EFFECT OF PLANT TRIBULUS TERRESTRIS EXTRACT ON REPRODUCTIVE PERFORMANCES OF RAMS

Elena Kistanova, H. Zlatev, V. Karcheva, A. Kolev

Abstract: Tribulus terrestris extract was added to the forage of 8 rams of Pleven Blackhead and Abaci breed once daily in dose 1.5g per head for a period of 40 days. Semen parameters and sexual behavior during semen collection were evaluated.

It was found that Tribulus terrestris extract improves semen quality of rams: the count of spermatozoids, time of viability and motility of sperms increase. The great number of born lambs after the use of treated rams for insemination confirms high fertility of their semen. All experimental rams manifested a good libido and active sexual behavior.

Key words: Tribulus terrestris extract, rams, semen quality, sexual reflexes

Introduction

The use of synthetic hormones for reproductive stimulation of livestock animals has numerous negative consequences. Use of hormones is sometimes ineffective and it quite often induces a lasting hypofunction of the hypothalamic-pituitary-gonadal axis. On the other hand, synthetic hormones and their derivates are accumulated in animal products such as meat and milk. This makes these products dangerous for human health.

In order to obtain high quality and safety for human health products it is very important to search for new ecologically clean reproductive stimulants, and above all - of plant origin. The formulation of a non-hormonal preparation with sufficient activity and without harmful side effects could contribute to overcoming problems in the sphere of animal reproduction.

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2 E.Kistanova, V. Karcheva, A. Kolev, Institute of Animal Science, Department of Reproduction, Kostinbrod, Bulgaria; H.Zlatev, Vemo Ltd, Sofia, Bulgaria
The plant *Tribulus terrestris* has been long time known in native medicine of eastern countries and Bulgaria. It has been used in the treatment of sexual deficiencies. *Tribulus terrestris* contains biologically-rich compounds as steroids, saponins, flavonoids, alkaloids and unsaturated acids, which are involved in promoting numerous physiological responses. (Hu et al., 2000; Yan et al., 1996) The most active components of this plant extract are saponins of the furostanol type, termed protodioscin. The effects of *Tribulus terrestris* preparation on human males and experimental animals are well known (Tomova et al., 1981; Viktorov et al., 1994; Cauthaman et al., 2002, 2003; Brown et al., 2000, 2001; Antonio et al., 2000).

Literature data on influence of this preparation on livestock animals’ reproduction is rather scanty. Viktorov et al. (1994) has noticed the positive effect of protodioscin on sexual behavior of male pigs with impotence.

The aim of our investigation was to study the effect of the *Tribulus terrestris* extract on reproductive performances of rams in breeding season.

**Material and Methods**

Experiment was designed with 8 rams of Pleven Blackhead and Abaci breed. The rams were divided in two equal groups: control and experimental. Both groups contained young (2-3 years old) and mature (6-8 years old) rams. The extract from *Tribulus terrestris* (producer Vemo Ltd, Bulgaria) contained furostanol saponins, estimated as 55% of protodioscin. It was administrated to experimental rams orally, once daily with forage in dose of 1,5g per head for a period of 40 days.

160 ejaculates were examined in the experiment: 20 ejaculates per ram. The sperm was collected with an artificial vagina. The following parameters of the semen were evaluated: volume, sperm motility, sperm count, term resistance at 39°C and morphological changes of spermatozoids. During the morphological analysis the following items were took into account: 1 – normal spermatoza; 2 – with injured acrosoma; 3 – with another injures (without heads, with cytoplasm droplets, with curled tails). Routine methods were used for the production and dye of the histological samples.
The sexual behavior of rams was studied during semen collection. The libido manifestation and the time of the reflex period of sexual reflexes were estimated.

Student t-test was used for statistical estimation of results.

Results and Discussion

Figure 1 shows the changes in semen parameters of rams during investigation period. The most considerable effect of *Tribulus terrestris* extract is observed on sperm concentration and survivability. Survivability of sperms at 37°C in treated rams is significantly higher that in the control group by 73% (P<0,01). It should be noticed that in some days the viability of spermatozoids in experimental rams (young and mature rams) was very long, more than 24 hours. Count of spermatozoids in 1cm³ after treatment was enhanced by 31% (P<0,05).

The parameters of sperm volume and spermatozoids motility are higher in experimental group too, but these differences are not significant. A great individual variability of these parameters among rams in both groups was observed.

Table 1 shows the morphological status of spermatozoids in both groups. *Tribulus terrestris* extract has not injured spermatozoids. Furthermore, the percent of acrosomal damages in the experimental group is lower than in the control group.

*Table 1. Morphological parameters of spermatozoids after treatment with Tribulus terrestris*

<table>
<thead>
<tr>
<th>PARAMETERS/PARAMETRI %</th>
<th>GROUPS/GRUPE</th>
<th>Control/kontrola</th>
<th>Experimental/ogled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal sperms/normalna sperma</td>
<td>79,7±3,2</td>
<td>81,9±8,1</td>
<td></td>
</tr>
<tr>
<td>Injured acrosomas/ oštećeni akrozomi</td>
<td>5,9±3,6</td>
<td>3,2±1,1</td>
<td></td>
</tr>
<tr>
<td>Other injures/ostala oštećenja</td>
<td>14,4±3,5</td>
<td>14,9±8,6</td>
<td></td>
</tr>
</tbody>
</table>

The obtained data is in accordance with the results of Viktorov *et al*(1994). They observed a pronounced stimulating effect on the
spermatogenesis after use the protodioscin for sexually mature rats for 30 days. The mean spermatozoa number per ml was higher by two millions in the treatment animals compared to the controls. Their spermatozoa were viable long time than in control group. The same effect is confirmed by our results too. The positive effect of Tribulus terrestris on reproductive system of normal and castrated rats is shown in the investigation of Cauthaman et al (2002, 2003). Our results about the morphological status of spermatozoids are in agreement with the data obtained from Arsyad (1996) in investigation of protodioscin effect on quality of sperms from males. Unfortunately, in the available literature, we have not found data about the use the Tribulus terrestris preparation in the study of spermatogenesis in livestock animals and therefore we can not compare our results.

Table 2. Time of the reflex period of sexual reflexes
Tabela 2. Trajanje reflexe perioda kod seksualnih refleksa

<table>
<thead>
<tr>
<th>Age of rams</th>
<th>Numbers of rams/Broj ovnova</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control/ kontrola</td>
<td>Mature/ Zreli</td>
</tr>
<tr>
<td>Time, sec Vreme, sek.</td>
<td>831</td>
</tr>
<tr>
<td>Experimental/ Oglad</td>
<td>25,3±9,2</td>
</tr>
<tr>
<td>Time, sec Vreme, sek.</td>
<td>933</td>
</tr>
<tr>
<td></td>
<td>14,1±4,1</td>
</tr>
</tbody>
</table>

By investigation of the animal sexual behavior the individual reaction to the extract was observed (Table 2). It should be noticed that all rams manifested a good libido. Time of sexual reflexes in both groups is more dependent on age than on the Tribulus terrestris effect. Young rams have shorter reflex period than mature ones not only in the control group, but in experimental as well. We observed a positive effect of Tribulus terrestris in mature rams, as the duration of sexual reflexes in experimental animal was shorter by 11,2 sec than in the control ram.

Our results do not corresponded to the results of Viktorov et al (1992) obtained from male pigs. These authors undertake that in the animals with poor libido and long reflex period of sexual reflexes, treated
with daily dose of 70mg/kg body mass for 10 days, recovery was recorded in 100% cases. In our experiment two rams with long reflex period of sexual reflexes didn’t improve it after treatment with daily dose of 1,5g per head for 40 days, although the semen parameters were enhanced. May be this parameter is more determined genetically and is more dependent on age than on environs conditions.

**Conclusion**

The *Tribulus terrestris* extract has a very positive effect on spermatogenesis of rams during breeding season. The semen quality of rams treated with daily dose 1,5g per head of this extract for 40 days improves as in young as in mature animals. The count of spermatozooids, time of viability and motility of sperms increased. The great number of lambs born after the use of the treated rams for insemination confirm good fertility of their semen. All experimental rams manifested a good libido and active sexual behavior.

**UTICAJ EKSTRAKTA BILJKE TRIBULUS TERRESTRIS NA REPRODUKTIVNE REZULTATE KOD OVNOVA**

*Elena Kistanova, H.Zlatev, V.Karcheva, A. Kolev*

**Rezime**


Cilj našeg ispitivanja je bio da se ispita uticaj ekstrakta biljke *Tribulus terrestris* na reproduktivne performance ovnova u sezoni parenja.
Ogled je izveden sa 8 ovnova rase plevenska crnoglava i abasi. Ekstrakt iz biljke *Tribulus terestris* (proizvođač - Vemo Ltd, Bulgaria) je sadržavao furostanol saponine procenjene kao 55% protodioscina. Ekstrakt je divan oglednim ovnovima jednom dnevno sa krmivom u dozi od 1,5g po ovnu u periodu od 40 dana. Ocenjivani su sledeći parametri semena: zapremina, pokretnjivost sperme, broj spermatozoida, otpornost na temperature na 39°C i morfološke promene na spermatozoidima. Seksualno ponašanje ovnova je takođe analizirano tokom sakupljanja semena.

Kvalitet semena ovnova tretiranih ekstraktom biljke *Tribulus terestris* je poboljšan kako kod mladih tako i kod zrelih životinja. Broj spermatozoida, trajanje životne sposobnosti, pokretnjivost sperme su se povećali. Veliki broj rođene jagnjadi potvrđuje visoku plodnost semena ovih ovnova. Svi ogledni ovnovi su manifestovali dobar libido i aktivno seksualno ponašanje.

**Reference**


Figure 1. Changes in semen parameters after treatment with Tribulus terrestris extract
Slika 1. promene u parametrima semena nakon tretmana sa Tribulus terrestris ekstraktom

CN- control group/kontrolna grupa
EX – experimental group/eksperimentalna grupa
A/ Semen volume/zapremina semena

B/ Sperm concentration/konzentracija sperme
C/ Sperm motility/pokretljivost sperme

D/ Survivability of sperm at 39°C/otpornost sperme na 39°C