GOITROGENIC EFFECTS OF EXTRACTED RAPESEED MEAL AND NITRATES IN SHEEP AND THEIR PROGENY

STRUMIGENNÍ ÚČINEK ŘEPKOVÉHO EXTRAHOVANÉHO ŠROTU A DUSIČNANŮ U OVCÍ A JEJICH POTOMSTVA

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ABSTRACT: Three groups of the breed Šumava sheep were fed meadow hay completed with extracted rapeseed meal, containing 4.2 mmol of glucosinolates, and 4 g of sodium nitrate per animal per day for 382 days. Moreover, two of the groups received parenterally 0.25 mg iodine and 0.15 mg sodium selenite per animal and per day at weekly intervals. The fourth group, also supplemented with iodine and selenium, was fed a diet without the addition of nitrate in which extracted rapeseed meal was replaced with oats meal. Biochemical analyses included determination of iodine concentrations in colostrum and milk of ewes and iodine, thyroxine and triiodothyronine concentrations in blood plasma of lambs from birth up to the age of 60 days. Thyroids of five necropsied lambs were weighed and examined by light and electron microscopy. Hypothyreosis with sporadic occurrence of congenital goitre and low concentrations of iodine and thyroxine in blood plasma were observed in the progeny of the ewes receiving in their diet extracted rapeseed meal and nitrate without iodine and selenium supplementation. The histological structure of the enlarged thyroid indicated 3rd stage of goitre. Electron microscopy revealed multilayer arrangement of follicles, cystic extension of cisterns of the endoplasmatic reticulum, numerous activated mitochondria, and pyknotic nuclei displaced towards the cytoplasmatic membranes of the thyrocytes. Biochemical analyses demonstrated low concentrations of iodine in colostrum and milk (57 ± 36 and 27 ± 15 µg/l, respectively) and of iodine and thyroxine in blood plasma of lambs (69 ± 24 µg/l and 101 ± 27 nmol/l, respectively). On the other hand iodine concentrations in colostrum and milk were significantly (P < 0.01) higher in the groups receiving the same doses of goitrogens, but supplemented with iodine alone (colostrum 334 ± 330 µg/l; milk 101 ± 41 µg/l), or iodine and selenium (colostrum 430 ± 520 µg/l; milk 70 ± 16 µg/l). Also higher were mean iodine and thyroxine concentrations in blood plasma of lambs born in these groups. The highest concentrations of iodine in milk and colostrum (2 083 ± 1 423 and 198 ± 8 µg/l, respectively) were found in the group fed the diet free of extracted rapeseed meal and nitrates and supplemented with iodine and selenium. The differences between this and any other group were highly significant (P < 0.01). Mean concentrations of thyroxine and triiodothyronine in blood plasma of lambs of the individual groups correlated with iodine concentrations in colostrum and milk of the dams. The differences among groups were nonsignificant. The experiment demonstrated goitrogenic effects of glucosinolates presented in extracted rapeseed meal and nitrate, which can be compensated by supplementation of iodine or iodine + selenium.

sheep; glucosinolates; supplementation; iodine; selenium; thyroid gland; goitre; thyroxine; triiodothyronine; blood; colostrum; milk

ABSTRAKT: V pokusu se 24 ovcíni, plemene šumavské ovece, ve věku 12 až 18 měsíců byl 382 dnů sledován účinek dlouhodobého příjmu řepkového extrahovaného šrotu a dusičnanů v dietě s cílem určit jejich vliv na štěnou žilu bahnic a potomstva a posílit kompenzační efekt jodu a selenu. Ovce byly umístěny v experimentální stáji fakulty. Byly rozděleny na čtyři skupiny po šesti kusech (tab. I). Základ celocelové krmné dávky dosáhl 1200 kg masa, napojící voda pocházela z úspěšněho zdroje. Ovce tři pokusných skupin (E1, E2, C1) přijímala v doplněk k krmné směsi s řepkovým extrahovaným šrotem v průměru 4,2 mmol glucosinoláty a 4 g NO₃⁻ na kus a den. V krmné směsi pro čtvrtou skupinu (C2) byl řepkový extrahovaný šrot nahrazen ovesním šrom bez přídavku NaNO₃ (tab. II). Suplementárním zdrojem jodu pro ovce ve skupinách E1, E2 a C2 byl roztok jidlu draslenského u selenu pak přípravek Selenit inj. ad. us. vet s účinnou látkou bezvodým selenidštanem sodním. Injekční aplikace se uskutečňovala v týdenních intervalech v důsledku odpovídajících příjmu 0,25 mg jodu a 0,15 mg selenu pro kus a den. Byl sledován zdravotní stav oveči a jejich potomstva, modifikovanou metodou dle Sandell-Kolthoffa stanovena koncentrace jodu v koštu a v mléce bahnic, zjišťována koncentrace jodu a hormonů thyroxin a trijodothyroninu

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INTRODUCTION

Along with the medical research of iodine deficiency in the human population, which has been included among the cardinal preventive projects of the World Health Organisation (Delange et al., 1997), agricultural scientists pay increased attention to sufficient supply of iodine to animals. In the Czech Republic, the significance of the relation between iodine deficiency and performance of farm animals is increasing due to frequent occurrence of goitre in young farm animals (Čada, 1988) data on further adverse effects of iodine deficiency published abroad (Groppel, 1991), and reports of iodopoenic disturbances occurring even after 40 years of iodine supplementation of table salt (Zamrazil et al., 1997). Among other suggestions, the benefits of inclusion of iodine into the natural food chains is emphasized. The most effective physiological way is to increase iodine content in natural foods including meat, milk, milk products and eggs.

Epidemiological analyses revealed several factors participating in the occurrence of clinical and subclinical hypothyrosis in animals (Herzig et al., 1999). The primary deficiency induced by insufficient iodine intake is due to feeding of unprocessed roughage and grain meals of exclusively local origin (Kursa et al., 1997). This fact is associated with historically known iodine deficiency in the environment of the Czech Republic, therefore there was occurrence of goitre in humans andcretinism in newborns (Pohůňková and Němec, 1988; Haťková, 1995; Stůrka, 1995).

The data presented in this paper were obtained within a research project encouraged by results of epidemiological studies and preventive actions aimed at the control of iodopoenic situations in animal herds in South, West, and Central Bohemia and in Moravia. Previous screenings have showed that, in terms of the current knowledge, the supply of iodine to animals was insufficient and thus conditions enhancing the effect of goitrogenes were prepared (Cndă, 1988; Herzig et al., 1999; Pisafiková et al., 1996).

The increasing interest in goitrogenic effects of rapeseed is justified by the current position of this species among other oil-producing crops and growing use of rape products (extracted rapeseed meal and rapeseed cake) as components of concentrates (Emanuelson, 1989; Šimek et al., 1999). Currently, data on the relation between the content of glucosinolates in rape and the activity of the thyroid gland gained in experiments in laboratory animals and results of experiments in swine (Schöne et al., 1997) are available. Ehlers et al. (1994) recommend to consider the variety of rape, to observe rules for dosage, and to ensure excessive saturation with iodine when ERM is fed to cattle.

Besides biochemical tests, the application of morphological and histological methods is inevitable for
the investigation of physiological events and pathological conditions of the thyroid gland (Picha et al., 1970). Their relevance to the diagnostics of thyreopathies was confirmed by Čada (1988) who disposed of a rich necropsy material and described the development of lesions characterising goitre due to maternal iodine deficiency.

Weighing and partial histological studies of the thyroid gland were extensively applied by Groppel (1991) and other members of his team (Schöne et al., 1997). An increase in the weight of the thyroid gland in pigs fed a concentrate containing 15% (Corino et al., 1991) of ERM was also demonstrated.

The objectives of the experiment were to find effects of long-term feeding of ERM and sodium nitrate on the thyroid gland of ewes and their progeny and to assess the compensatory effect of iodine and selenium supplementation. This model experiment was based on the recent occurrence of spontaneous goitre in ruminants in several areas of the Czech Republic.

MATERIAL AND METHODS

Tests of effects of goitrogenic agents were done in 24 ewes of the breed Sumava sheep aged 12 to 18 months. The sheep were purchased from a breeding herd in the district of Prachatice which is an area with increased occurrence of goitre. The animals were divided into four groups of six (E1, E2, C1, C2). The design of the experiment, composition of feed ration, and data on iodine and selenium supplementation are given in Tab. I. Composition of concentrate is given in Tab. II.

The sheep were housed in groups in a deep-litter barn, fed twice a day, and had free access to crockery troughs with potable water supplied from a public source.

In all the groups, the major component of the ration was meadow hay harvested in the area of origin of the sheep. Hay was completed with the concentrates A and B prepared in the experimental feed mill of the Central Agricultural Control and Testing Institute, Lysá nad Labcem, Czech Republic. Vitamin additives Combindal A forte, Combindal D forte, and Combindal E forte were administered in drinking water. Moreover, feeding carrot was offered and iodine-free lick salt was available.

The sheep of the groups E1, E2, and C1 received daily the concentrate B containing extracted rapeseed meal (ERM - 82.5%) and sodium nitrate (NO3 - 1.9%). The dose was 287 g per animal and day and corresponded to a daily intake of 4 g of sodium nitrate and 4.2 mmol of glucosinolates per animal and day. The content of glucosinolates was determined by gas chromatography after derivatisation to silyl compounds (Zukalová and Vašák, 1978). The group C2 received the concentrate A in which ERM was replaced with oats meal and urea. The experiment was started by a 7-day adaptation period during which the animals received one half of the concentrates. Additional sources of iodine and selenium for the sheep of the groups E1, E2, and C2, administered from 1st to 264th day of the experiment, were a potassium iodide solution and Selevit inj. ad us. vet. containing amhydrous sodium selenite as the active substance. The supplements were administered subcutaneously at weekly intervals in doses corresponding to 0.25 mg of iodine and 0.15 mg of selenium per animal.

The ewes were bred naturally from day 81 to day 187 using two breeder rams tested for fertility. The rams were kept alternatively with the individual groups always for a two-week period. Highly pregnant ewes were separated into individual boxes and kept there with their lambs 3 to 5 days after parturition. This period was prolonged in ewes which gave birth to twins.

The ewes were weighed before the experiment, on experiment day 220, and after the experiment. Considering the differences in the initial live weight, the relative growth rate was calculated using the formula (Karakoz, 1968).

\[ Q = \frac{Y_t - Y_0}{Y_0} \]

The lambs were weighed immediately after birth and at the age of 10 days. Blood samples were collected from v. jugularis immediately after birth, 24 and 48 hours thereafter and on post-partum days 10, 30 and 60, always between 07.00 and 10.00 a.m.

<table>
<thead>
<tr>
<th>Group</th>
<th>Feed ration (animal/day)</th>
<th>Goitrogen</th>
<th>Supplementation</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>hay 1.4 kg concentrate B 287 g, ERM NaNO3</td>
<td>iodine 0.25 mg as a KI solution once per week s.c.</td>
<td>selenium 0.15 mg as Selevit once per week s.c.</td>
</tr>
<tr>
<td>E2</td>
<td>hay 1.4 kg concentrate B 287 g, ERM NaNO3</td>
<td>iodine 0.25 mg as a KI solution once per week s.c.</td>
<td>selenium 0.15 mg as Selevit once per week s.c.</td>
</tr>
<tr>
<td>C1</td>
<td>hay 1.4 kg concentrate B 287 g, ERM NaNO3</td>
<td>iodine 0.25 mg as a KI solution once per week s.c.</td>
<td>selenium 0.15 mg as Selevit once per week s.c.</td>
</tr>
<tr>
<td>C2</td>
<td>hay 1.4 kg concentrate A 287 g, ERM NaNO3</td>
<td>iodine 0.25 mg as a KI solution once per week s.c.</td>
<td>selenium 0.15 mg as Selevit once per week s.c.</td>
</tr>
</tbody>
</table>

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Iodine concentration was determined in the first colostrum sample collected immediately after parturition and in milk samples collected at the end of the experiment (day 382), i.e. 61 to 140 days after parturition.

Iodine in blood plasma of lambs and in colostrum and milk was determined after alkaline digestion using the Sandell-Kolthoff spectrophotometric method as modified by Bednář et al. (1964). T₄ and T₃ were determined by radioimmunoassay using the commercial kit supplied by Immunotech (Prague).

One lamb of each group and another female lamb showing clinical goitre (8B) were killed on day 13 and necropsied. Samples of the thyroid gland, collected immediately after the killing, were examined using light and transmission electron microscopy. The weight of the glands was determined using laboratory balance. The samples for light microscopy were fixed in 10% formaldehyde and processed by the conventional paraffin technique. Approximately 5-µm-thick sections were stained with haematoxylin-eosin. Samples for transmission electron microscopy were collected from the lambs Nos. 7, 8B, 13A and 21. The samples were fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer for 12 h and then washed in cacodylate buffer and post-fixed with 1% osmium tetroxide. After treatment in ascending ethanol series, the samples were embedded in Durcupan. Ultrathin sections were viewed at 80 kV in the electron microscope Philips 420.

The significance of among-group differences in mean concentrations of iodine in colostrum and milk, and in mean concentrations of thyroxine, triiodothyronine, and iodine in blood plasma were analysed by the t-test using the STAT-plus software (Matoušková et al., 1992).

RESULTS

The results of the three weightings (days 1, 220, and 380) are shown in Tab. III. It is evident that the growth of the ewes receiving ERM + nitrate (groups E1, E2, and C1) was slightly more rapid during the first phase of the experiment. Towards the end of the experiment, the development was influenced by individual differences in metabolic demands associated with lactation. The most marked decrease in live weight (group E2) was observed in ewes suckled twins.

In terms of the basic fertility parameters, the reproductive performance in the groups E1, E2, and C1 with the pregnancy rate 100% was good. Pregnancy was uncomplicated in all the ewes. The first lamb was born on experiment day 249, i.e. 168 days after the beginning of the mating period. The last lamb was born in the group C1 on experiment day 321, i.e. 140 days after the termination of the mating period.

<table>
<thead>
<tr>
<th>Component</th>
<th>A (kg)</th>
<th>B (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium carbonate CaCO₃</td>
<td>4.8000</td>
<td>4.8000</td>
</tr>
<tr>
<td>Sodium chloride NaCl</td>
<td>1.8000</td>
<td>1.8000</td>
</tr>
<tr>
<td>Zinc oxide ZnO</td>
<td>0.0087</td>
<td>0.0087</td>
</tr>
<tr>
<td>Copper sulphate CuSO₄·5H₂O</td>
<td>0.0018</td>
<td>0.0018</td>
</tr>
<tr>
<td>Flour</td>
<td>9.0000</td>
<td>9.0000</td>
</tr>
<tr>
<td>Sodium nitrate NaNO₃</td>
<td>0.0000</td>
<td>1.9000</td>
</tr>
<tr>
<td>Urea</td>
<td>1.5000</td>
<td>0.0000</td>
</tr>
<tr>
<td>Oats meal</td>
<td>82.9000</td>
<td>0.0000</td>
</tr>
<tr>
<td>Extracted rapeseed meal</td>
<td>0.0000</td>
<td>82.5000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial mean weight</td>
<td>E1 + ERMT₄ + NO₃ supp 1 + Se n = 6</td>
</tr>
<tr>
<td>Relative growth rate on experiment day 220 (%)</td>
<td>41.0</td>
</tr>
<tr>
<td>Mean weight on experiment day 220 (kg)</td>
<td>54.5</td>
</tr>
<tr>
<td>Relative growth rate on experiment day 380 (%)</td>
<td>35.1</td>
</tr>
<tr>
<td>Mean weight on experiment day 380 (kg)</td>
<td>55.4</td>
</tr>
<tr>
<td>Number and % of pregnant ewes</td>
<td>6/100</td>
</tr>
<tr>
<td>Days from the beginning of the mating period</td>
<td>194.6 ± 25.3</td>
</tr>
<tr>
<td>Gross natality</td>
<td>1.15</td>
</tr>
<tr>
<td>Net natality</td>
<td>1.15</td>
</tr>
<tr>
<td>Number of twin births</td>
<td>1</td>
</tr>
<tr>
<td>Lamb sex – males</td>
<td>4</td>
</tr>
<tr>
<td>– females</td>
<td>3</td>
</tr>
<tr>
<td>Mean birth weight of lambs</td>
<td>4.6 ± 0.5</td>
</tr>
</tbody>
</table>
IV. Absolute and relative weight and histological pattern of the thyroid gland, and postnatal thyroxine (nmol/l T\(_4\)), triiodothyronine (nmol/l T\(_3\)), and iodine (µg/l) concentrations in blood plasma of lambs of the groups receiving goitrogens and differing in iodine and selenium supplementation

<table>
<thead>
<tr>
<th>Group</th>
<th>Lamb No.</th>
<th>Sex</th>
<th>Birth weight (kg)</th>
<th>Weight on day 11 (kg)</th>
<th>Clinical finding</th>
<th>Thyroid gland weight (g)</th>
<th>Thyroid gland relative weight (%)</th>
<th>Histological pattern</th>
<th>T(_4)</th>
<th>T(_3)</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>2</td>
<td>male</td>
<td>4.9</td>
<td>9.0</td>
<td>normal</td>
<td>0.9</td>
<td>0.010</td>
<td>normal hist. structure</td>
<td>159</td>
<td>7.8</td>
<td>115</td>
</tr>
<tr>
<td>E2</td>
<td>13A</td>
<td>male</td>
<td>3.5</td>
<td>6.2</td>
<td>normal</td>
<td>1.7</td>
<td>0.027</td>
<td>normal hist. structure</td>
<td>134</td>
<td>4.6</td>
<td>89</td>
</tr>
<tr>
<td>C1</td>
<td>7</td>
<td>male</td>
<td>4.8</td>
<td>8.7</td>
<td>normal</td>
<td>1.9</td>
<td>0.022</td>
<td>normal hist. structure</td>
<td>195</td>
<td>10.3</td>
<td>121</td>
</tr>
<tr>
<td>C1</td>
<td>8B</td>
<td>female</td>
<td>3.2</td>
<td>*3.3</td>
<td>goitre</td>
<td>4.4</td>
<td>0.133</td>
<td>various stages of parenchyma maturation, collapsed follicles, most of the colloid vacuolized, abundant interstitium = 3rd stage of goitre</td>
<td>46</td>
<td>2.1</td>
<td>45</td>
</tr>
<tr>
<td>C2</td>
<td>21</td>
<td>male</td>
<td>4.1</td>
<td>8.2</td>
<td>normal</td>
<td>1.0</td>
<td>0.012</td>
<td>differentiated pattern</td>
<td>252</td>
<td>7.3</td>
<td>111</td>
</tr>
</tbody>
</table>

* on postpartum day 3

V. Individual concentrations of thyroxine (nmol/l T\(_4\)), triiodothyronine (nmol/l T\(_3\)), and iodine (µg/l) in littermates with different manifestations of hypothyrosis (group C1, No. 8B, 8A), in necropsied and clinically health lambs with normal histological structure (group E1, E2, C2, No. 2, 13A, 21), and their group means in lambs from birth to day 60

<table>
<thead>
<tr>
<th>Group</th>
<th>Lamb No.</th>
<th>Clinical finding</th>
<th>Iodine</th>
<th>T(_4)</th>
<th>T(_3)</th>
<th>Group mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Age (days)</td>
<td>Group mean</td>
<td>Age (days)</td>
<td>Group mean</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 1 2 10 30 60</td>
<td>(\overline{x})</td>
<td>0 1 2 10 30 60</td>
<td>(\overline{x})</td>
</tr>
<tr>
<td>C1</td>
<td>8B</td>
<td>goitre</td>
<td>45 26 57 35</td>
<td>46 40 37</td>
<td>6.5 2.6</td>
<td>6.4 5.3</td>
</tr>
<tr>
<td></td>
<td>8A</td>
<td>indifferent</td>
<td>52 37 53 32 38 33</td>
<td>53 34 39 40 91 46</td>
<td>7.8 7.2</td>
<td>8.1 8.4</td>
</tr>
<tr>
<td>E1</td>
<td>2</td>
<td>normal</td>
<td>115 111 122</td>
<td>159 111 75 78</td>
<td>134 132 116 101</td>
<td>134 132 116 101</td>
</tr>
<tr>
<td>E2</td>
<td>13A</td>
<td>normal</td>
<td>89 80 54 144</td>
<td>134 132 116 101</td>
<td>134 132 116 101</td>
<td>134 132 116 101</td>
</tr>
<tr>
<td>C2</td>
<td>21</td>
<td>normal</td>
<td>111 136 206 236</td>
<td>252 176 156 73</td>
<td>7.3 7.8</td>
<td>8.3 7.3</td>
</tr>
</tbody>
</table>

* postpartum day 3
Iodine concentrations C1 vs. E1 (P < 0.05) Differences in mean concentrations between T\(_4\) and T\(_3\) were insignificant
C1 vs. E2 (P < 0.05)
C1 vs. C2 (P < 0.01)
VI. Effect of extracted rapeseed meal and nitrates on mean concentrations of iodine in colostrum and milk of groups differing in iodine and selenium supplementation

<table>
<thead>
<tr>
<th>Group</th>
<th>E1 ERM ± NO₃ suppl. I + Se n = 6</th>
<th>E2 ERM ± NO₃ suppl. I n = 6</th>
<th>C1 ERM ± NO₃ no suppl. n = 6</th>
<th>C2 no goitrogenic suppl. I ± Se n = 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colostrum up to 6 h after parturition</td>
<td>430</td>
<td>334</td>
<td>57</td>
<td>2 083</td>
</tr>
<tr>
<td>Milk within day 61 and 140 of the lactation period</td>
<td>70</td>
<td>101</td>
<td>27</td>
<td>198</td>
</tr>
</tbody>
</table>

Significance of differences among group means:
- Colostrum: C1 vs. E1, E2, C2 (P < 0.01) Milk: C1 vs. E1, E2, C2 (P < 0.01)
- C2 vs. E1 (P < 0.05) C2 vs. E1 (P < 0.01)
- C2 vs. E2 (P < 0.01) C2 vs. E2 (P < 0.01)

A substantial difference in net birth rate was found between the groups receiving the goitrogenic agents and additional iodine and selenium (E1, E2) and the control group C1 (goitrogenic agents without additional iodine and selenium) on one side and the group fed the diet without goitrogenic agents (C2) on the other side. The highest number of live lambs was born in the group E2 (goitrogenic agents + iodine). Pregnancy rate in the group fed the conventional ration (C2) was lower than the breeding objective of the breed Sumava sheep. It should be noted that one sheep of this group was infertile in spite of being in a very good condition and did not show any apparent deviation from the normal state of health including gonadal organs.

Clinical findings in the thyroid gland

No clinical abnormalities were detected by ausculation and palpation of the thyroid glands except for the ewes 7, 8, and 10 of the group C1. The latter three ewes developed a slight enlargement making the thyroid gland palpable on experiment day 56. The enlargement showed rather a decreasing than an increasing tendency.

Typical goitre developed in the lambs. One female lamb (No. 8B) of the group C1, born with a low viability and live weight, was lethargic and developed thyroid enlargement palpable as two distinct painless, oval, rigid, and elastic structures with sizes of 3–4 x 2 cm. Since the lamb did not respond to supportive therapy and the clinical state rather deteriorated, it was decided to kill it at the age of 3 days. Indefinite findings on thyroid glands persisting up to the end of the experiment were further recorded in the lamb No. 8A and the well-developed lamb No. 12 of the group C1. A change of the ration led to extinction of the enlargement. Slight enlargements in another two well-doing lambs (Nos. 9 and 10) disappeared spontaneously without any change in the ration.

Thyroid gland functions

Individual concentrations of T₃ and T₄ in the lambs for which clinical and necropsy data, including thyroid gland weights and microscopic findings, were available for comparison (Tabs. IV and V) indicated an insufficient supply of iodine to the thyroid gland in the lambs of the group C1. Such deficiency was evident from the thyroid hyperplasia in the lamb No 8B, absolutely low concentrations of T₃ (46 to 37 nmol/l) and T₄ (2.6–1.9 nmol/l), and a very low concentration of iodine in blood plasma on the 1st and 3rd day after birth (45 to 26 μg/l). All the values differed considerably from those found in lambs No. 2, 13A, and 21 of the other groups (E1, E2, C2) in which the concentrations of T₃, T₄, and plasmatic iodine ranged from 73 to 252 nmol/l, from 4.6 to 8.3 nmol/l, and from 80 to 236 μg/l, respectively (Tab. V).

Relevant to epi-pathological considerations is the fact that, in the lamb No. 8A – a littermate of the lamb 8B mentioned in the preceding paragraph – the concentrations of plasmatic iodine and T₄ were very low, while the concentration of T₃ did not differ from those found in the other groups and the palpatory finding was indefinite. The poor stability of the thyroid gland of the lamb 8A is documented also by fluctuations of functional parameters up to day 60 after birth when low concentrations of plasmatic iodine and T₄ were found, concentrations of T₃ were variable. Individual findings corresponded to the results of biochemical function tests of the thyroid gland carried out in the groups during the postnatal development (Tab. V).

Mean concentrations of iodine in colostrum and milk in the individual groups of ewes are given in Tab. VI from which the effects of differences in feed composition and iodine supplementation on the metabolism of iodine are apparent. Feeding of the goitrogenic ration (without addition of iodine and selenium) in the group C1 resulted in extremely low excretion of iodine by the mammary gland both at the beginning and towards the end of the lactation period. The iodine concentrations
in colostrum (57 ± 36 µg/l) and milk (27 ± 15 µg/l) were far below the physiological values. In this respect, the differences between C1 and any other group were highly significant (P < 0.01).

Iodine concentrations in colostrum and milk of ewes of the experimental groups fed the ration containing goitrogens and supplemented with iodine and selenium or iodine alone, i.e. 430 ± 520 µg/l for colostrum and 70.0 ± 16.0 µg/l for milk in the group E1, and 334 ± 330 µg/l for colostrum and 101 ± 41 µg/l for milk in the group E2, reached the levels indicating a satisfactory supply of iodine. The highest concentrations of iodine in colostrum (2 083 ± 1 423 µg/l) and in milk (198 ± 8 µg/l) in the late lactation phase were found in the group C2 fed the conventional ration and supplemented with iodine and selenium. In spite of a high variation, the concentrations differed highly significantly (P < 0.01) from those found in the other groups.

Necropsy, histological and TEM findings

As shown in Tab. IV, no gross lesions were found upon necropsy of four clinically normal lambs (Nos. 2, 13A, 7, 21). All the lambs were in a good nutritional state and no enlargement or asymmetry of thyroid lobes were seen.

Asymmetry of the thyroid gland due to enlargement of the left lobe, moderate oedema of the surrounding tissues and dilatation of jugular veins was found in the lamb No. 8B. Further findings included disseminated atelectatic lung lesions, moderate liver swelling, greenish-brown discolouration of kidneys with enlarged pelvices, infiltration of fibrous and lipid tissues surrounding the kidneys, and brownish-grey thin pasty ingesta with large milk coagula in the abomasum.

Absolute weight of the thyroid gland of the lamb 8B corresponded to 2.5-fold to 5-fold of the absolute weight of a normal gland.

Normal histological structure of thyroid glands of lambs of the groups E1 and E2, fed the ration containing the goitrogens and supplemented with iodine + selenium and iodine alone, respectively, indicated a sufficient supply of iodine to the ewes. The structure of the thyroid parenchyma of the lamb 8B (group C1 fed the ration with ERM and nitrate) with clinically apparent goitre corresponded to 3rd stage of goitre. Thyrocytes of the morphologically normal glands of the lambs 7, 13A, and 21 were arranged usually in a single layer and their cytoplasm contained activated mitochondria, lysosomes and abundant rough endoplasmatic reticulum with moderately enlarged colloid-containing cisterns (Fig. 1). In the enlarged thyroid gland of the lamb 8B, thyrocytes formed a multilayer structure, colloid-containing cisterns of the endoplasmatic reticulum were cystically enlarged, numerous activated mitochondria and pyknotic nuclei were displaced to the cell periphery, and cytoplasmatic and nuclear membranes disintegrated here and there (Fig. 2). Such lesions along with the low T4 and T3 concentrations indicated thyroid hyperplasia and hypofunction as a result of iodine deficiency in the dam.

Dominant in the histological pattern (90%) of the thyroid gland of the clinically normal lamb No. 21 of the group C2 fed a conventional ration and supplemented with iodine and selenium was a well developed structure with regular follicles and homogeneous dark stained colloid-containing sporadic macrovacuoles.

Two intercurrent extrathyroidal diseases were recorded during the experiment. One ewe (No. 4 in the group E1) developed mastitis with subsequent hypogalactia which affected the growth and development of her twin lambs. The ewe No. 20 of the group C2 with an asymptomatic history died suddenly on experiment day 281, i.e. 194 days after the beginning of the mating period. At necropsy, the death cause was identified as acute heart failure associated with the approaching partur-
tion, and dilatation of forestomachs overfilled with ingesta corresponding in composition to the ration. The weight of her two dead foetuses was extremely high (4,120 and 3,950 g) for twins of the breed Sumava sheep.

DISCUSSION

The ovine thyroid gland has become the subject of many studies owing to its relative sensitivity to the supply of iodine and effects of goitrogenic agents. Clinical manifestations of iodine deficiency in sheep are similar to those seen in other farm animals (Barberán and Valderrábano, 1987). Sheep as a suitable animal for experimental studies of deficiency diseases, in particular of their effects on the development of foetuses, were recommended also by Potter et al. (1982), Nasser et al. (1986), Alexander et al. (1990), Wu et al. (1992).

The concentration of 21.51 µmol of glucosinolates per 1 g of ERM, which made up 82.5% of the concentrate B, indicates that the rape cultivars grown currently in the Czech Republic still contain considerable amounts of progoitrogens, in particular the progoitrin alkylglucosinolate (17.74 µmol per 1 g) as a precursor of goitritn with a strong goitrogenic effect described by Zech et al. (1993), Schöne (1999) and other authors. Goitrin is known to inhibit the production of T4 and transfer of iodine in the thyroid gland. This thyreostatic effect was demonstrated experimentally in dairy cows (Ehlers et al., 1994) and also in our experiments in sheep. The length of the Ehlers’ and our experimental periods was approximately the same (14 vs. 13 months) and the similarity of results pertained to the low content of iodine in the milk of the experimental groups in which the competitive effect of glucosinolates in the thyroid gland was not compensated by increased supply of iodine, and to the development of a metabolic situation with a hypothyreotic tendency. The more dramatic course of hypothyreosis manifested by the development of goitre in the progeny of the ewes receiving goitrogens in the feed was apparently due to a higher proportion of ERM in the ration, higher content of glucosinolates in ERM, cumulative effects of sodium nitrate, and a lower content of iodine in the basic ration. Parameters of performance were also similar. While both Emanuelsen (1994) and Ehlers et al. (1994) reported a tendency towards higher milk yield and higher content of milk proteins, the results of our experiments indicated a favourable physical condition of ewes and birth rate and birth weight of lambs.

Both the results of laboratory examinations and clinical and post-mortem findings indicate the severity of iodopaenia developing in the group C1, as well as the effect of the supplementation in the groups E1 and E2. In this respect, valuable information was obtained from the monitoring of iodine content in milk, which is generally regarded as a reliable indicator of iodine supply (Kaufmann et al, 1998; Herzog et al., 1999).

A fact to be regarded in among-species comparative considerations is that the concentrations of iodine in sheep and goat colostrum and milk are higher than in cow’s milk. Therefore, concentrations below 79 µg/l for ovine colostrum and 62 µg/l for ovine milk should be interpreted as signs of deficiency (Groppel, 1991). A similar experience was reported by Auzolas and Caple (1984) who observed goitre in lambs fed ovine milk containing 45 to 98 µg iodine per litre. Our data are consistent with the results of Mason (1976) who observed goitre in 80% of lambs born to ewes receiving 30 µg of iodine per animal per day whose milk contained 45 µg iodine per litre. On the other hand, all lambs born to ewes receiving 80 or 120 µg of iodine per animal per day whose milk contained 95 and 131 µg of iodine per litre, respectively, were free from goitre.
In our experiments, the mean concentration of iodine in milk of the iodine-supplemented ewes was above the lower margin of the physiological range, was higher in the groups E2 and C2, and markedly reflected the state of health of the ewes and lambs, which were free from goitre, and the corresponding concentrations of T4 and T3 in blood plasma of lambs. On the other hand, the significantly lower concentration of milk iodine in the ewes of the group C1 indicated pathophysiological consequences of the absence of iodine supplementation allowing glucosinolates and nitrate ions to inhibit the transport of iodine into the thyroid gland. Such effect of glucosinolates resulting in low iodine concentration in milk and also lower concentration of T4 in blood plasma of cows fed rapeseed cake was described by Papas et al. (1979).

The high content of iodine in colostrum and its individual variations are well-known facts. Nevertheless, the increased amount of iodine excreted in milk of ewes fed a ration free of goitrogens (group C2) cannot be overlooked and is indicative of normal iodine metabolism if the intake is sufficient. The same applies also to milk secreted in the late lactation phase. The more conspicuous is the highly significant difference between the groups C2 and E1. Although the supplementation in both the groups was the same, ewes of the group E1 excreted in colostrum and milk 2.8 to 4.8 times less iodine due to the long-term intake of ERM and nitrate ions. It is important, however, that, thanks to the compensatory effects of supplementation, the mean iodine concentrations of 70 μg/l (E1) and 101 μg/l (E2) were high enough to meet physiological requirements of sucking lambs. The sufficient supply was apparent from the concentrations of iodine, T4, and T3 in blood plasma of lambs expressed in terms of group means of six analyses done from birth to the age of 60 days. The highest values of the three analyses were found in the first hours after birth.

The interest in the role of selenium in the pathogenesis of thyroid disorders is encouraged on one side by the fact that selenium deficiency is associated with an excess peroxide which can damage thyrocytes (Corvilain et al., 1993), and on the other side by the presence of selenium in molecules of enzymes controlling the production of thyroid hormones, their transformation and eventual destruction in target tissues. They include selenoprotein 5'-deiodinase consisting of three enzymes of which Types I and III contain selenium in their molecules. The three enzymes represent an interconnected regulatory system which is responsible for deiodination of thyroxine and prompt provision of a sufficient amount of the active hormone triiodothyronine (Anonym, 1996; Köhrle, 1996).

In our experiment, the selenium supplementation was motivated by papers recommending such treatment for areas where naturally low concentrations of selenium in blood of the human population are recorded (Contempre et al., 1991). Such treatment was recommended also in veterinary literature (Todorova et al., 1997), particularly for animals receiving goitrogens in their rations (Donald et al., 1993). Relations between metabolism of selenium and goitrogenic effect of cyanogenetic glycosides and functional changes in the thyroid gland reported (Gutzwiller, 1993). Bireš et al. (1996) found low concentrations of selenium in blood serum and goitre in goats in Slovakia.

Further information from areas with notorious occurrence of myopathies due to selenium deficiency (George et al., 1966; Langlands et al., 1981; McCoy et al., 1997) is consistent with our experience indicating that nutritional myodystrophy in lambs occurred in areas which, to a certain extent, overlapped areas of occurrence of goitre (Kursa et al., 1997). The assumption that endemic selenium deficiency diseases of animals resulted from low selenium content in soil, crops and animal tissues (Anke et al., 1983; Kursa and Kroupová, 1975) is supported by the recently demonstrated correlation between low levels of selenium in the human population of South Bohemia and metabolism of iodine and thyroid hormones (Kvíčala et al., 1997).

In our experiment, the effect of selenium was apparent from a comparison of iodine, T4, and T3 concentrations in blood plasma of the progeny of the groups E1 and E2. Both were fed a ration containing the goitrogens (ERM and nitrate) and were supplemented with iodine, but the group E1 received additional selenium in a dose used in sheep also by Todorova et al. (1997). All the parameters of the thyroid function were higher in lambs of the group E1. A similarly conceived experiment in sheep grazing in an area affected by the occurrence of selenium deficiency diseases and supplemented with iodine + selenium or iodine alone was carried out by Donald et al. (1993) who used potassium thiocyanate as the goitrogenic agent. The difference in the supplementation had no apparent effect on the thyroid function parameters in lambs born by the ewes receiving the goitrogenic agent.

Worth mentioning is also the finding of an insignificant decrease of T4 and increase of T3 in sheep fed a diet with a low content of selenium and supplemented with 0.3 ppm sodium selenite (Bik and Kondracki, 1997).

The most apparent manifestation of iodine deficiency is the enlargement of the thyroid gland resulting from hyperplasia due to the effect of thyrotrphin activating the thyroid gland by a feedback mechanism in order to compensate hypothyrosis (Podoba and Langer, 1993). Nevertheless, the decisive criterion for assessing the severity of the insufficiency is the histopathological pattern (Seffner and Groppel, 1986; Čada, 1988).

The results of histological and, as the case may be, histometrical examination are valuable particularly in cases when a limited number of samples is available or if clinically complicated cases are to be explained. Although only five thyroid glands were weighed and examined histologically within our study, we consider the results useful, because they confirmed the clinical findings and the clarification causes of differences in the
biochemical parameters as described and interpreted above. From results of extensive epidemiological studies in the human population of the United States, Gaitan et al. (1989) concluded that the polyfactorial character of pathogenesis of goitre and complex interactions of intrinsic (immunological and genetic) factors and specific extrinsic factors (environment) must be considered in the analyses and assessment of the effects of goitrogenic agents. Such conclusion may also explain the fact that advanced goitre with histopathological finding typical of 3rd stage developed in our experiments in only one lamb (group C1 receiving ERM + nitrate without supplementation). Individual differences in responses of the thyroid gland to thyrostatic effects of sodium hypochlorite were reported also by Bekeová et al. (1998) who assume that such differences are due to both intrinsic factors and current environmental conditions.

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