EFFECT OF THE *Spirulina platensis* INCLUDED IN THE MAIN DIET ON THE BOAR SPERM QUALITY

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Invited paper

Abstract: Microalgae *Spirulina platensis* accumulates many chemical components which are suitable for all higher organisms as food and forage raw material. There are a lot of vitally important for the organisms minerals and macro-elements such as iron, calcium, sodium, potassium, copper, magnesium, phosphorus, selenium, vitamins, carotin, nucleic acids, enzymes and other active substances. That should explain the value of Spirulina as a feed additive for the agricultural animals. In the present work the influence of the microalgae *Spirulina platensis* on the quantitative and qualitative characteristics of boars' sperm was studied. The experiment was carried out with 6 boars from Danube white breed in the experimental animal base of the Agricultural Institute - Shumen. The time of the experiment was divided in the control and experimental periods. During the control period the animals received the main diet in accordance with Bulgarian state standard BDS- 1642-96. In the experimental period to the main diet was added 7 ml/ per head the fresh biomass of microalgae preserved by melasa (final quantity - 1,4 mg *Spirulina platensis*). The obtained results shown that the addition of Spirulina improves the sperm parameters in boars: the volume of the ejaculates increased with 30 ml in experimental period (306 ml against 276 in control, P<0,05) as well as the spermatozoa concentration enhanced with 27 mln/ml. The total dehydrogenases activities estimated by reaction with methylene blue as well as LDH activity were higher in the experimental period. Also the survivability of the spermatozoids at 24, 48 and 72 hour in the experimental period was more than in control.

Key words: microalgae *Spirulina platensis*, boar, sperm quality, dehydrogenases activity
Introduction

The use of AI in swine breeding will continue to increase and in the future, nearly all swine producers will employ this technology. The most part of semen utilized in AI programs is in a liquid (fresh) form and is utilized within several days after collection. It has been well-demonstrated that farrowing rates and litter sizes achieved using liquid semen can be equal or better than those resulting from natural mating systems (Flowers and Alhusen, 1992). The insemination with frozen semen generally results in low fertility and fecundity (Szczęśniak-Fabiańczyk et al., 2003). In this case is very actually to obtain the boars semen with high quality parameters and good fertilizing capacity.

During the last years in accordance with the development of biological farm system the producers have been looked for the natural active compound for the improvement of the reproductive performances of live stock animals. Wen-Hung Lin et al. (2007) shown that the diet included traditional Chinese mushrooms Cordyceps militaris leads to improving of sperm production and sperm quality in subfertile boars from Duroc and Landrace breeds. The other ecologically pure source for the correction of the reproductive function in domestic animals is biopreparation from the Spirulina platensis - BioR. According to the Rudic et al. (2008), the administration of the BioR injectable solution induces the increasing of the ejaculate volume (up to 25%), provides an increasing of the concentration and spermatic cell density (up to 20%) and intensifies the sperm mobility (up to 30%) in the sire bulls and boars.

An interest in Spirulina focused mainly on its rich content of vitality important compounds. Spirulina is 60-70% protein by weight and contains a rich source of vitamins, especially vitamin B12 and provitamin A (β-carotene), and minerals such iron, calcium, sodium, potassium, copper, magnesium, phosphorus, selenium. One of the few sources of dietary γ-linolenic acid (GLA), it also contains a host of other phytochemicals. That makes microalgae Spirulina suitable for all higher organisms as food and forage raw material.

In the present work the effect of the full biomass microalgae Spirulina platensis on the quantitative and qualitative characteristics of boars’ sperm was studied.

Materials and Methods

Experiment design

The experiment was conducted with 6 sex matured boars of Danube white breed from the animal experimental base of the Agriculture Institute – Shumen,
Bulgaria. The time of the experiment was divided in the control and experimental periods. During the control period the animals received the main diet in accordance with Bulgarian state standard BDS- 1642-96. The chemical ingredients of the obtained forage are presented in Table 1. During the experimental period to the main diet was added 7 ml/ per head the fresh biomass of microalgae preserved by melasa (final quantity - 1,4 mg *Spirulina platensis*). All animals were provided ad libitum access to water and were inspected for aspects concerning their health, welfare and body conditions.

Table 1. Chemical composition of received food

<table>
<thead>
<tr>
<th>Parameters</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>86.89</td>
</tr>
<tr>
<td>Organic matter</td>
<td>81.39</td>
</tr>
<tr>
<td>Water</td>
<td>13.11</td>
</tr>
<tr>
<td>Crude protein</td>
<td>17.97</td>
</tr>
<tr>
<td>Crude fat</td>
<td>7.27</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>5.59</td>
</tr>
<tr>
<td>Nitrogen free extractive compounds (NFE)</td>
<td>49.50</td>
</tr>
<tr>
<td>Trace minerals</td>
<td>5.50</td>
</tr>
<tr>
<td>Calcium</td>
<td>1.14</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.77</td>
</tr>
</tbody>
</table>

**Sperm collection**

The sperm was collected by artificial vagina one time per week from each boar during the control and experimental periods. Total six ejaculates per animal per period were obtained. The semen collection in the experimental period started since 40-th day of Spirulina supplementation (time needed for full cycle of new spermatozoids development and maturation in boars).

**Semen quality evaluation.**

Volume (without first fraction) was determined by using a graduated glass vessel with precision of 0.01 ml warmed up 38°C. The sperm concentration (10⁶/ml) was estimated by spermdensitometry "ZI-78" after dilution with 0,9% of sodium chloride solution according to the apparatus table. The motility of spermatozoa after ejaculates obtaining was subjectively evaluated (using a scale
from 0 to 100 % as an average result from 10 visual fields) with a light microscope at a magnification of 400X.

The survivability of sperm was measured by two ways - thermal resistance to the 39ºC and to the 15ºC. In the first way an undiluted sperm was kept in the water bath at 39ºC seven hours. The motility of life sperm was estimated with a light microscope every hour until 3-rd hour, every 30 minutes until 5-th hour and every 15 minutes until 7-th hour. In the second way the sperm was diluted with extender DZN (Bulgaria) in rate 1:1 and was kept at 15ºC. The percent of live and motile spermatozoa was evaluated at 24, 48 and 72 hours. Before evaluation the samples were warmed up 36 ºC.

**Biochemical parameters of sperm**

The total dehydrogenase activity of spermatozoids was estimated by methylene-blue reduction method (Mayer et al., 1993). Briefly, the equal quantity of sperm and 0.01% of methylene blue solution was mixed at 40 ºC. Dehydrogenase activity was measured with time of methylene-blue discoloration.

The activity of the spermatozoids’ lactate dehydrogenase (LDH) in the water and tritons’ extracts was estimated by the spectrophotometric method of Wroblewski and LaDue (1955) after centrifugation and removing of sperm plasma.

The evaluation of the ability of spermatozoa to produce reactive oxygen species (ROS) was done by using nitroblue tetrazolium (NBT) staining (Navid et al., 2003). NBT staining test was done for whole ejaculate by adding equal volumes of 0.1% of NBT solution and incubated for 30 minutes at 37ºC. After samples centrifugation the smears were prepared from the pellet and air-dried. Total of 100 spermatozoa per smear were scored as follow: formazan presents in mid piece and acrosome ++, only in mid piece +.

**Statistical analysis**

The data from the experiments were developed statistically by using the computer program Statistica (Stat Soft Inc., Ver.6.0). Significance of mean differences was estimated by Student's t-test.

**Results and Discussion**

Table 2 reflects the obtained results related to the characteristics of boar semen collected during the pre- and post-treatment period. It was established the significant enhancement (P<0,05) in semen volume and sperm motility after addition of the Spirulina to the main diet of animals. Our results are in agreement with data obtained of Rudic et al. (2008) injected to the boars the extract from
Spirulina platensis. In contrast to their results we obtained significant but not such high enlargement of the investigated parameters. In our experiments the semen volume increased up to 11% (by Rudic et al., (2008) - 25%), motility - up to 5% (by Rudic et al. (2008) - 30%). The sperm concentration according their results enlarged up to 20%, we had only 9% increasing, but without statistical significance. That allows supposing the most power effect of extract BioR on the boar spermatogenesis than full biomass microalgae *Spirulina platensis*.

Table 2. Sperm characteristic of the investigated boars

<table>
<thead>
<tr>
<th>Parameters, number of animals - 6 ejaculates</th>
<th>Volume, ml</th>
<th>Concentration $10^6$/ml</th>
<th>motility, %</th>
<th>survivability, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre/treatment period</td>
<td>276.0±37.9</td>
<td>300.0±18.4</td>
<td>75.6±2.7</td>
<td>69.1±4.0a</td>
</tr>
<tr>
<td>post/ treatment period</td>
<td>306.5±41.7</td>
<td>327.5±15.4</td>
<td>79.3±2.5*</td>
<td>74.9±2.6*aa</td>
</tr>
</tbody>
</table>

* - significant differences between pre- and posttreatment periods (P<0.05)

a,aa - significant differences between hours in both group (P<0.05 and P<0.01)

The most visible beneficial effect of Spirulina was observed on the survivability of boar sperm. In post treatment period after 72 hours storage at 15 ºC was kept up to 20% (P<0.05) more live and mobile spermatozoa (Table 2) than in control period. The thermal resistance of sperm to the 39 ºC in post treatment period also is higher (Figure 1). Since 4.30 hours storage the differences in live and mobile spermatozoa between samples from control and post treatment period are significant (P<0.05). Up to 3 times more active spermatozoa was survived until 7-th hour of storage in samples from post treatment period (5,05±2,8 % in control against 16,0±4,5% in post treatment period, Figure 1).
This positive effect of Spirulina should be explained with increasing of the enzymatic activity in the spermatozoa during the post treatment period. The enzymatic system involving in certain biological oxidation contains the enzymes called dehydrogenases. The data in Table 3. show that the total dehydrogenase activity as well as the lactate dehydrogenase activity in boar sperm from post treatment period was higher then in control period.

Table 3. Dehydrogenises activity in boar sperm

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Total activity of DH, in time of discoloration, sec</th>
<th>Activity of LDH, U/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>water extract</td>
</tr>
<tr>
<td>pre/treatment period</td>
<td>452.8±37.5</td>
<td>60.5±8.3</td>
</tr>
<tr>
<td>post/ treatment period</td>
<td>338.6±12.7</td>
<td>157.6±31.7</td>
</tr>
</tbody>
</table>

* -significant differences between pre- and posttreatment periods (P<0.05)

It is known that the total dehydrogenase activity correlate with sperm concentration and sperm motility (Beck and Salisbury, 1943). In our experiments we confirmed these results, the correlation coefficients between total DH activity...
and sperm motility was $r=0.78 \ (P<0.05)$, sperm concentration - $r=0.52 \ (P>0.05)$. However, in post treatment period we observed also the close correlation between DH activity and semen volume ($r=0.8; \ P<0.05$). LDH plays an important metabolic role in sperm capacitation and fertilization (Duan, and Goldberg, 2003). In case that LDH activity is more than two times higher in the post treatment period (Table 3), the beneficent effect of Spirulina could be realized in increasing of the fertilizing ability of sperm. In confirmation of this supposition are the results of Granaci (2007), which shown that the administration of boars with the Spirulina extract BioR was contributed to increase the farrowing rates for sows and the total number of piglets born.

The nitroblue tetrazolium test (NBT) was shown that in pre treatment period in more than 70% of spermatozoa observed high production of ROS in mid piece and acrosome (Figure 2A).

In post treatment period the percent of spermatozoa with visible ROS production in acrosome rapidly decreased (Figure 2B). It varies between 15 and 25% in depends of individual specificity. Spermatozoa, as cells living under aerobic conditions, from one side need $O_2$ for life supporting, from other- its metabolites such as ROS can modify their functions and endanger their survival (De Lamirande and Gagnon, 1995).
The generation of ROS was associated with a loss of motility, disruption of membrane integrity and impaired mitochondrial function in spermatozoa (Ball et al., 2002). Mammalian sperm has two main defense systems against the ravages of ROS. One is the enzyme, superoxide dismutase (SOD), which catalyzes removal of the $O_2^-$ anion. The other defense system consists of the enzyme, glutathione peroxidase (GPX), which requires intracellular reduced glutathione (Storey, 2008). Seminal fluid contains endogenous antioxidants such as superoxide dismutase, catalase and some reductases. These inactivate ROS and thus protect sperm cells (Faustini et al., 2004). Seminal oxidative stress develops as a result of an imbalance between ROS-generating and -scavenging activities (Sikka et al., 1996; Sharma and Agarwal, 1996). In boar seminal plasma was detected the extracellular Cu/Zn SOD, which plays an important physiological role in counteracting oxidative stress in spermatozoa (Kowalowka et al., 2008). In our experiment, the addition of Spirulina to the boars' diet, probably, increase the level of SOD in seminal plasma, because the constituents present in Spirulina are phycocyanin, b-carotene, polyunsaturated acids and superoxide dismutase (Jetley et al., 2004). On the second hand, the Spirulina pigment phycocyanin has demonstrated antioxidant activity. It scavenges peroxyl radicals (Romay et al., 1998; Herrero et al., 2004). Our experiment shown, that the antioxidative properties of Spirulina play positive role in the defense against oxidative stress in boar semen. The ROS production in
the boar spermatozoa decreased in the post treatment period. The spermatozoa of boars received Spirulina demonstrate higher survivability during the storage at temperature above zero (15°C) and thermal resistance at 39°C.

**Conclusion**

Enriching of the main diet of boars with supplementation of full biomass Spirulina platensis leads to improving the sperm quality. It was established the significant enhancement (P<0.05) in semen volume (up to 11%) and sperm motility (up to 5%) in post treatment period. The tendency for increasing of sperm concentration (up to 9%, P>0.05) was observed. The investigations of the biochemical parameters were shown the higher activity of total DH as well as LDH in the spermatozoa of boars received Spirulina. These spermatozoa demonstrated better survivability during the storage at temperature above zero (15 °C) and thermal resistance at 39°C. The antioxidative properties of Spirulina reduce semen oxidative stress. The NTB test pointed at the lower ROS production in sperm during the post treatment period.

In accordance with obtained results, *Spirulina platensis*, could be suggested as a rich source of vitality important components for the improving of boar semen quality, especially, obtained for the artificial insemination.

**Uticaj Spiruline platensis u obroku na kvalitet sperme nerastova**

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**Rezime**

Poslednjih godina, u skladu sa razvojem bioloških sistema u poljoprivredi, proizvođači su u potražnji za prirodnim aktivnim jedinjenjem kojim bi se poboljšale reproduktivne performanse domaćih životinja. Interes za spirulinu je usmeren uglavnom na njen sadržaj jedinjenja od važnosti za vitalnost. Spirulina je 60-70% protein po težini i predstavlja bogat izvor vitamina, posebno vitamina B12 i provitamina A (β-karoten), kao i minerala kao što su gvožđe, kalcijum, natrijum, kalijum, bakar, magnezijum, fosfor i selen. Takođe je jedan od izvora dijetetske γ-linolenske kiseline (GLA), i sadrži različite fito hemikalije. To čini mikro algu spirulinu pogodnom za sve više organizme, koji je mogu koristiti kao hranu i krmnu sirovinu.
U ovom radu je ispitivan uticaj pune mase mikro alge *Spirulina platensis* na kvantitativne i kvalitativne karakteristike sperme nerastova. Ogled je izveden na 6 nerastova rase dunavska bela svinja, u oglednoj stanici Poljoprivrednog instituta u Šumenu. Period ispitivanja je podeljen u kontrolni i ogledni period. Tokom kontrolnog perioda životinje su hranjeni obrokom prema bugarskom standardu BDS-1642-96. U oglednom periodu glavnom obroku je dodato 7 ml po grlu sveže biomase mikro alge čuvane u melasi (konačna količina - 1,4 mg *Spirulina platensis*).

Obogaćivanje glavnog obroka za nerastove dodavanjem pune biomase *Spirulina platensis* je dovelo do poboljšanja kvaliteta sperme. Utvrđeno je značajno povećanje (P<0,05) zapremine semena (do 11%) i pokretljivosti spermatozoida (do 5%) u periodu nakon tretmana. Trenutna tendencija povećanja koncentracije sperme (do 9%, P>0,05) je utvrđena. Ispitivanje biohemijskih parametara je pokazalo veću aktivnost ukupnog DH kao i LDH u spermatozoidima nerastova koji su dobijali spirulinu. Ovi spermatozoidi su pokazali bolje preživljavanje tokom skladištenja na temperaturi iznad nule (15ºC) kao i termičku otpornost na 39ºC. Antioksidativne osobine spiruline umanjuju oksidativni stres semena. NTB test je pokazao nižu proizvodnju ROS u spermatozoidima tokom perioda posle tretmana.

U skladu sa dobijenim rezultatima, *Spirulina platensis* se može preporučiti kao bogat izvor jedinića važnih za vitalnost i poboljšanje kvaliteta semena nerastova, posebno za veštačko osemenjavanje.

**References**


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