**DIETARY SUPPLEMENTATION OF RHUS CORIARIA (SUMACH) MODERATELY AFFECTS THE RABBIT SPERMATOZOA MOTILITY**

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Abstract: Appropriate nutrition and feeding belong among the factors that ensure successful and beneficial animal production. The most suitable forage for the rabbits as herbivores is the feed of crop origin. It is important that the feed contains all the essential nutrients, fiber, mineral substances and vitamins. In present study, the effect of sumach addition into feeding ration of rabbits was observed with special interest in spermatozoa motility properties. Male rabbits (n = 25) of New Zealand white breed were used in this experiment. The rabbits were divided into five different groups. One control group (C) and four experimental groups (S1, S2, S3, S4). Sumach (Rhus coriaria) fruit was added to the complex feeding ration in milled and parched form in four different concentrations: 0.50%; 0.75%; 1.00% and 1.50% and fed for the period of 90 days. Results of the CASA analysis show that sumach dietary supplementation affects the spermatozoa motility, however with no statistical significance. Based on the results of this study, moderate effect of sumach on male reproduction depends on additive concentration and duration of intake. The first 20 days of the experiment showed very homogeneous results. Analyses conducted at the Day 40 showed both positive and negative effect of sumac. Concentrations 0.50% and 1.00% seemed beneficial. At the Day 60 and the Day 80 of the experiment decreased values of CASA parameters in all concentrations were monitored. Evaluations carried out at the terminal collection day (Day 90) reported enhanced motility, progressive motility and velocity curved line in rabbit groups administrated with addition of sumach in concentrations of 0.75% and 1.00%.

**Key Words:** rabbit, Rhus coriaria, sumach, spermatozoa motility, CASA

**INTRODUCTION**

The intensification of rabbit breeding puts the emphasis on physiological, health and behavioural demands due to high sensitivity of rabbits to environmental conditions (Casamassima et al. 2017). There is a worldwide trend in administration of antioxidants with natural origin to the farm animals in order to improve the animal welfare and possibly to create a functional food (Vizzarri and Corino 2016). It is highly believable that the way to achieve this goal leads through the use of phytogenics, feed additives derived from herbs and plants with natural growth promoting factor (Gálik et al. 2013).

Sumach is the commonly used name for genus Rhus, the plant recognized due to its wide range of beneficial properties as follows: antimicrobial (Nasar-Abbas and Halkman 2004), antioxidant (Kosar et al. 2007), antimutagenic (Chakraborty et al. 2009), antidiabetic (Mohammadi et al. 2010), antifungal (Mccutcheon et al. 1994), antiinflamatory (Fourie and Snyckers 1983), etc. These plants originate from temperate and tropical regions and are able to adapt to non-agricultural conditions in marginal regions (Rayne and Mazza 2007). Sumach found its use also in traditional Arabic Palestinian...
medicine for the treatment of variety of diseases and has been used since the antiquity (Kizil and Turk 2010). Sumach is nowadays extensively used alone or in combination with other spices as a condiment (Sagdúc and Ozcan 2003). Abu-Reidah et al. (2015) analyzed sumach for the content of phenols and other phytochemicals and report that *Rhus coriaria* is rich in tannins, (iso)flavonoids and terpenoids and in total is composed of 211 constituents.

Target of this study was to evaluate addition of sumac in the rabbit feeding mixture on spermatozoa motility parameters in various time periods.

**MATERIAL AND METHODS**

**Experimental design**

Six months old adult male rabbits (*n* = 25) of New Zealand White breed were subjected to this study. Animals were divided in four experimental groups (S1, S2, S3, S4) and one control group (C). The animals contained within the experiment were housed in air-conditioned halls in individual one-storey metal cages. The light mode was set to 14 hours. All groups were administrated complex granular feed KK V1 *ad libitum* and the drinking water of optimal temperature was unlimitedly supplied via automatic drinker. The feed was administered in form of pellets. The complete feed mixture was composed of: 23% lucerne fodder, 18% sunflower scrap, 18% sugar beet, 10% wheat, 9% wheat bran, 5% molasses, 5% olive starch, 3.5% 3.2%, soybean oil 1.7%, lignobond 1%, monocalcium phosphate 0.3%, sodium chloride 0.3%. The chemical composition is provided in Table 1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>926.26</td>
<td>g/kg</td>
</tr>
<tr>
<td>Crude protein</td>
<td>192.06</td>
<td>g/kg</td>
</tr>
<tr>
<td>Fat</td>
<td>36.08</td>
<td>g/kg</td>
</tr>
<tr>
<td>Fibre</td>
<td>135.79</td>
<td>g/kg</td>
</tr>
<tr>
<td>Non-nitrogen compounds</td>
<td>483.56</td>
<td>g/kg</td>
</tr>
<tr>
<td>Ash</td>
<td>78.78</td>
<td>g/kg</td>
</tr>
<tr>
<td>Organic matter</td>
<td>847.49</td>
<td>g/kg</td>
</tr>
<tr>
<td>Calcium</td>
<td>9.73</td>
<td>g/kg</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>6.84</td>
<td>g/kg</td>
</tr>
<tr>
<td>Magnesium</td>
<td>2.77</td>
<td>g/kg</td>
</tr>
<tr>
<td>Sodium</td>
<td>1.81</td>
<td>g/kg</td>
</tr>
<tr>
<td>Potassium</td>
<td>10.94</td>
<td>g/kg</td>
</tr>
<tr>
<td>Metabolizable energy</td>
<td>12.35</td>
<td>MJ/kg</td>
</tr>
</tbody>
</table>

Commercial feed dedicated for experimental groups was enriched with sumach (*Rhus coriaria*) in various concentrations: 0.50% in S1 group, 0.75% in S2 group, 1.0% in S3 group and 1.50% in S4 group. The same diet was applied for the whole duration of experiment (90 days). Control group received feed without sumach addition (Table 2).

In this animal study, institutional and national guidelines for the care and use of animals were followed, and all experimental procedures involving animals were approved by The State Veterinary and Food Institute of Slovak Republic, no. SK CH 29004.
Table 2 Overview of the feed treatments with Rhus coriaria

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration of Rhus coriaria (%)</th>
<th>Number of animals</th>
<th>Duration of experiment (days)</th>
<th>Amount of Rhus coriaria added (g/100kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>-</td>
<td>5</td>
<td>90</td>
<td>0</td>
</tr>
<tr>
<td>S1</td>
<td>0.50</td>
<td>5</td>
<td>90</td>
<td>600</td>
</tr>
<tr>
<td>S2</td>
<td>0.75</td>
<td>5</td>
<td>90</td>
<td>850</td>
</tr>
<tr>
<td>S3</td>
<td>1.00</td>
<td>5</td>
<td>90</td>
<td>1000</td>
</tr>
<tr>
<td>S4</td>
<td>1.50</td>
<td>5</td>
<td>90</td>
<td>1600</td>
</tr>
</tbody>
</table>

CASA analysis

Motility analyses were realized using the CASA method with SpermVision software (Minitub, Tiefenbach, Germany) and the microscope Olympus BX 51 (Olympus, Japan). After semen collection the samples were pipetted into Makler counting chamber (10 μm, Sefi-Medical Instruments, Germany). Measurements of spermatozoa motility were performed immediately after the semen collection. The following spermatozoa characteristics were assessed: motility (MOT), progressive motility (PRO) and velocity curved line (VCL). Every single output of the CASA system is the result of 7 diverse sub-measurements of 7 different fields of Makler Counting Chamber (Tirpák et al. 2015).

Statistical analysis

For the determination of the effect of feed additive on spermatozoa motility, ANOVA and Dunnett’s comparative test were applied using GraphPad Prism 5 (GraphPad Software Inc., USA). All statistical tests were carried out at levels of significance at P < 0.05, P < 0.01 and P < 0.001.

RESULTS AND DISCUSSION

Inceptive evaluation of spermatozoa motility was realized 20 days since the beginning of the experiment. Animals in experimental groups S1 and S3 had decreased spermatozoa motility while the groups S2 and S4 had higher motility than those in the group C. Results obtained after 40 days suggest slightly positive effect of sumach supplementation on spermatozoa motility in rabbits fed with concentrations 0.50% (S1) and 1.00% (S3) of additive. Notable differences were observed at testing days 60 and 80 where the experimental groups showed lower motility compared to the control. The last sampling (Day 90) revealed elevated motility in experimental groups S2 and S3. All the differences noted during the experiment were insignificant (P > 0.05).

Twenty days of sumach administration caused the enhanced percentage of progressively motile spermatozoa in all experimental groups, rising along with higher additive concentration. Spermatozoa assessed on the Day 40 of present research showed higher amount of spermatozoa with progressive movement in groups S1 and S3. In the case of progressive motility, the control groups emerged to be the most efficient when evaluated at Day 60 and 80. Percentage of progressively motile spermatozoa was monitored at the end of the experiment in rabbits within groups S2 and S3. Statistical significance this was not detected (P > 0.05) in this parameter.

Rabbits fed with an additive-free feeding ration provided semen with spermatozoa of higher velocity during first 60 days of testing. Sumach concentrations (0.75% and 1.00%), represented by groups S1 and S3, demonstrated the positive effect of dietary supplementation on the velocity of the spermatozoa at the end of the trial. As for the velocity curved line, all observed differences absented the statistical significance (P > 0.05).
Figure 1 Spermatozoa motility (MOT; %) in different semen extenders at various stages of the experiment

Legend: C – control group (without the sumach addition); S1 (0.50%), S2 (0.75%), S3 (1.00%), S4 (1.50%) – experimental groups with sumach addition. Significant difference: *P < 0.05; **P < 0.01; ***P < 0.001.

Figure 2 Spermatozoa progressive motility (PRO; %) at various stages of the experiment

Legend: C – control group (without the sumach addition); S1 (0.50%), S2 (0.75%), S3 (1.00%), S4 (1.50%) – experimental groups with sumach addition. Significant difference: *P < 0.05; **P < 0.01; ***P < 0.001.

Figure 3 Velocity curved line (VCL; μm/s) of spermatozoa at various stages of the experiment

Legend: C – control group (without the sumach addition); S1 (0.50%), S2 (0.75%), S3 (1.00%), S4 (1.50%) – experimental groups with sumach addition. Significant difference: *P < 0.05; **P < 0.01; ***P < 0.001.

Vizzarri and Corino (2016) and Casamassima et al. (2017) claim that growing interest in dietary supplementation of natural extracts shows promising outcomes in various aspects of rabbit breeding. The literature lacks the information about sumach effect on reproduction. However, there is a lot of information which help to explain and backup our findings. Capcarová et al. (2010) studied the effect
of sumach supplementation on internal milieu of adult male rabbits. Performed blood analysis revealed enhanced antioxidant activity and decreased level of cholesterol. Oral administration of sumach to type 2 diabetic rats showed mild antihyperglycemic properties coming out of several mechanisms. Concentration of low density lipoproteins was rapidly decreased while the high density lipoproteins were strongly increased. Glutathione peroxidise activity was not affected, however the superoxide dismutase and catalase activity was found positively significant following sumach application (Mohammadi et al. 2010). Sumach was administrated also to rats and humans in order to monitor DNA damage in treated and untreated individuals. Reduction of DNA damage along with antioxidant activity in experimental groups indicates direct scavenging effect of sumac. Induction of glutathione S-transferase (GST), composed of two isozymes (GST-α and GST-π) suggest sumach protective properties against genotoxic carcinogens which are detoxified by these enzymes (Chakraborty et al. 2009).

CONCLUSION

Addition of Rhus coriaria as dietary supplement to rabbit feed did not show any significant effect on spermatozoa motility properties in any tested concentration (0.50%, 0.75%, 1.00%, 1.50%). Administration of this substance resulted in decrease of motility parameters (motility, progressive motility and velocity curved line) after 20, 40, 60 and 80 days of treatment. CASA assessment revealed the improvement of all motility parameters in rabbits fed with sumach additive (0.75% and 1.00% of active compound) for 90 days. Based solely on these results, it is not convenient to declare neither positive nor negative effects of sumach on male reproduction.

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