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THE EFFECT OF A SUPPLEMENT OF CHROMIUM (PICOLINATE) ON THE LEVEL OF BLOOD GLUCOSE, INSULIN ACTIVITY AND CHANGES IN LABORATORY EVALUATION OF THE EJACULATE OF BREEDING BOARS

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Abstract

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The experiment was based on feeding the organic form of chromium (picolinate) and the assessment of its effect on the level of blood glucose, insulin activity and changes in the laboratory values of the ejaculate (sperm motility, ejaculate volume, sperm concentration and per cent of pathological sperm) in breeding boars. The experiment involved 40 boars divided into two equal groups. Boars of the experimental group (n = 21) received 181.81 µg of chromium per kg of feed ration (FR) administered perorally, in the control group (n = 19) chromium intake was not increased.
The chromium supplement significantly ($P < 0.05$) increased cell absorption of blood glucose in the experimental group of boars as against the control group. There was no difference in insulin activity between the two groups.
Changes in laboratory values of the ejaculate were evaluated and no significant differences were discovered in any of the parameters. During the experiment the sperm concentration and motility were absolutely the same in boars of both groups; it can therefore be concluded that increasing the level of chromium in the feed ration of boars of the experimental group had no direct effect on these parameters. It was the same in the case of the ejaculate volume which gradually decreased between periods 1 and 4, in boars receiving chromium by 12.8 % and in the control group by 8.1 %. The beneficial effect of chromium was seen in the reduced count of pathological sperm in boars in periods 3 and 4 of tests as against boars of the control group. In spite of the considerable 26.3 % difference between the groups this decrease was not statistically significant. Data evaluation revealed a significant correlation ($P < 0.01$) between the number of samplings per boar and sperm concentration. A correlation ($P < 0.05$) was also detected between the ejaculate volume and sperm concentration

boar, ejaculate, blood, chromium, picolinate, glucose

Chromium (Cr) as an essential trace element in animal nutrition (Schwarz and Mertz, 1959; cit., Underwood and Suttle, 1999) is indispensable for normal carbohydrate (Mertz, 1975; Amoikon *et al.*, 1995; Evock-Clover *et al.*, 1993), lipid (Balk *et al.*, 2007; Cefalu and Hu, 2004; Uusitupa *et al.*, 1992) and protein metabolism (Evans and Bowman, 1992; Roginksi and Mertz, 1969). Cr increases the effect of insulin, the count of insulin receptors on the cell surface and the sensitivity of cells to insulin (Anderson, 1997; Anderson, 1998) by which means the cell absorption of blood glucose increases (Mooradian and Morley, 1987). The demand for Cr increases under conditions of higher stress – e.g. during fatigue, injury, reproduction load, various forms of metabolic, physical and emotional stress

as well as effects of the environment (Anderson, 1994; cit., Anderson *et al*., 1997; Wright *et al*., 1994; Lindemann, 1999). At present a legislative ban has been imposed in EU countries on the administration of Cr (Pechová and Pavlata, 2007).

Up to the present time, research activities exploring the effect of chromium on the reproductive ability of pigs have been successfully performed with sows (Lindemann *et al*., 1995; Page *et al*., 1993; Amoikon *et al*., 1995). The objective of the present study was to discover if the recommended daily rate of chromium had a direct impact on the blood glucose level, insulin activity and changes in the laboratory values of the ejaculate of breeding boars.

MATERIAL AND METHODS

The experiment was conducted at the boar insemination station (BIS) in Velké Meziříčí and involved 40 breeding boars divided into two equal groups according to age and breed. The following breeds were used for the experiment: Duroc, Czech Improved White, Landrace and the paternal breeds SL 38 (Pn x DU), SL 48 (LW x Pn). The experimental animals were housed individually and had *ad-libitum* access to water. The boars ware 1 to 3 years old and average weight of boars was 300 kg. The boars ware stable in individual box.

All the animals were fed 3.3 kg of the basic feed ration (Tab. I) containing 62 µg of Cr per kg. The total uptake of Cr by boars of the control group (n = 19) was 205 µg/day, what is only 31% of the recommended daily supply according to Close and Cole (2003). In addition to that boars of the experimental group (n = 21) received organically bound chromium applied per orally as chromium picolinate in the form of pills at a rate of 181.81 µg per kg of the feed mixture. These pills were administered during morning feeding.

To demonstrate spermatogenesis (ca 42 days) the experiment was established to last 95 days. Monitoring was commenced in the first half of December 2010 and was terminated in mid-March 2011. The experiment was divided into four periods lasting 18, 31, 28 and 18 days, respectively. Samples of boars' sperm were taken according to the current demands for the production of insemination doses considering the health condition and age of the boar; minimally 3 times a month. The veterinarian monitored the health condition of the animals.

Macroscopic and microscopic evaluation of the ejaculate was performed in the laboratory of the insemination station. The ejaculate volume was assessed using a graduated cylinder. Sperm motility was determined microscopically within 15 minutes of sampling using sperm that had been gently stirred. The evaluation of motility was done by microscopic method. Subjectively It was assessed straighforward movement behind head. Sperm concentration was determined by photometry using the Spekol 11 instrument. The per cent of pathological sperm was determined microscopically from the first sampling in the month.

Blood samples for analyses were taken on day 43 of the experiment from six boars of each group, 2 hours before morning feeding. Prior to sampling the boars were not physically burdened or stressed. Blood was taken from the *vena jugularis externa* into plastic sampling bottles. Immediately after collection the blood samples were placed in a packing cooler and were analysed within 2 hours at the latest. Samples of plasma were obtained by centrifugation during 16 000 speed per minute.

For the determination of glucose 200 µl of the reagent, glucose (Groner, Germany) was drawn with a pipette into plastic burettes and then 10 µl of the blood sample was added. Absorbance was measured for 10 minutes at γ = 505 nm. For calculations we used the values of the absorbance of the reagent and absorbance values after 10 minutes of incubation with the sample. The values were subtracted and the result was recalculated according to the calibration curve for the content of glucose.

The immuno-enzymatic method (EIMA) was used to determine the activity of insulin. The measurements were performed with the automatic analyser AIA 600 II intended for measurements of immunochemical parameters in biological fluids. To analyse insulin we used 50 µl of the analysed blood plasma of boars mixed with 100 µl of the diluting solution. The samples were incubated at 37 °C. The sample was then rinsed with a wash solution and after adding the 4-methylumbelliferyl phosphate (4MUP) substrate into the test cup we measured the fluorescence intensity of the insulin. The concentration was calculated according to the performed calibration curve.

The results were evaluated statistically using the Statistika programme and the differences between the means were evaluated by Student's t – test.

I: *The composition of the feed ration for boars*

Component	% in feed ration
Barley grain	36.00
Wheat grain	20.36
Oat grain	20.00
SBM (soybean meal)	14.50
EKPO T	3.00
BergaFat	2.10
Calcium carbonate	1.50
Monodicalciumphosphate	1.20
Mineral vitamin premix for boars	0.50
Sodium chloride	0.40
Magnesium oxide	0.15
L-Lysine HCl	0.14
L - Threonine	0.09
Methionine DL	0.06

BergaFat – palm oil; EKPO T – biscuit meal

RESULTS

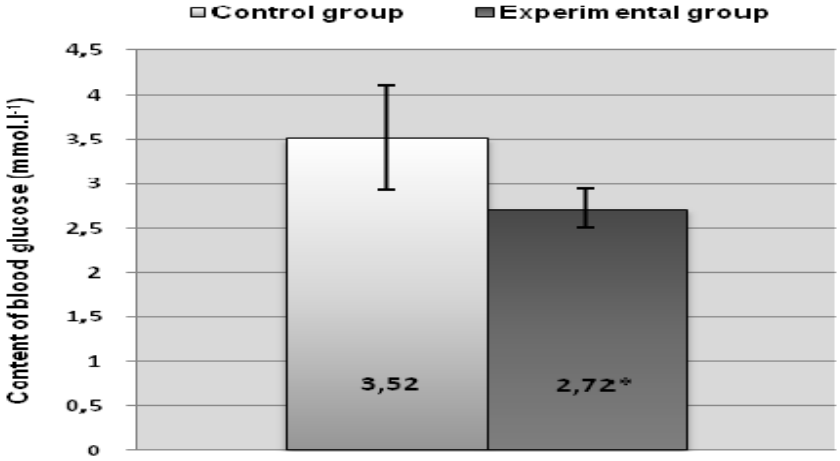
In the course of the experiment we evaluated the effect of the chromium supplement on the level of glucose and activity of insulin in the blood of breeding boars. Fig. 1 and 2 show the average values of the monitored parameters, their standard deviations and statistical correlations. The average values of blood glucose were statistically significantly lower ($P < 0.05$) in boars of the experimental group than in boars of the control group; the difference between the two groups was 22.73%. No difference was detected in the activity of insulin between the two groups.

During the experiment we also collected data from laboratory evaluations of the ejaculate of boars. Tab. II shows the average value of the monitored parameters, their standard deviations and statistical correlations. From the table it is evident that the intervention into the feed ration of the boars did not significantly change any of the monitored parameters. Nonetheless we can notice that the chromium supplement, the effect of which was observed in periods 3 and 4 of the experiment, reduced the incidence of pathological sperm in the boars as against boars which did not receive this trace element. In periods 3 and 4 the incidence of pathological sperm was respectively by 26.3% and 10.0% lower in breeding boars of the experimental group and in the control boars. During the experimental monitoring the trend in the concentration and motility of the sperm was absolutely the same in both groups of boars (Fig. 3, 4) which indicates that increasing the chromium level in the feed ration of boars of the experimental group had no direct effect on these parameters. The average value of sperm motility in the initial sampling of the breeding animals of the control and experimental group was 70.53 ± 2.71% and 68.49 ± 5.29%, respectively, and during the monitoring period the sperm motility of boars of both groups did not change by more than 1.3%. In both groups the difference in the concentration of sperm between the individual periods did not exceed 61.5 thousand sperm.mm^{-3}. In terms of the volume of the ejaculate we saw a linear decrease in this parameter in boars which received the chromium supplement, i.e. by as much as 36.94 ml between periods 1 and 4. An almost identical tendency was also seen in boars of the control group; it decreased by 22.98 ml between periods 1 and 4.

Evaluations of the obtained data showed a highly significant correlation ($P < 0.01$) between the average

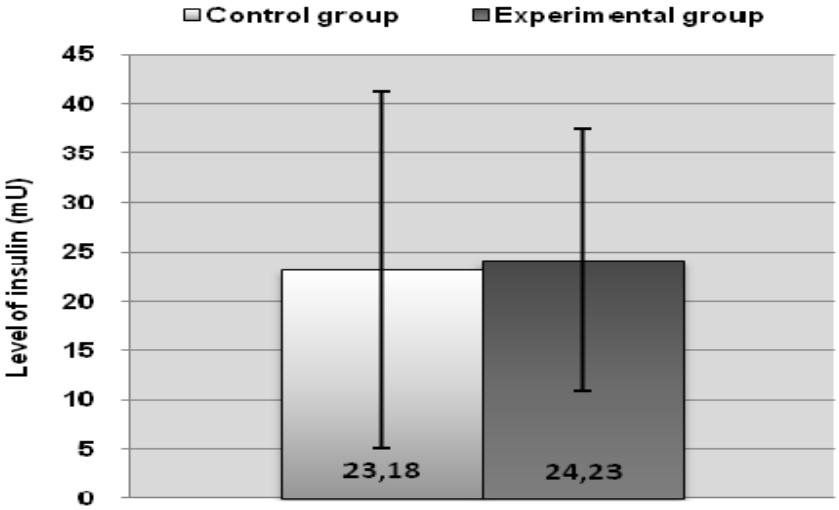
II: *Representation of changes in laboratory values of the boar ejaculate*

Period	Group	Number of taking	Average number of taking/boar	Parameters of ejaculate			
				Volume (ml)	Motility of sperm (%)	Concentration of sperm (tis/mm^3)	Pathological sperm (%)
I.	Control	53	2.78	284.87 ± 69.09	70.53 ± 2.71	387.24 ± 142.00	8.30 ± 6.15
	Experimental	56	2.66	288.25 ± 129.09	68.49 ± 5.29	462.22 ± 133.67	9.48 ± 4.75
II.	Control	64	3.36	264.77 ± 78.21	70.53 ± 1.53	443.53 ± 125.62	8.51 ± 4.83
	Experimental	70	3.33	284.66 ± 119.78	68.73 ± 7.29	523.69 ± 169.62	9.16 ± 4.88
III.	Control	61	3.21	264.99 ± 79.88	69.53 ± 8.13	423.40 ± 150.57	11.71 ± 7.16
	Experimental	61	2.90	267.20 ± 112.73	67.22 ± 9.01	502.22 ± 148.34	8.63 ± 4.68
IV.	Control	42	2.21	261.89 ± 88.24	71.40 ± 4.02	368.20 ± 117.85	10.40 ± 7.70
	Experimental	51	2.42	251.31 ± 101.76	68.89 ± 6.80	427.38 ± 133.45	9.36 ± 6.10

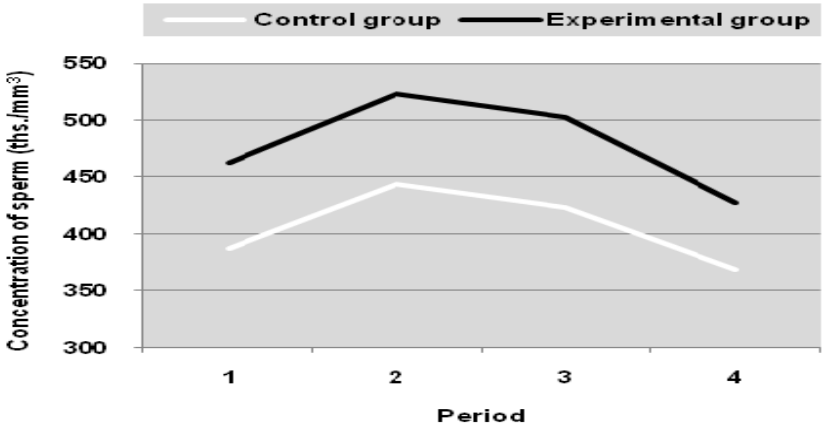


1: *Representation of the average level of blood glucose in boars included in the experiment *(P < 0.05)*

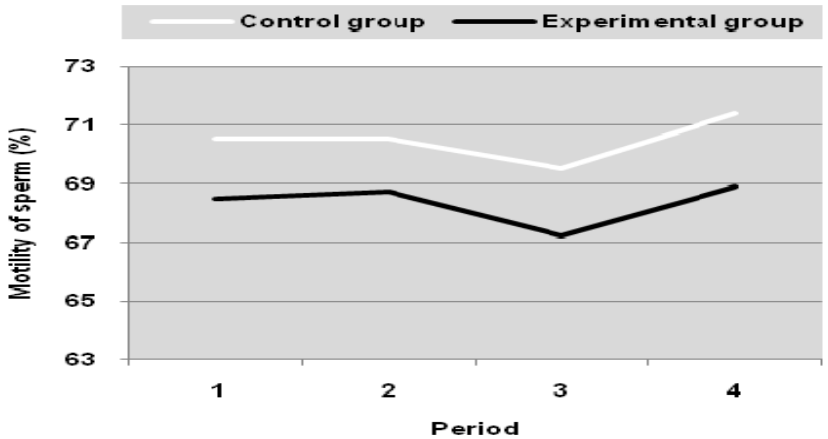
number of sperm collections per boar and the concentration of the sperm (Fig. 5). A correlation was discovered between the volume of the ejaculate and the sperm concentration as Fig. 6 shows. In both the control and experimental group of animals the correlation between these variables was statistically significant ($P < 0.05$). Fig. 6 shows that the sperm concentration decreased with the increasing volume



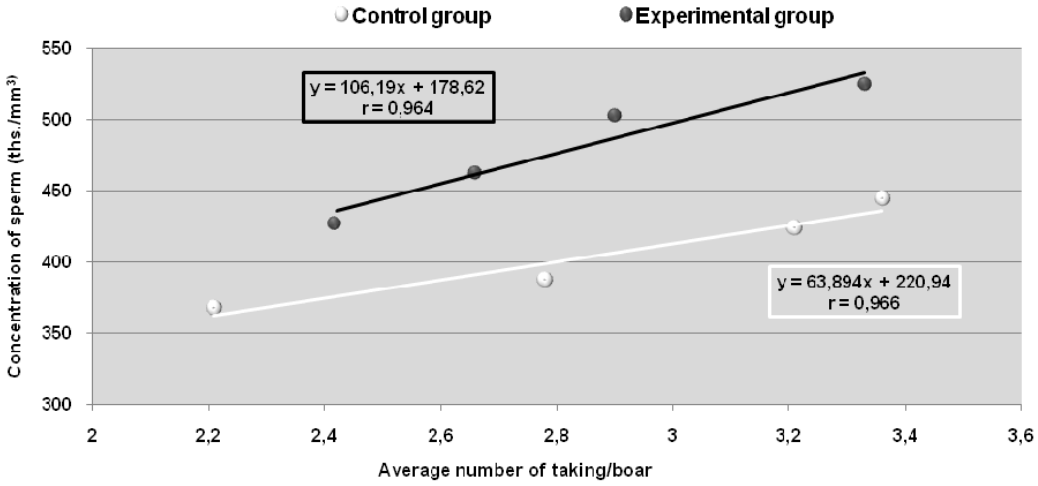
2: *Representation of the average activity of insulin in boars included in the experiment*



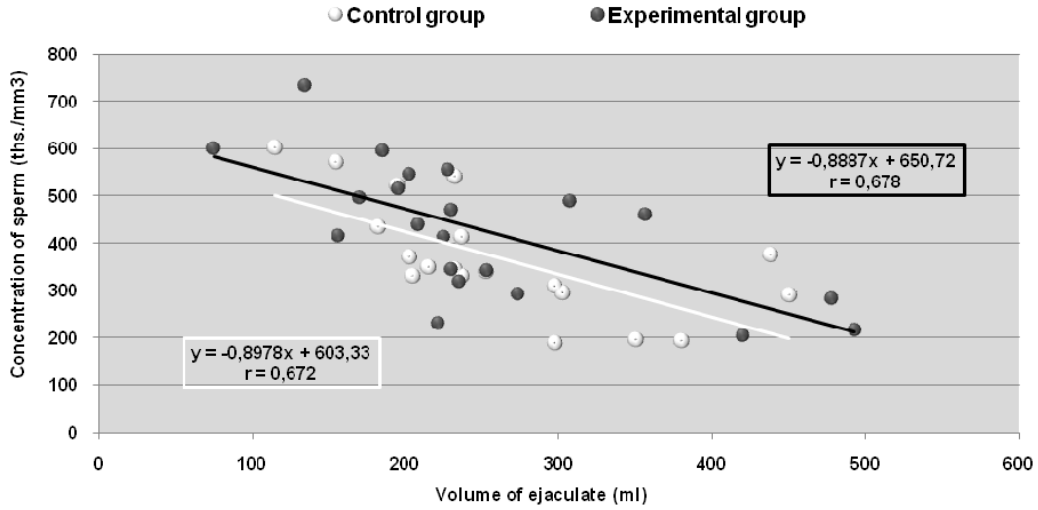
3: *Representation of the sperm concentration in boars during the experiment*



4: *Representation of sperm motility in boars during the experiment*



5: *Correlation between the average number of collections per boar and sperm concentration in boars during the experiment*



6: *Correlation between the volume of the ejaculate and sperm concentration in boars during the experiment*

of the ejaculate. In the experimental group a 10 ml increase in the volume of the ejaculate reduced the sperm concentration by 8.89 thousand sperm. mm³; similarly in the control group every 10 ml higher ejaculate volume decreased the sperm concentration by 8.98 thousand sperm/mm³.

DISCUSSION

In their study Roginski and Mertz (1969) proved that chromium has an effect on the metabolism of carbohydrates and on the hormone insulin. Insulin acts in such a way that it attaches to specific receptors on the cell surface and Cr can either increase the number of insulin receptors or increase the affinity of insulin receptors, and/or a combination of both (Anderson, 1987; cit. Page *et al.*, 1993). Evans and Bowman (1992) reported that Cr picolinate increased the rate of internalisation of insulin into bone muscles of rats and increased glucose absorption. This increase should be consistent with the detected reduction in the concentration of insulin in the blood. Page *et al.* (1993) reported that in their study the serum concentration of insulin was not affected by chromium; Amoikon *et al.* (1995) reached the same conclusions. Neither in our study did the insulin activity change in boars receiving chromium; the experimental group showed the same value as boars of the control group. Chromium deficiency in humans consuming normal food increased the level of glucose and insulin in the blood (Mertz, 1998; Jeejeebhoy *et al.*, 1977). In their study Balk *et al.* (2007) confirmed that a supplement of chromium reduced the glucose level by 1 mmol/l in a group of individuals afflicted by diabetes of type 2. In humans not suffering from diabetes, chromium had no effect on the concentration of blood glucose. Anderson (1998) confirmed these conclusions and added that the dose recommended for an individual afflicted by diabetes of type 2 is 200 µg/day. In his publication Lukaski (1999) reported that a dose of 250 µg/day of trivalent chromium

administered for two weeks to humans with a high level of blood glucose reduced the level of blood sugar to the physiological threshold. Anderson (1998) compiled a survey of a number of studies implying that a supplement of chromium improved glucose tolerance and reduced the concentration of plasma glucose also in people not suffering from diabetes. In our experiment a supplement of chromium significantly reduced the level of blood glucose in boars.

Dsehmukh *et al.* (2009) evaluated the motility, sperm concentration and morphological changes on sexual organs after supplementing doses of 0, 4, 15 and 60 µg of chromium per kg of feed ration of trivalent chromium for rats. These authors discovered that during the entire experimental period no changes in the quality of the ejaculate were detected which could be ascribed to increased rates of chromium. The best results in motility and sperm concentration were achieved at a dose of 4 µg of chromium per kg of feed ration of chromium; these results however were not statistically significant. In a similar experiment with rats Anderson and Polansky (1981) discovered that the overall sperm count in rats fed a feed ration containing less than 100 µg of chromium per kg of feed ration of chromium decreased. Along with this also successful fertilisation decreased by 25% as compared with the group which received a supplement of chromium in the feed ration. Anderson (1988) also discussed the negative impact of chromium insufficiency on fertility and sperm count in laboratory rats. Gall *et al.* (2003) tested a supplement of chromium picolinate in 153 boars divided into two groups. The boars received 2.27 kg of feed ration with a supplement of 200 µg of chromium per kg of feed ration of chromium, similarly as in our experiment. The boars were sampled on average three times a week and the experiment lasted 135 days. This research team also reached the conclusion that supplementing the feed ration of the boars with chromium has no radical effect on reproductive parameters. In their publication Close and Cole (2003) stated that it is suitable to supplement the feed ration of boars in reproduction with chromium to improve the reproduction parameters. According to these authors chromium supplementation increased the concentration of sperm and overall fertility in male laboratory rats. They further mentioned that a 200 µg of chromium per kg of feed ration supplement of chromium had a positive effect on the overall reproductive potential of breeding boars. According to Wilson *et al.* (2004) supplementing the feed ration of boars with chromium may also have a positive effect on overcoming stressful situations and improving reproduction parameters. In our studies we discovered that chromium had a positive effect on decreasing the incidence of pathological sperm in boars of the experimental group compared to boars of the control group; in periods 3 and 4 the quality of the ejaculate increased considerably.

According to the study of Foote *et al.* (1959); cit. Foote (1978) more frequent ejaculations increased the total count of sperm ejaculated in one unit of time. If sperm was collected on a daily basis the concentration was 178 thousand/ml, sperm collected once in 3 days gave a concentration of 269 thousand sperm/ml. Audet *et al.* (2009) conducted an experiment with 50 Duroc boars. When sperm was collected more frequently (3 times a week) the sperm concentration decreased by 30% as compared to sperm in boars collected 3 times in two weeks. In our experiment the sperm concentration increased with the increasing frequency of ejaculation (Fig. 5) and can be attributed to a very low use of the boars (maximally once a week) for sperm collections.

CONCLUSION

In an experiment conducted with 40 boars it was observed that feeding the organic form of chromium had an impact on the level of blood glucose, on the activity of insulin and on changes in laboratory values of the ejaculate of breeding boars.

In our study an addition of chromium to the feed ration for boars did not result in significant changes in the studied parameters, with the exception of blood glucose. Even so we noticed that chromium had a beneficial effect on reducing the incidence of pathological sperm in boars receiving chromium picolinate in comparison with boars of the control group. The effect of chromium under conditions of stress and more detailed investigation of factors influencing the effect of chromium on overall health and reproduction of breeding boars will have to be the subject of further research.

SUMMARY

The experiment was based on feeding the organic form of chromium (picolinate) and the assessment of its effect on the level of blood glucose, insulin activity and changes in the laboratory values of the ejaculate (sperm motility, ejaculate volume, sperm concentration and per cent of pathological sperm) in breeding boars.

The 40 boars were divided into two equal groups. The experiment involved 40 boars divided into two equal groups. Boars of the experimental group (n = 21) received 181.81 µg of chromium per kg of feed ration (FR) administered perorally, in the control group (n = 19) chromium intake was not increased.

The average values of blood glucose were statistically significantly lower ($P < 0.05$) in boars of the experimental group than in boars of the control group; the difference between the two groups was 22.73 %. No difference was detected in the activity of insulin between the two groups.

Changes in laboratory values of the ejaculate were evaluated and no significant differences were discovered in any of the parameters. During the experiment the sperm concentration and motility were absolutely the same in boars of both groups; it can therefore be concluded that increasing the level of chromium in the feed ration of boars of the experimental group had no direct effect on these parameters. It was the same in the case of the ejaculate volume which gradually decreased between periods 1 and 4, in boars receiving chromium by 12.8 % and in the control group by 8.1 %. The beneficial effect of chromium was seen in the reduced count of pathological sperm in boars in periods 3 and 4 of tests as against boars of the control group. In spite of the considerable 26.3 % difference between the groups this decrease was not statistically significant.

Evaluations of the obtained data showed a highly significant correlation ($P < 0.01$) between the average number of sperm collections per boar and the concentration of the sperm. A correlation was discovered between the volume of the ejaculate and the sperm concentration. In both the control and experimental group of animals the correlation between these variables was statistically significant ($P < 0.05$). The sperm concentration decreased with the increasing volume of the ejaculate.

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