THE EFFECT OF VARIOUS FORMS (ORGANIC, INORGANIC) AND LEVELS OF SELENIUM ON THE LABORATORY VALUES OF THE EJACULATE OF BREEDING BOARS IN SUMMER SEASON

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Abstract

The experiment was based on feeding the organic and inorganic forms of selenium and the assessment of their effects on the laboratory values of the ejaculate (total count of sperm, sperm motility, ejaculate volume, sperm concentration and per cent of pathological sperm) in breeding boars. The experiment involved 78 boars divided into four equal groups. The individual groups received feed mixtures with the supplement of 0.3 and 0.6 mg Se/kg of feed mixture in organic form, and 0.3 and 0.6 mg Se/kg of feed mixture in inorganic form. The experiment lasted 20 weeks, during which the average daily temperature was 21°C. In the last 30 days of our study the boars were exposed to higher ambient temperatures.

The selenium supplement significantly decreased the sperm concentration in boars supplemented by 0.3 mg Se/kg of feed mixture ($P < 0.01$) in inorganic form; this group of boars also had a lower total count of sperm produced ($P < 0.01$). The group of boars receiving the inorganic selenium supplement of 0.6 mg/kg of feed mixture had a lower sperm concentration ($P < 0.05$); however ejaculate volume increase ($P < 0.01$) occurred at the same time. In our experiment the groups of boars receiving the selenium supplement of 0.6 mg/kg of feed mixture in both organic and inorganic form had the most equal values of the total count of sperm produced. It can be assumed this rate of selenium is capable of eliminating the impacts of heat stress on breeding boars to a certain degree.

Key Words: Boar, ejaculate, selenium, heat stress

Selenium is an essential element for pigs in reproduction. For many years selenium was regarded as a substance with toxic effects in livestock. Essentiality of selenium was discovered in 1957. This element plays an important role in the correct sequence of physiological functions, especially in high-production animals (SCHWARZ and FOLTZ, 1957; cit., UNDERWOOD and SUTTLE, 1999). Selenium occurs in all cells and body tissues; its content in an organism varies according to the amount of the element in the feed ration (KIM and MAHAN, 2001). Selenium is a component of glutathione peroxidase enzyme, which counts among the most important antioxidants in animal bodies (SMITH, 1979; KOLLER *et al.*, 1984). Shortage of selenium in the diet can cause sperm deformities and infertility in males (WU, 1979; HAWKES, 2001). For the present, modern genotypes of breeding boars the recommended rate of selenium is 0.3 mg/kg feed mixture (NRC, 1998).

Currently it is possible to decide between organic and inorganic forms of selenium. The objective of this experiment was a comparison of various levels and forms of selenium and their effects on the laboratory values of the ejaculate of breeding boars.

Material and Methods

The experiment proper was conducted at the boar insemination station (BIS) in Velké Meziříčí and involved 78 boars, which were divided into four equal groups according to age and breed. The age of the boars varied from 1 to 3 years. The following breeds were used for the experiment: Duroc, Czech Improved White, Landrace and the sire breeds SL 38 (Pn x DU), SL 48 (LW x Pn). The experimental animals were housed individually (2 x 2m) and had ad-libitum access to water. All the animals were fed 3.3 kg of the basic feed mixture (Tab. 1 and 2) containing 0.02 mg Se/kg of feed mixture (FM). The experiment involved four groups of animals. The first group (S1) of boars (n = 18) were fed 0.3 mg Se/kg FM in organic form. The second group (S2) of animals (n = 20) were fed 0.3 mg Se/kg FM in inorganic form. To the third group (S3) of boars (n = 21) selenium was administered in the rate of 0.6 mg Se/kg FM in organic form. The last, fourth group (S4) of experimental animals (n = 19) were fed 0.6 mg Se/kg FM in inorganic form. Sodium selenite was fed as inorganic source of selenium. Yeast supplemented with selenium (Sel-Plex - Alltech's) was used for complementing of selenium in organic form.

To demonstrate spermatogenesis (ca 42 days) the experiment was established to last 20 weeks. Monitoring was commenced in mid-April 2011 and was terminated at the end of August 2011. The experiment was divided into five periods lasting 19, 31, 30, 31 and 31 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 ejaculate was collected from the boars by means of a phantom.

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; straight-line forward motion after the head was evaluated. 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. The ambient temperature development was monitored by means of the Dataloger device located 1.5 m above ground in the central part of the pigsty. The temperature was recorded in hourly intervals.

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

Table 1. The composition of the feed mixture for boars

Component	% in feed mixture
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 0.5%	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 assessed the effect of supplementation of various levels and forms of selenium (organic and inorganic) on the laboratory values of the ejaculate of breeding boars. During the experiment we collected data from laboratory evaluations of the boars' sperm. Tab. 3 and 4 show the average values of the monitored parameters, their statistical deviations and statistical correlations. From the tables it is evident that statistical significance in sperm motility was not found in any of the monitored groups of animals.

With the S1 group of boars (organic form of selenium – 0.3 mg/kg FM) a slight decrease in sperm concentration by 7.7% was observed at the end of the fifth period of the experiment. In the animals of the S2 experimental group (inorganic form of selenium – 0.3 mg/kg FM) a gradual decrease in sperm concentration was seen from the third period, by as many as 15.2% ($P < 0.01$) at the end of the fifth period. Throughout the entire experimental period there were no significant changes in sperm concentration in the S3 group of boars (organic form of selenium – 0.6 mg/kg FM); at the end of the experiment the decrease was only 3.2%. With the last, fourth S4 group of experimental animals (inorganic form of selenium – 0.6 mg/kg FM) a gradual decrease in sperm concentration in the ejaculate occurred; in the fourth period this decrease amounted to 11.9% ($P < 0.05$) and in the fifth period to 10.8% - this value did not significantly differ from the initial state. Simultaneously with the decreasing sperm concentration the ejaculate volume increased; in the fourth and fifth period we measured an increase by 13.8% ($P < 0.05$) and 15.9% ($P < 0.01$) respectively. Therefore the total quantity of sperm produced remained unchanged.

The volume of ejaculate in the S1 group of boars did not show significant changes, at the end of the experiment the increase was only 6.3%. Likewise the second S2 group of boars did not reach any changes; the ejaculate volume in these animals was practically at the same level throughout the duration of the experiment. We recorded no significant difference with the S3 group of animals either; in the last period there was 5.1% increase.

The total count of sperm in the boars of the S1 group decreased by 5.6%. A gradual decrease in the total count of sperm was seen in the S2 group of animals; in the fifth period the decrease amounted to 18.5% ($P < 0.01$). The S3 and S4 groups of boars had the most equal total count of sperm produced throughout the experiment with no significant differences.

The last indicator of ejaculate quality to be evaluated was the per cent of pathological sperm. In the first S1 group of boars an increasing trend was observed during the experiment, at its end the total increase by 26.4% occurred. In the S2 group of boars no significant difference in the per cent of pathological sperm was discovered. In the boars of the S3 group an increase by 20.4% was observed as from the third period, by 36.6% ($P < 0.05$) in the fourth period, and by 24.4% in the last period. The last S4 group of animals also showed an increasing trend in the development of the per cent of pathological sperm, which was most marked in the fourth and fifth periods, albeit without statistical significance.

Ambient temperature was evaluated during the experiment. The highest temperatures were measured during the fifth period (August), when the temperature during the individual days was from 25 to 30°C between 3 p.m. and 7 p.m. The greatest changes depending on the temperature development were observed with sperm concentration. Our monitoring revealed that with increasing temperature the sperm concentration decreased in S1, S2 and S4 groups of boars. On the contrary, boars of the S3 group had minimum changes in the development of the sperm concentration over the entire period of the experiment. The average temperature values in the individual periods and the sperm concentration values in the boars of the experimental groups are shown in Fig 1.

Table 2. The composition of premix for boars (0.5%)

Parameter	Unit	Quantity
Vit.A	U.I.	3,000,000
Vit.D3	U.I.	400,000
Alpha-Tocopherol	Mg	20,020
Vit.B1	Mg	500
Vit.B2	Mg	1,200
Vit.B6	Mg	800
Vit.B12	Mg	6
Vit.K3	Mg	600
Vit.C	Mg	16,000
Biotine	Mg	70
Folic acid	Mg	200
Niacinamide	Mg	8,000
Calcium pantothenate	Mg	4,000
Choline chloride	Mg	55,200
Betaine	Mg	26,500
Lysine in the form of L-Lysine monohydrochloride	g	225.79
Butylhydroxi-toluene	Mg	400
Ethoxyquin	Mg	179.82
Cu - in the form of copper sulfate pentahydrate	Mg	2,882.82
Zn – in the form of zinc oxide	Mg	19,976.02
Mn – in the form of manganese oxide	Mg	19,759.89
Fe – in the form of iron carbonate	Mg	23,624.51
Co – in the form of cobalt sulphate heptahydrate	Mg	91.35
I – in the form of potassium iodide (KI)	Mg	229.20
Carrier ad. – wheat meal, calcium carbonate	Kg	1

Table 3. Representation of changes in the laboratory values of the ejaculate in boars of the groups S1 (0.3 mg Se/kg FM organic form) and S2 (0.3 mg Se/kg FM inorganic form)

Group	Period	Average number of samplings per one boar	Ejaculate indicators				
			Total count of sperm (bill.)	Sperm motility (%)	Ejaculate volume (ml)	Sperm concentration (ths/mm^3)	Pathological sperm (%)
S1	I.	2,3	99,0 ± 30,8	72,2 ± 3,3	255,7 ± 77,4	412,4 ± 145,9	8,8 ± 5,3
	II.	3,2	102,4 ± 28,1	73,1 ± 3,9	242,9 ± 73,8	450,8 ± 159,6 *	7,3 ± 5,6
	III.	3,5	101,7 ± 27,4	69,3 ± 7,4	262,7 ± 85,9	423,6 ± 161,6	9,0 ± 7,5
	IV.	3,3	93,9 ± 22,4	72,0 ± 6,5	270,3 ± 94,2	385,1 ± 145,1	10,6 ± 7,2
	V.	3,4	93,5 ± 30,7	73,7 ± 4,7	271,7 ± 103,8	380,7 ± 170,9	11,1 ± 7,6
S2	I.	2,2	105,7 ± 21,2	72,0 ± 3,5	269,2 ± 134,5	405,2 ± 135,4	9,3 ± 5,8
	II.	3,2	100,1 ± 24,8	71,5 ± 4,9	244,7 ± 63,2	431,8 ± 137,9	7,2 ± 4,4
	III.	3,0	93,8 ± 25,4	71,1 ± 9,6	260,2 ± 74,6	388,9 ± 153,5	8,4 ± 6,3
	IV.	3,1	94,7 ± 35,8	72,5 ± 3,4	267,2 ± 68,4	370,0 ± 149,3	8,2 ± 6,6
	V.	3,7	86,2 ± 21,5 **	70,7 ± 9,9	264,0 ± 67,3	343,6 ± 111,8 **	9,3 ± 7,9

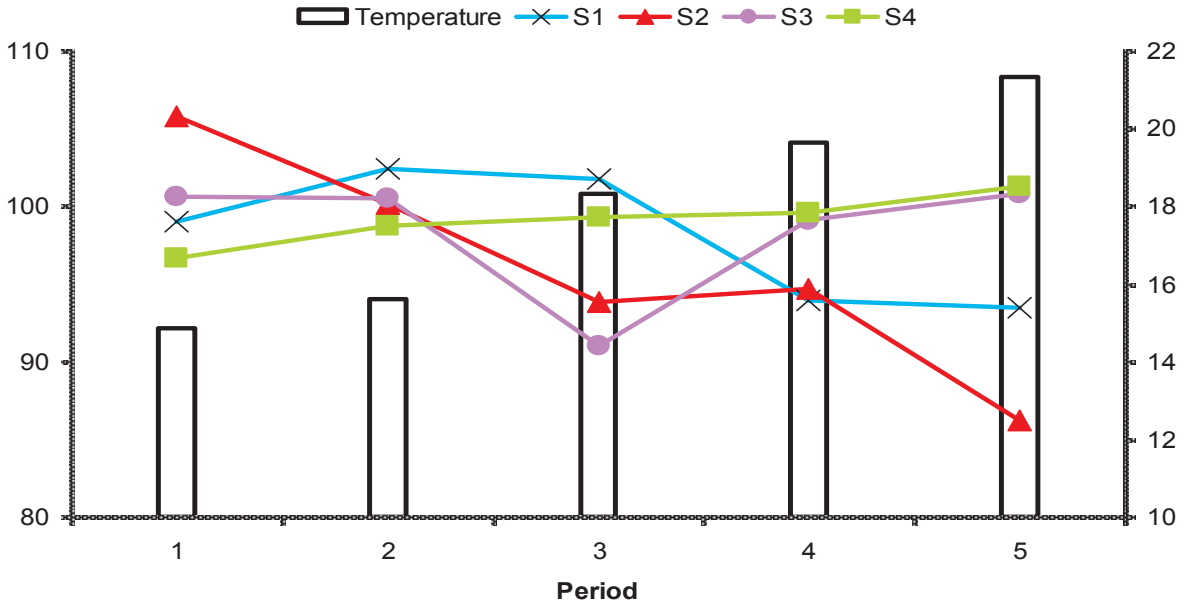
^{x} - Symbol expressing statistically significant changes (as against period I, i.e. the start of the experiment) $P < 0.05$ ^{x}; $P < 0.01$ ^{xx}

Table 4. Representation of changes in the laboratory values of the ejaculate in boars of the groups S3 (0.6 mg Se/kg FM organic form) and S4 (0.6 mg Se/kg FM inorganic form)

Group	Period	Average number of samplings per one boar		Ejaculate indicators			
			Total count of sperm (bill.)	Sperm motility (%)	Ejaculate volume (ml)	Sperm concentration (ths/mm^3)	Pathological sperm (%)
S3	I.	2,4	100,6 ± 27,0	72,2 ± 2,8	234,3 ± 72,5	452,4 ± 138,0	7,9 ± 5,0
	II.	3,1	100,5 ± 26,3	72,9 ± 4,0	232,9 ± 70,0	453,3 ± 123,6	6,3 ± 3,6
	III.	3,2	91,0 ± 16,3	73,1 ± 4,0	226,0 ± 87,2	444,6 ± 145,3	9,5 ± 7,4
	IV.	3,5	99,1 ± 25,4	70,5 ± 13,0	241,5 ± 86,4	443,6 ± 137,0	10,7 ± 5,9 *
	V.	3,6	100,8 ± 32,0	68,6 ± 14,7	247,0 ± 84,5	437,6 ± 160,6	9,8 ± 6,8
S4	I.	2,1	96,6 ± 24,4	72,5 ± 3,2	255,2 ± 83,1	407,9 ± 133,4	7,0 ± 3,9
	II.	3,3	98,7 ± 28,1	73,5 ± 3,9	272,4 ± 75,6	386,9 ± 152,7	6,3 ± 4,8
	III.	3,3	99,2 ± 33,5	72,0 ± 4,2	263,9 ± 94,8	406,9 ± 163,8	7,2 ± 4,8
	IV.	3,2	99,6 ± 32,3	71,7 ± 7,8	290,4 ± 85,1 *	359,4 ± 125,0 *	9,3 ± 5,9
	V.	3,4	101,2 ± 34,0	70,3 ± 9,2	295,8 ± 95,0 **	363,9 ± 148,1	9,3 ± 4,9

^{x} - Symbol expressing statistically significant changes (as against period I, i.e. the start of the experiment) $P < 0.05$ ^{x}; $P < 0.01$ ^{xx}

Figure 1. The development of the total count of sperm produced (bill.) in the boars of the experimental groups depending on the ambient temperature (°C)



Discussion

MARIN-GUZMAN *et al.* (1997) conducted an experiment with 192 adult cross-bred boars ([Landrace x Yorkshire] x Duroc) divided into two groups for 16 weeks. The first group of animals served as control group without selenium supplement, the second, experimental group had selenium supplemented in the feed ration in the amount of 0.5 mg/kg FM in organic form (yeasts fortified with selenium). The authors recorded an increase in ejaculate volume by 25.7% ($P < 0.05$), sperm concentration by 14.7% and sperm motility by 31.3% ($P < 0.01$) as against the control group of boars. Three years later also MARIN-GUZMAN *et al.* (2000) carried out a similar experiment, in which they compared a group supplemented with 0.5 mg Se/kg FM (in organic form) with the control group of boars receiving no selenium supplement. Selenium was fed as early as of the 28th day of age of the animals. The objective of the researchers was to find out what would be the impact of this feed intervention on the spermatogenesis in the individual life periods of the boars. During the experiment the boars were killed and their reproductive systems properly analysed. In boars receiving selenium supplement in their diet the sperm concentration was significantly increased in the 9th month ($P < 0.05$) and the 18th month ($P < 0.01$). In the 5th and 6th months of the boars' age there was no significant difference between the groups. KOLODZIEJ *et al.* (2005) also arrived at the conclusions that the 0.5 mg Se/kg FM supplement (in organic form) caused a significant rise in the sperm concentration by 29.7% ($P < 0.05$) and a decrease in the per cent of pathological sperm by 46.7% ($P < 0.05$) as compared to the control group, in the feed ration of which selenium was only contained from native sources (0.2 mg/kg FM). On supplementing organically bound selenium in the amount of 0.5 mg/kg FM GROENEWEGEN *et al.* (2006) ascertained an increase in sperm concentration by 11.1% in relation to a higher production of insemination rations (by 9.7%). No significant differences in the motility and ejaculate volume were found by this collective of authors. As our experiment did not involve a group of boars receiving no selenium supplement, and during the experiment the animals were exposed to temperature stress, the differences in the motility and concentration of sperm were not as significant. A similar experiment to ours was carried out by LÓPEZ *et al.* (2010). Group of boars A received the supplement of 0.4 mg Se/kg FM (inorganic form) in their feed ration. Group of boars B were supplemented with selenium in the amount of 0.4 mg Se/kg (organic form). Group of animals B had significantly higher sperm concentrations ($P < 0.05$) as against group A. We did not succeed in corroborating this result; with both groups of animals supplemented with the organic form of selenium no increase in sperm concentration was observed. The evaluation of the sperm motility by the above collective of authors showed a lower sperm motility with boars of group B, albeit without statistical significance. From our monitoring these conclusions are not quite evident; in the S1 group of boars we observed an increase in sperm motility by 2.0%; on the contrary the motility in the boars of group S3 was by 5.2% lower without statistical significance. In the research by JACYNO *et al.* (2005) three groups of boars were supplemented with selenium in the amounts of 0.2 mg/kg FM in inorganic form (group I), and 0.2 and 0.4 mg Se/kg

FM in organic form (groups II and III). In their evaluation of the ejaculate volume the researchers observed an increase ($P < 0.01$) in both groups supplemented with the organic form of selenium. Also in our experiment the ejaculate volume increased ($P < 0.01$), yet the increase was found in the group of boars receiving selenium in inorganic form (0.6 mg/kg FM). Furthermore, this collective of authors recorded no differences in sperm motility; the values ascertained by them between the experimental groups (72.1 – 73.3%) come close to our results, in which the motility between the groups was from 68.6 to 73.7%. The results of JACYNO *et al.* suggest an increase in sperm concentration and a decrease in per cent of pathological sperm in the groups of boars II ($P < 0.01$) and III ($P < 0.05$) as against group I. After their 13-week experiment FERNANDEZ *et al.* (2008) came to the conclusion that supplementation with organic selenium at the rate of 0.5 mg/kg FM significantly increased motility and concentration of sperm in comparison with the group of boars that were receiving only 0.025 mg Se/kg FM from native sources. After the completion of their experiment two boars from each group were castrated and their testicles microscopically assessed. The group of animals receiving selenium in their diet had more developed seminiferous tubules than the group of boars without selenium supplementation.

ECHEVERRÍA-ALONZO *et al.* (2009) compared the effect of selenium on the reduction of heat stress impacts on boars to find out that in the summer season the ejaculate volume was decreased ($P < 0.05$) in the group receiving the supplement of 0.5 mg Se/kg FM but the sperm concentration and motility increased (without statistical significance) as against the group with no selenium supplementation. As this collective of authors worked with a group of boars receiving no selenium supplement, the researchers observed increases both in sperm concentration and motility. JACYNO and KAWECKA (2002) monitored what would be the effect of supplementing 0.2 mg Se/kg in inorganic form (control group) and organic form (experimental group) to the basic diet of boars in summer (June to September) and winter (January to April) months. We are not quite in agreement with these authors in the conclusions that ejaculate volume and sperm motility were not significantly different in any of the groups. In our experiment the ejaculate volume in the animals of the S4 group was significantly higher ($P < 0.01$) in the 5th period. In the case of sperm concentration in the summer season JACYNO and KAWECKA recorded a value lower by 12.1% in the control group in comparison with the experimental animals. In the winter months in the boars of the experimental group the concentration was significantly higher by 26.1% ($P < 0.05$). The per cent of pathological sperm was not substantially different in the summer months; in the winter period this value was significantly higher ($P < 0.01$) in the boars of the control group. Our experiment was also conducted in warm summer months, therefore the sperm concentration results we measured agree with the results of these authors, who performed their monitoring in the period from June to September.

MÁCHAL *et al.* (2007) monitored the development of qualitative and quantitative values of ejaculate from February to June. In the first three months of their evaluation no significant changes occurred in the values of the ejaculate. In the last month (June) with higher temperatures they observed a decrease in sperm concentration. Sperm motility and ejaculate volume were not affected. SMITAL (2008) assessed qualitative and quantitative values of ejaculate in 2712 boars in various seasons of the year, and arrived at a conclusion that in the summer season the quality of the sperm produced dropped, on the contrary in autumn and winter months the laboratory values of the ejaculate (per cent of abnormal sperm, sperm concentration, motility, and survival) were favourable. WATTERMANN and BAZER (1985) state that an increase in temperature lowers the quality of the ejaculate and inhibits the biosynthesis of androgens and spermatids at the very start of their development. WATTERMANN, WELLS and JOHNSON (1972); WATTERMANN *et al.* (1976) exposed boars to the temperature of 31^{o}C 24 hours a day throughout a period of 11weeks. In the boars exposed to temperature stress the sperm motility and concentration were lower ($P < 0.01$) as against the control group of boars that were exposed to the 20^{o}C temperature whole day. The ejaculate volume remained unchanged. WATTERMANN and DESJARDINS (1979) put the boars to the same temperature stress as the authors mentioned before, and examined the impact of high temperature on the production of sex hormones. It ensues from their results that temperature stress reduces the level of testosterone and luteinizing hormone in boars ($P < 0.05$) and decreases sperm concentration by more than 50%. Our measured results show that already short-term temperature deviations towards 30^{o}C are capable of a significant decrease in sperm concentration in the groups of boars that received inorganic form of selenium at the rate of 0.3 mg/kg FM.

Conclusion

In the experiment involving 78 boars we compared the effect of organic and inorganic forms of selenium on the changes in the laboratory values of the ejaculate of breeding boars.

The selenium supplement significantly decreased the sperm concentration in boars supplemented by 0.3 mg Se/ kg of feed mixture ($P < 0.01$) in inorganic form; this group of boars also had a lower total count of sperm produced ($P < 0.01$). The group of boars receiving the inorganic selenium supplement of 0.6 mg/kg of feed mixture had a lower sperm concentration ($P < 0.05$); however ejaculate volume increase ($P < 0.01$) occurred at the same time. In our experiment the groups of boars receiving the selenium supplement of 0.6 mg/kg of feed mixture in both organic and inorganic form had the most equal values of the total count of sperm produced. It can be assumed this rate of selenium is capable of eliminating the impacts of heat stress on breeding boars to a certain degree.

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