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EFFECT OF SUPPLEMENTATION WITH INORGANIC AND ORGANIC SELENIUM ON SPERM QUALITY AND QUANTITY IN NORTH-EAST BULGARIAN MERINO RAMS

Rossen Stefanov[1], Mihail Chervenkov[2], Georgi Anev[3], Nevena Maksimović[4], Madlena Andreeva[1], Teodora Ivanova[5], Aleksandar Milovanović[6]

[1]Institute of biology and immunology of reproduction, BAS, Sofia, Bulgaria
[2]Faculty of veterinary medicine, University of Forestry, Sofia, Bulgaria
[3]Experimental station of agriculture, Targovishte, Bulgaria
[4]Institute of animal husbandry, Zemun, Belgrade, Serbia
[5]Institute of biodiversity and ecosystem research, BAS. 23, Acad. G. Bonchev St., Sofia, Bulgaria
[6]Scinetific veterinary institute, Novi Sad'', Novi Sad, Serbia
Corresponding author: stefanovrossen@gmail.com
*R. Stefanov and M. Chervenkov contributed equally to this work
Original scientific paper

Abstract: Selenium is a trace element, which stimulates antioxidant defenses and improves reproductive functions in human and animals, under the form of selenoproteins. The objective of the study was to evaluate the effect of selenium, supplemeted as inorganic or organic form in the diet of stud rams, on some of their semen parameters. The experiment was performed with 15 clinically healthy rams from North East Bulgarian merino breed. The animals were divided in three groups (5 per group). The rams from first experimental group (G1) received a diet with supplementation of 4,0mg sodium selenite (Na_2SeO_3) per animal per day, while the animals of the second experimental group (G2) obtained diet with 1.83g L-selenomethionine (Sel-Plex, Alltech, USA) per animal per day. Eventually, each animal from the G1 and G2 received 1.83g selenium per day. The control group (GC) received a diet without supplementation of selenium. The principal composition of the diet in each group was the same. The ejaculates were obtained via artificial vagina. The evaluated parameters were volume and pH of the ejaculates and motility, concentration and *in vitro* survivability of the spermatozoa at 39˚C for 360 min.

It was found that the supplementation of ram studs diet either with inorganic and organic selenium led to increase in the volume of the

ejaculates, motility and survivability of the spermatozoa. The pH of the freshly obtained semen was not affected by selenium treatment.

Key words: ram, selenium, diet, ejaculate, sperm quality

Introduction

The different products which can be obtained from sheep, namely meat, milk, wool and furs are the reason why the sheep breeding is one of the major sectors of animal husbandry worldwide. The development of this sector implies more intensive use of reproductive techniques in order to obtain animals with better productive traits. Some of the most popular techniques used to improve the fertility in sheep are artificial insemination and cryopreservation of semen. Central role in their successful application plays the quality of ram semen. There are many factors which influence the semen quality and feeding is one of them. The composition of the ram diet can improve or worsen the quality and quantity of their ejaculates subsequently their spermatozoa *(Kheradmand et al., 2006; Brown, 2004).*

Selenium (Se) is one of the microelements with important biochemical functions. It is a component of the enzyme glutathione peroxidase that protects the cells from accumulation of peroxide oxidation products *(Surai, 2002).* Under the form of selenoprotein it stimulates antioxidant protection and promotes reproductive activity. Low sperm Se content is associated with abnormal sperm morphology and motility in humans and several animal species *(Saaranen et al., 1989; Marzec-Wróblewska et al., 2012).* The addition of various selenium containing compounds has led to increased growth of young animals and improved productivity and health status *(Dimanov et al., 1982; Profirov et al., 1981; Dimanov et al., 1992).* Applied individually or in combinations with other additives like vitamins (A, E, D) or other microelements (i.e. Co, Zn, etc.) Se was reported to have positive effect on reproductive performance, including semen parameters, of different animal species and humans (*Sikka et al., 1995 ;Scott et al., 1998, Kendall et al., 2002; Surai, 2002; Zubair et al., 2015).* Recent data showed that Se supplementation could be useful even in improvement of the quality of dog semen with lowered fertility (*Domosławska et al., 2015*). *El-Sheshtawy et al. (2014)* reported that administration of Se increase both sperm cell concentration and percentage of alive sperms and decrease sperm abnormalities and acrosomal damage in

Baladi goat bucks. *Marin-Guzman et. al. (1997)* found that selenium accelerates the maturation of spermatozoa in the epididymis and reduces the amount of sperm with cytoplasmic droplets.

The form of Se (organic or inorganic) was also found as important factor for the outcome of the supplementation. *Lopez et al.* (2010) showed that addition of organic Se to the regular rations of boars lead to increased sperm concentration compared to inorganic Se but reduced some motility parameters and resistance to oxidative stress. Specifically in Sanjabi rams organic selenium (alone and in combinations with zinc) was recently reported to improve semen characteristics (*Ghorbani et al., 2018*). On the other hand inorganic selenium was found to decrease the percentage of sperm defects but without direct influence on ram sperm volume, total motility, concentration and membrane integrity in Brasil rams (*Piagentini et al., 2017*).

The above mention data along with the insufficient knowledge about the effect of Se on the reproductive performance of local Bulgarian sheep breeds were major clues to test the effect of selenium, supplemeted as inorganic or organic form in the diet of stud rams from North East Bulgarian merino breed (NEBM) focusing on the quality and quantity of the obtained semen.

Materials and methods

Experimental animals and diet

The experiment was performed with 15 rams from North East Bulgarian merino breed - Shumen type in The Experimental Station of Agriculture – Targovishte, Bulgaria. The animals were divided into three groups of five – a control (GC) and two experimental (G1 with addition of inorganic selenium and G2 with organic selenium supplementation. Each group consisted of rams aged 3.5 to 6.5 years of age and 90 to 110 kg of body weight. All rams were clinically healthy, without external and internal parasites and grown according to generally accepted standards for animal welfare (*Council Directive 98/58/EC*).

Throughout the experimental period the daily rations consisted of quality feed providing 100-110 g of protein digestible in the intestines, as 50% of the energy was supplied by concentrated feed.

Semen assessment

Evaluation of the ejaculates was performed at the Laboratory for artificial insemination in the Experimental station of agriculture, Targovishte, Bulgaria. The following parameters were assessed: volume of the ejaculate (in ml) - with a graduated pipette, accurate to 0.01ml; sperm motility (in %) – under microscope (Carl Zeiss, Jena, Germany) at magnification of 400 x, by a trained technician; pH of semen by pH meter (Denver Instruments, USA); sperm count in 1 ml – by using of Thoma counting chamber.

The *in vitro* survivability of the spermatozoa was assessed as follows: The ejaculates were diluted in semen extender 6A in ratio 1:3 and incubated in thermostat at 39 ˚C for 360 minutes. Since only the spermatozoa which are alive possess the ability to move, we use that as indicator for sperm survivability. Evaluation of the sperm motility was performed on 10^{th} and 360^{th} minute of incubation by the method described above.

Design of the experiment

The groups were formed 21 days prior the experiment, so that rams can be adapted to the same rearing conditions.

The study was divided into two periods - adaptive and experimental. The adaptive period continues for 21 days, during which the groups were formed, and the animals were allowed to accustom to the same living conditions. During this period the daily rations were not supplemented with selenium. In the experimental period, rams from first experimental group (G1) received a diet with supplementation of 4,0mg sodium selenite (Na_2SeO_3) per animal per day, while the animals of the second experimental group (G2) obtained diet with 1.83g L-selenomethionine (Sel-Plex, Alltech, USA) per animal per day. Eventually, each animal from the G1 and G2 received 1.83g selenium per day. The control group (GC) received a diet without supplementation of selenium. The experimental period was divided into two sub periods – 1^{st} sub period - from the 1^{st} day to the 30^{th} day of selenium addition; 2^{nd} sub period - from the 30^{th} day to the 45^{th} day of selenium addition.

The semen collection in the adaptive and experimental periods was performed once a week by artificial vagina from a trained technician. After obtaining the semen was transferred to the laboratory and processed as it was described in *SEMEN ASSESSMENT*.

Statistical analysis

The statistical analyses were performed by software R.2.8.1. Values are presented as mean ± standard deviation. The effect of the different type of selenium supplementation on semen parameters was assessed for every period by multiple comparisons between treatment groups using Student-Newman-Keuls method (SNK). For all statistical procedures performed, p values < 0.05 were considered significant.

Results and discussion

One of the important parameters which affect the semen quality is pH *(Semkov et al., 1989; Zhou et al., 2015).* The results of pH measurement of the different groups are presented at *Table 1*. It was found that the average pH of the ram ejaculates of both the control group and the experimental groups throughout the entire period of the study was in close range from 6.46 to 6.60. *Bartoov et al.(1980)* found that optimal pH for the normal function of ram semen mitochondria is in the range of 6.0 to 6.5 and as it is well known, the mitochondria are responsible for many of the sperm functions including their motility and subsequently fertilizing ability *(Piomboni et al.,2011).*

Table 1. Effect of Se supplementation on pH in ram ejaculates

Periods	Groups		
	Control (GC)	Inorganic selenium suppl. (G1)	Organic selenium suppl. (G2)
	mean± SD	mean± SD	mean± SD
Adaptation	6.53±0.07a	6.6±0.03a	6.46±0.04a
1st experimental sub period	6.46±0.03a	6.44±0.04a	6.47±0.03a
2nd experimental sub period	6.42±0.03a	6.39±0.03a	6.39±0.06a

The results are represented as mean ± SD. Values followed by different letter were significantly different by SNK (p=0.05).

All pH values of the GC and the experimental groups, at the adaptation and experimental period were at the normal range for the North East Bulgarian merino breed. The results indicate that the addition of

organic and inorganic selenium in the rations of the rams did not lead to a significant change in the pH value of the ejaculates. Even more at the end of the second experimental period the pH of the Se treated groups were lower (6.39) compared to the GC (6.42). In experiment conducted with rams under heat stress in sub-tropical environment of Egypt was found that supplementation of inorganic Se (sodium selenate) in their diet leads to significant decrease of semen pH, percentages of dead spermatozoa, sperm abnormalities and acrosomal damage and increase in sperm motility, sperm-cell concentration as well as improvement of other physiological parameters *(Marai et al., 2009*).

In the adaptive period the mean ejaculate volume in GC and in G2 was equal – 1.07 ml, while in the G3 was a little bit higher- 1.19, but without statistical significance (*Table 2*).

Table 2 Effect of Se supplementation on ram ejaculate volume.

Periods	Groups		
	Control (GC)	Inorganic selenium suppl. (G1)	Organic selenium suppl. (G2)
	mean± SD (ml)	mean± SD (ml	mean± SD (ml)
Adaptation	1.07±0.07a	1.19±0.07a	1.07±0.06a
1st experimental sub period	1.03±0.05a	1.27±0.06b	1.22±0.06b
2nd experimental sub period	1.10±0.05a	1.46±0.09b	1.33±0.14b

The results are represented as mean ± SD. Values followed by different letter were significantly different by SNK (p=0.05).

After the addition of inorganic and organic Se to the diet of the animals from the experimental groups (G1 and G2), the volume of their ejaculates become significantly higher ($p<0.05$) in comparison to the control group.

It is interesting to note that while the average increase in the volume of the ejaculate in the two experimental groups (in absolute values) was the same at the end of the experimental period (about 0.26ml), the effect of organic selenium addition was more pronounced at the 1st experimental sub period compared to the inorganic Se supplementation. The obtained results suggest that the addition of both organic and inorganic Se to the main diet

increase the ejaculate volume in rams. The effect was more rapid with organic selenium.

Similarly to us *Mahmoud et al., (2013)* found significant increase in ejaculate volume in rams after application of combination of Se and vitamin E. In experiment with Barbari bucks, the supplementation of their diet with combination of zinc and selenium also lead to significant increase of ejaculate volume *(Kumar et al.,2014).* On the other hand *El-Sheshtawy et al.,(2014),* didn't found significant difference in the ejaculate volume of bucks injected with Se, vitamin E or combination of both compared to non-treated group.

Another important factor for determining the quality of semen material in farm animals is sperm survivability at 39°C for 360 min. At *Table 4* are presented the data from the sperm resistance at 39°C in the supplemented with Se and non-supplemented groups. During the adaptation period, the average survival rate of the ram spermatozoa at the 10^{th} and the 360^{th} min of incubation in GC and G1 are similar. In G2 the survival rate is significantly higher than in GC and G1, at both measurements.

During the entire experimental period, the groups which received Se supplemented diet shows significantly higher sperm survival rate in comparison to the control group, both at the 10^{th} and at the 360^{th} minute of incubation at 39°C. The results in the first experimental sub period (from the 1^{st} to the 30^{th} day of Se supplementation) are similar in G1 and G2, but in the second experimental sub period (from the 30^{th} to the 45^{th} day of Se supplementation), the animals which were supplemented with organic selenium, displayed a significantly higher survival rate (45%) after 360 min of incubation at 39°C, in comparison to those which were supplemented with inorganic selenium (33%).

Table 3. Effect of Se supplementation on ram sperm motility

Periods	Groups		
	Control (GC)	Inorganic selenium suppl. (G1)	Organic selenium suppl. (G2)
	mean± SD (%)	mean± SD (%)	mean± SD (%)
Adaptation	48.61±4.33a	47.63±3.52a	64.38±3.29b
1^{st} experimental sub period	56.33±3.18a	67.43±0.60b	68.33±0.91b
2^{nd} experimental sub period	53.5±4.87a	71.5±0.54b	70±1.62b

The results are represented as mean ± SD. Values followed by different letter were significantly different by SNK (p=0.05).

The presented results demonstrated that the additional intake of inorganic and organic selenium has a positive effect on the survival of sperm cells during incubation for 360 min at 39˚C.

The effect of selenium supplementation on sperm motility is presented in *Table 3*. The average percent of motile spermatozoa in GC during the adaptation and trial period varies within narrow limits and ranges from 48.61% to 56.33%.

There is a statistically significant difference between the percent of motile spermatozoa in G1 (47.63%) and G2 (64.38%) in the adaptive period, which eventually disappeared after the addition of inorganic or organic selenium to the ram's diet. In both experimental sub periods the percent of motile spermatozoa is significantly higher in the groups with inorganic (67.43% for the 1st and 71.5% for the 2nd) and organic (68.33% for the 1st and 70% for the 2nd) Se supplementation, compared to the group without Se supplementation (56.33% for the 1st and 53.5% for the 2nd). This result suggests that Se supplementation as inorganic or organic form has positive effect on sperm motility.

Table 4. Effect of Se supplementation on ram sperm survivability at 39˚C for 360min

Periods	Groups					
	Control (GC)		Inorganic selenium suppl. (G1)		Organic selenium suppl. (G2)	
	10 min (% motile spermatozoa)	360 min (% motile spermatozoa)	10 min (% motile spermatozoa)	360 min (% motile spermatozoa)	10 min (% motile spermatozoa)	360 min (% motile spermatozoa)
Adaptation	48.33±4.33b	5.28 ±2.00e	46.39 ±4.33b	5.94 ±2.00e	61.88 ±4.33a	16.14 ±3.33d
1st experimental sub period	57.25±6.21a	4.0 ±0.92e	65.4 ±1.00a	12.32 ±2.43d	65.5 ±1.20a	16.30 ±2.43d
2nd experimental sub period	53.0±3.15a	3.8 ±0.61e	71.5 ±0.96a	33.0 ±2.38c	70.5 ±1.12a	45.00 ±2.38b

The results are represented as mean ± SD. Values followed by different letter were significantly different by SNK (p=0.05).

Sperm motility is accepted as a major factor for assessment of semen quality and fertilization ability *(David et al., 2015),* which means that Se supplementation to the NEBM ram's diet may have a beneficial effect on the reproductive performance of the animals.

In experiment conducted by *Piagentini et al. (2017)* was found that Se supplementation to ram's diet significantly improve the morphology, but not the motility of the sperm. The difference between the results of *Piagentini et al.(2017)* and those obtained in our research can be contributed to different factors like the different climate (subtropical in their case, moderate in ours), breed differences or the way of semen collection (we use artificial vagina and they used electroejeculation). On the other hand there is a lot of data which shows that Se alone or in combination with Zn or vitamin E improves not only the morphology but also the sperm motility in rams (*Kendall et al., 2000; Marai et al., 2009; Ghorbani et al., 2018*). Moreover it was found that the injective application of selenium alone or in combination with vitamin E increase the sperm motility in bucks (*El-Sheshtawy et al., 2014),* and rams *(Mahmoud et al., 2013).* None the less it was found that addition of selenium to semen of ram, water buffaloes and human has positive effect on sperm parameters after freezing and thawing procedures *(Seremak et al., 1999; Dorostkar et al., 2012; Rezaeian et al., 2016).* In experiments with boars was found that addition of selenium improves the sperm quality including motility and fertilization rate *(Marin-Guzman et. al., 1997).* The same authors also stated that the insufficiency of Se in boar's diet is even more detrimental for semen quality than the insufficiency of vit E *(Marin-Guzman et. al., 1997).*

Conclusion

The addition of selenium as organic or inorganic form to the diet of the North East Bulgarian merino rams has positive influence on the ejaculate volume, sperm motility and sperm survival rate after incubation at 39˚C for 360 min, without negative effect on pH of the ejaculates.

The represented data combined with the findings of the other studies on that topic, suggested that selenium supplementation in the main diet can be used for improving the reproductive traits of ram studs.

Uticaj dodavanja neorganskog i organskog selena na kvalitet i količinu sperme ovnova severoistočne bugarske merino rase

Rossen Stefanov, Mihail Chervenkov, Georgi Anev, Nevena Maksimović, Madlena Andreeva, Teodora Ivanova, Aleksandar Milovanović

Rezime

Selen je element u tragovima, koji stimuliše antioksidantske odbrane i poboljšava reproduktivne funkcije kod ljudi i životinja, u obliku selenoproteina. Cilj studije je bio da se proceni efekat selena, dopunjenog u neorganskom ili organskom oblik u ishrani ovnova, na neke parametre semena. Eksperiment je obavljen sa 15 klinički zdravih ovnova severnoistočne bugarske merino rase. Životinje su podeljene u tri grupe (5 po grupi). Ovnovi prve eksperimentalne grupe (G1) dobijali su obrok sa dodatkom 4,0 mg natrijum selenita (Na_2SeO_3) po grlu dnevno, dok su životinje druge eksperimentalne grupe (G2) hranjene obrokom sa 1,83 g L-selenometionina (Sel- Plek, Alltech, SAD) po grlu dnevno. Na kraju, svaka životinja iz G1 i G2 dobijala je 1,83g selena dnevno. Kontrolna grupa (GC) dobila je obrok bez dodatka selena. Glavni sastav obroka u svakoj grupi bio je isti. Ejakulati su dobijeni preko veštačke vagine. Ocenjivani su sledeći parametri: volumen i pH ejakulata i pokretljivosti, koncentracija i preživljavanje in vitro spermatozoida na 39°C tokom 360 min.

Utvrđeno je da dodatak obroku neorganskog i organskog selena doveo do povećanja zapremine ejakulata, pokretljivosti i preživljavanja spermatozoida. Na sveže dobijeno seme nije uticao tretman sa selenom.

Ključne reči: ovan, selen, ishrana, ejakulat, kvalitet sperme

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