc and the effect of these zinc forms on the reproductive indicators (ejaculate volume, concentration of sperms and percentage of pathologic sperms in ejaculate) in breeding boars. The experiment included sixty-six boars, which were divided into two equable groups by age and breeds. In 115 days of experiment duration, the volume of ejaculate increased in both groups. Boars receiving the organic zinc form showed a highly significantly increased ($P < 0.001$) average volume of ejaculate from 207.5 ± 66.56 ml to 289.5 ± 81.28 ml. Boars receiving the inorganic zinc form exhibited the volume of ejaculate increased by 59.52 ml ($P < 0.05$) in the last two weeks of the experiment while the average volume in the initial sampling was 229.62 ± 102.64 ml.

The concentration of sperms in the group receiving the organically fixed zinc slightly decreased from 407.44 ± 126 thous./ mm^3 to 400.67 ± 200.86 thous./mm^3. By contrast, the group of boars whose feeding mixture contained the inorganic form of zinc reached in the last two weeks of the experiment an increased concentration of sperms from 402.63 ± 180.26 thous./mm^3 to 424.10 ± 149.81 thous./mm^3.

The percentage of pathologic sperms did not show any demonstrable difference between the two groups. Both groups exhibited a mild increase in this indicator. The experimental group with the supplement of organic zinc showed an increase from 10.18 ± 8.46 % to 11.67 ± 7.68 % and the experimental group with the supplement of inorganic zinc showed an increase from 9.4 ± 8.4 % to 9.92 ± 6.68 %.

The obtained sperm served to produce under identical conditions on average by 20.97 insemination doses (ID) more from the beginning of the experiment in the group of animals receiving organic zinc form. The group receiving inorganic zinc form exhibited an increase by 20.2 ID.

Our experimental results demonstrated that feeding the organic zinc form has a beneficial influence on the volume of ejaculate and hence on the production of insemination doses. Other studied reproduction parameters (concentration and percentage of pathologic sperms) did not show any significant differences resulting from the feeding by different zinc forms.

Key Words: boar, ejaculate, zinc, organic form, inorganic form

The work dealt with the effect of feeding different forms of mineral elements (organic and inorganic) on the reproductive indicators of breeding boars, namely with the influence of zinc, which directly affects the reproduction.

According to ZEMAN (2004), mineral substances in the form of chelates improve the reproduction capacity in sows, cloven-hoof quality, support the immune system and semen quality in boars.

New products of fodder biotechnologies as well as sources of macro and microelements in organically fixed forms are developed for application in feeding rations. These sources represent nutrition factors that may favourably influence utilization of nutrients (digestibility, retention, balance) and enhance production quality and quantity. Because their availability for organisms is higher than that of inorganic salts of elements, their excretion from the body is lower and hence a lower risk exists of increased environment contamination. Mineral substances bound to the organic transport component of "chelate" favourably affect the digestibility of nutrients. The complex of zinc has a favourable effect on increased fertility in both males and females (ŠIMEK, 2001). Zinc participates in the development of Leydig cells, conditions the response to luteinizing hormone and supports the production of testicular hormones (HASKETH, 1982; cit., CLOSE and COLE, 2003). The highest zinc concentrations can be found in the eye choroid and in the prostate (JELÍNEK, KOUDELA et al., 2003). Zinc is necessary for the correct functioning of reproductive organs but according to ESTIEEN and HARPER (2005), only a limited number of experimental studies exist so far about the effect of zinc on the quality of ejaculate in breeding boars.

The aim of our experiment was to find out what is the effect of supplementary zinc form in the feeding ration on the results of reproduction.

Material and Methods

The experiment was conducted at the Insemination station for boars in Velké Meziříčí. It included sixty-six boars divided into two groups, which were comparable in terms of the age and breeds of animals. Breeds of boars included in the experiment were Duroc, White Improved, Landrace and paternal breeds SL 38 (Pn x DU), SL 48 (LW x Pn).

Both groups of animals were fed the basic feeding mixture (see Tab. 1); the mixture contained no zinc in the basic premix. The element was supplemented in the basic feeding ration to the control group (n = 33) in the inorganic form (as zinc oxide) and to the experimental group (n = 33) in the organic form (zinc fixed to amino acid – methionine). The complex developed through the hydrolyzation of soya protein by means of specific enzymes and water-soluble zinc sulphate. This mixture of amino acids and peptides together with ions was injected under high pressure to the drying cylinder where moisture residues were evaporated and proteinates were created of high zinc content. Zinc was supplemented in the premix to the control and experimental groups with the feeding ration at 99.88 mg/kg of fodder mixture. Without the premix of fodder raw materials, the basic feeding ration contained 26.25 mg Zn per kg of fodder mixture. The boars received 3.3 kg of fodder mixture per day. The premix was applied to animals every day with the morning feed. Carriers in the experimental premix were feed meal and lime meal.

The studied reproduction parameters (ejaculate volume, concentration and percentage of pathologic sperms) were assessed directly in the laboratory of the insemination station for boars in Velké Meziříčí. The volume of ejaculate was established by using the measuring cylinder. The concentration of sperms in ejaculate was expressed as a count of sperms in 1 mm^3.

The concentration was ascertained photometrically by using Spekol 11. The volume of 9 ml 1M HCl was scooped by a charger for small amounts of liquid into a thin-walled testing tube, 0.25 ml of sample from the stirred native ejaculate was added by using a varipipette and the sample was mixed by hand. The testing tube was inserted into the Spekol extension and the measured value was read. Sperm density was established according to the calibration table for the used Spekol.

The percentage of pathologic sperms was established from the first sampling in the month. The procedure was as follows: a drop of sperm was applied by means of a glass stick on the glass slide, a ground smear glass was approximated to the sperm drop at an angle of 45 ^{o} so that the drop could spread upon the contact with the glass along its edge and the smear itself was carried out in a thin layer by draught. The morphological assessment of abnormal sperms on the prepared spermgram in several vision fields – all abnormal sperms were counted individually), dying and evaluation of the spermgram was made by the district veterinarian.

The boars were subjected to semen sampling minimally once a week with respect to their health condition and age. The health of animals was monitored by the veterinarian.

For the manifestation of spermatogenesis, the duration of the experiment was set up to 115 days. The experiment started in mid-August and ended in the first decade of December. Resulting data were valuated for each month separately. The results were statistically processed by the Statistika software and differences between the averages were assessed by means of Student t-test.

Table 1. Composition of feed mixture for boars

Component	% proportion
Barley seed	36.00
Wheat seed	20.36
Oat seed	20.00
Soybean meal	14.50
EKPO T	3.00
Bergafat	2.10
Calcium carbonate	1.50
Monodicalcium phosphate	1.20
Premix of minerals and vitamins for boars	0.50
Natrium chloratum	0.40
Oxide magnesium	0.15
L-Lysine HCl	0.14
L - Threonine	0.09
Methionine DL	0.06

Bergafat – palm oil; EKPO T – biscuit meal

Results

The animals did not exhibit any health problems during the experiment. The experiment was carried out in line with the established methodology.

In the first experimental month, the average volume of ejaculate amounted to 207.50 ± 66.56 ml in the control group and to 229.62 ± 102.64 ml in the experimental group (see Tab. 2). The first changes in the volume of ejaculate were recorded in the second experimental month. An increase was observed both in the control group to 244.82 ± 112.49 ml (i.e. by 6.61%) and in the experimental group, in which the average ejaculate volume reached 236.95 ± 94.46 ml (i.e. increase by 14.19%). The feeding intervention shows in the reproduction indicators of boars after some 42 days (ZEMAN, 2004); this is why the ejaculate volume was increasing only slowly in the second month. A similar trend of the increasing volume of ejaculate in both the control group and the experimental group continued in the subsequent third period of study. If we look closer, the control group that was administered inorganic trace elements showed on average 257.77 ± 110.87 ml of ejaculate, i.e. an increase by 12.25% from the beginning of the experiment. The experimental group with the supplement of organically fixed zinc recorded in the same period an increase of the average ejaculate production to 252.93 ± 77.48 ml, i.e. an increase by 21.91% ($P < 0.05$) from the beginning of the experimental period. In the fourth period of study, the volume of ejaculate in boars of the control group was on av. 284.14 ± 103.83 ml. As compared with the value recorded at the beginning of the experiment, an increased occurred in the inorganic form of zinc by 23.93% ($P < 0.05$). The average amount of ejaculate per sampling in the experimental group was 263.71 ± 65.33 ml. As to development in the production of ejaculate, the experimental group showed an increase by 27.21% ($P < 0.01$) as compared with the control group. In the last period of study, the group of animals supplemented with inorganic form of zinc did not exhibit any essential increase of ejaculate volume. At the end of the experiment, we measured 289.14 ± 81.28 ml, i.e. an increase of ejaculate volume from the beginning of inorganic zinc administration by 25.92% ($P < 0.05$). The experimental group showed the highest value in this last period of study (289.50 ± 81.28 ml, i.e. an increase by 39.51%. The fact was highly statistically significant ($P < 0.001$).

Table 2. Statistical comparison of ejaculate volume (ml) with the organic and inorganic forms of zinc

Period	Organic form of zinc			Inorganic form of zinc		
	Average and standard deviation	No. of ejaculate taking	Average no. of ejaculate taking for a boar	Average and standard deviation	No. of ejaculate taking	Average no. of ejaculate taking for a boar
I	207,50 ± 66,56	61	1,84	229,62 ± 102,64	67	2,03
II	236,95 ± 70,95	120	3,63	244,82 ± 112,49	142	4,30
III	252,97 ± 77,48*	111	3,36	257,75 ± 110,87	125	3,78
IV	263,97 ± 65,33**	113	3,42	284,58 ± 103,83*	121	3,66
V	289,50 ± 81,28***	39	1,18	289,14 ± 122,48*	40	1,21
Total	250,74 ± 76,44	444	13,43	261.09 ± 111,30	495	14,98

* - the symbol is to express statistically significant changes within the column (as compared with the first period, i.e. at the beginning of the experiment) $P < 0.05$ *; $P < 0.01$** ; $P < 0.001$ ***

As to the concentration of sperms, the values recorded in the control group and in the experimental group at the beginning of the experiment were 402.63 ± 180.26 thous./mm^3 and 407.44 ± 126.37 thous./mm^3, respectively (see Tab. 3). In the second period, the value recorded in the control group of animals increased to 416.93 ± 158.47 thous./mm^3 and the experimental group showed an increasing trend too (417.07 ± 160.17 thous./mm^3). In the third period, the control group of animals recorded the highest concentration of sperms (468.39 ± 177.77 thous./mm^3), which was an increase by 16.33 % from the beginning of the experiment. On the other hand, the experimental group of animals showed only a slight increase in this reproduction indicator (420.57 ± 161.16 thous./mm^3).

In the fourth period of study, the control group exhibited a decreasing trend (446.10 ± 149.57 thous./mm^3). The experimental group showed in this last but one period of study 439.0 ± 150.03 thous./mm^3, which was an increase by 7.74 % as compared with the first period. In the last period of study, the boars supplemented with the inorganic form of zinc exhibited the average amount of sperms at 424.53 ± 149.81 thous./mm^3. As compared with the period at the beginning of the experiment, this was an increase by 5.43 %. The experimental group showed the amount of sperms at 400.67 ± 200.86 thous./mm^3 at the end of the experiment. The development of the concentration of sperms in the experimental period is presented in Table 3.

Table 3. Comparison of the concentration of sperms (thous./mm^3) in the groups of boars receiving organic and inorganic forms of zinc

Period	Organic form of zinc			Inorganic form of zinc		
	Average and standard deviation	No. of ejaculate taking	Average no. of ejaculate taking for a boar	Average and standard deviation	No. of ejaculate taking	Average no. of ejaculate taking for a boar
I	407,44 ± 126,37	61	1,84	402,63 ± 180,26	67	2,03
II	417,07 ± 160,17	120	3,63	416,93 ± 158,47	142	4,30
III	420,57 ± 161,16	111	3,36	468,06 ± 177,77	125	3,78
IV	439,00 ± 150,03	113	3,42	446,10 ± 149,57	121	3,66
V	400,67 ± 200,86	39	1,18	424,53 ± 149,81	40	1,21
Total	417,26 ± 162,45	444	13,43	431,91 ± 165,62	495	14,98

In the last studied indicator – percentage of pathologic sperms, the control group of animals supplemented with the inorganic zinc form started the experimental period at 9.4 ± 8.4 %. The experimental group with the organic zinc form started the experiment at 10.18 ± 8.46 % of pathologic sperms. In the second period of study, a decreasing trend started to show both in the control group (8.64 ± 7.66 %) and in the experimental group (6.3 ± 3.54 %). The decrease in the experimental group was significant at $P < 0.05$ as compared with the first period of study. In the third period of study, the decreasing trend in the control group (7.07 ± 5.18 %) continued while the experimental group exhibited a slight increase of the percentage of pathologic sperms (6.79 ± 5.66 %). In the fourth period of study, the increase was recorded both in the control group (9.08 ± 5.68%) and in the experimental group of animals (9.08 ± 5.68 %). In the last period of study, both groups showed the highest percentage of pathologic sperms for the entire experimental interval (control group 9.92 ± 6.68 %; experimental group 11.67 ± 7.68 %). If we compare the control group (8.8 ± 6.87 %) and the experimental group (8.92 ± 6.9 %) of animals, it is obvious that no statistically significant difference existed between the two groups. The development of the occurrence of pathologic sperms is shown in Table 4.

The development of the production of insemination doses (ID) in the control and experimental groups is shown in Table 5. The results indicate that the experimental group increased the number of IDs by 20.97 pcs. The control group supplemented with the inorganic form of zinc showed a similar trend in the number of produced IDs (increase by 20.20 pcs).

Table 4. Statistical assessment and comparison of the percentage of pathologic sperms in boars receiving organic and inorganic forms of zinc

Period	Organic form of zinc			Inorganic form of zinc		
	Average and standard deviation	No. of ejaculate taking	Average no. of ejaculate taking for a boar	Average and standard deviation	No. of ejaculate taking	Average no. of ejaculate taking for a boar
I	10,18 ± 8,46	61	1,84	9,4 ± 8,4	67	2,03
II	6,30 ± 3,54*	120	3,63	8,64 ± 7,66	142	4,30
III	6,79 ± 5,66	111	3,36	7,07 ± 5,18	125	3,78
IV	9,67 ± 6,57	113	3,42	9,08 ± 5,68	121	3,66
V	11,67 ± 7,68	39	1,18	9,92 ± 6,68	40	1,21
Total	8,92 ± 6,90	444	13,43	8,80 ± 6,87	495	14,98

* - the symbol is to express statistically significant changes within the column (as compared with the first period, i.e. at the beginning of the experiment) $P < 0.05$ *;

Table 5. Comparison of the theoretical production of insemination doses in groups with organic and inorganic forms of zinc (comparison only two experimental periods)

Period	Number of ID (organic source of zinc)	Number of ID (inorganic source of zinc)
I.	56.36	61.63
V.	77.33	81.83
Difference	20.97	20.20

ID – insemination doses

Discussion

In our experiment, we recorded an increasing trend in the volume of ejaculate by contrast to ALTHOUSE et al. (2000) who did not reach any material quantitative or qualitative increase of reproduction indicators in breeding boars even with a graded level given by standard. MESÁROŠ et al. (2006) succeeded on the other hand in affecting some quantitative and qualitative reproduction indicators (concentration of sperms, motility, percentage of pathologic sperms) in their experiments after a single application of 10 mg Zn/kg live weight in breeding boars by injection. In our experiment, we failed to confirm these values as doses administered to our experimental animals were lower than doses applied by the mentioned author.

LIAO et al. (1985) experimentally administered zinc to Duroc boars (n = 17) at 32, 89, 146 or 197 mg/kg of feed mixture. The semen of boars was sampled on average once in five days. Similarly as in our experiment, the team of researchers failed to prove the decreased number of morphologically modified sperms in assessing the percentage of pathologic sperms. In the concentration of sperms, LIAO et al. (1985) recorded the highest values upon the administration of 89 and 146 mg Zn/kg of feed mixture. In our survey, boars were supplemented 126.13 mg Zn/kg of feed mixture in organic and inorganic forms and in both groups we achieved –similarly as the above mentioned authors- satisfactory results of sperm concentrations. The fact was supported by experiments conducted by VIERA et al. (2008) who fed breeding boars (n = 8) a diet with zinc originating only from native resources (30.14 mg Zn/kg of feed mixture). Having been on this diet with insufficient zinc for 100 days, the experimental animals showed degenerative changes on Leydig cells and a high percentage of pathologic sperms. When the feeding ration was supplemented with 0.2 mg/kg live weight, the spermatogenesis returned to normal values within 60 days. MÁCHAL et al. (2007) compared the contents of selected microelements in the blood of boars in dependence on the concentration of microelements in the semen. Their results indicate that the concentration of sperms decreased with the increasing volume of ejaculate. The finding was not corroborated in our experiment. With the increasing amount of ejaculate, the experimental group showed only a negligible decrease of sperms concentration and the control group even showed a mildly increased concentration of sperms at the same trend of ejaculate volume development.

In two former experiments lasting three months with the administration of 126.13 mg Zn/kg of feed mixture and 128 mg Mn/kg of feed mixture to the control group (inorganic form) and to the experimental group (organic form), HORKÝ, JANČÍKOVÁ and ZEMAN (2010, 2011) arrived at similar conclusions as we in our experiment. The volume of ejaculate increased by 19% in the group supplemented with the organic form of zinc and manganese. The feeding of inorganic form of these microelements did not result in any more important differences. The percentage of pathologic sperms and sperms concentration did not exhibit any material changes as compared with the control group either. The results indicate that –similarly as in our experiment- the most distinct changes were recorded in the volume of ejaculate. SMITAL (2002) studied the quality of ejaculate and found out that the reproduction parameter is within the genetically conditioned framework affected not only by nutrition but also by the factors of external environment including the effect of season.

Experiments with the organically fixed source of zinc were conducted with other farm animal species too. KUMAR et al. (2006) for example carried out an experiment with bull breeders, in which they studied various levels and sources of zinc. Similar to our results, they found the vastest difference in the volume of ejaculate. The average amount of ejaculate was highest in the group that was supplemented with the organic form of zinc. The concentration of sperms was significantly higher ($P < 0.01$) in the group fed with organically fixed zinc, too. JANKOWSKI et al. (2002) experimented with turkeys, which were divided into ten groups (n = 5). The experimental birds were applied organic and inorganic zinc forms at various levels. Organic form of zinc did not significantly affect the studied indicators of reproduction. The volume of ejaculate did not significantly differ in any of the experimental groups. The highest values were measured in the organic form of zinc. The trend was also recorded in our experimental measurements of ejaculate amounts.

Conclusion

In the experiment that was conducted with 2 x 33 boars, we studied the effect of feeding organic and inorganic forms of zinc on selected reproduction indicators in breeding boars. The animals were divided into two equal groups, which were subject to the measurement of ejaculate amount, concentration of sperms and percentage of pathologic sperms.

In the organically fixed zinc, we recorded an increase of ejaculate volume from 207.5 ± 66.56 ml to 289.5 ± 81.28 ml, which represented an increase by 39.51 % ($P < 0.001$). The group with the inorganic zinc form exhibited an increased volume of ejaculate too – from 229.62 ± 102.64 ml to 289.14 ± 122.48 ml, i.e. an increase by 23.93 % ($P < 0.05$). The evaluation of the concentration of sperms revealed a slight decrease in the experimental group from 407.44 ± 126.37 thous./mm^3 to 400.67 ± 200.86 thous./mm^3 while the control group exhibited a slight increase from 402.53 ± 180.26 thous./mm^3 to 423.53 ± 149.81 thous./mm^3. In the last studied parameter of reproduction – percentage of pathologic sperms, we did not record any essential differences. Both groups showed a mild increase of this reproduction indicator – the experimental group from 10.18 ± 8.46 % to 11.67 ± 7.68 % and the control group from 9.4 ± 8.4% to 9.92 ± 6.68 %.

Our experimental results show that the organic form of zinc (Zn-methionine) had the most significant influence on the volume of ejaculate. No material differences were recorded in other studied parameters of sperms concentration and percentage of pathologic sperms in the ejaculate.

As to the increased production of insemination doses, the experimental group showed an increase by 20.97 pcs and the control group showed an increase by 20.20 pcs.

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