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Histometric and biochemical properties of the thyroid gland in sheep with high iodine supplementation

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Abstract

The aim of this study was to evaluate histometric and biochemical properties of the thyroid gland of sheep supplemented with high doses of iodine. The study was conducted on ewes (n = 12) and gimmers (n = 12) of Sumava mountain sheep; each group was subdivided into two groups (group A and B) of six animals. Feed of group A was supplemented with 3 mg iodine/kg of dry matter; group B was given 5 mg iodine/kg dry matter. The iodine in feed mineral supplement was in the form of calcium iodide. The ewes were at first carrying, subsequently lambing, lactating and finally remained barren. The experiment ended after 11 months, when all animals were slaughtered and a sample of the thyroid gland was taken for histometric examination and determination of iodine content by modified colorimetric method. Prior to the slaughter, blood samples were collected for determination of thyroidal hormones and the thyroid-stimulating hormone in blood serum. Thyroid glands of sheep from group B showed higher thyroid weight, larger follicles, higher percentage of large follicles and lower follicular cells compared to groups A. Normal or lower content of triiodothyronine and thyroxine, lower content of their free fractions and bordering or elevated concentrations of thyroid-stimulating hormone were detected in blood serum of all four groups. This trend can signalize the tendency of lowering activity of the thyroid gland. The results of this long-term study show impacts of higher iodine intake on the structure and function of the thyroid gland in sheep.

Morphometric, thyroidal hormones, PCNA, epithelium, thyrocytes

The main function of the thyroid gland is the production of triiodothyronine (T_3) and thyroxine (T_4) hormones. Optimum intake of iodine influences function and structure of the thyroid. The height of the follicular cells, size of the follicles and amount and character of the colloid inside the follicles all depend on the activity of the thyroid (McGavin et al. 2001; Capen et al. 2006). Hypothyroidism caused by iodine insufficiency leads to higher production of thyroid-stimulating hormone (TSH) and subsequent proliferation of follicular cells and colloid depletion. Iodine in surplus, on the other hand, causes lowering of TSH production and accumulation of the colloid inside the follicles causing their growth. The lowered content of TSH further lowers the activity of thyrocytes and causes a decrease in epithelial cell height (Shan et al. 2009). The prolonged administration of large doses of iodine markedly reduces iodine trapping by the thyroid, thus causing antithyroidal or goitrogenic effects in many domestic and experimental animals (Radostits et al. 2000). Current importance of studying the activity of the thyroid gland in farm animals in connection with various amounts and forms of iodine in feed or varying iodine concentrations in the environment is shown in many recently published studies (Kursa et al. 2010; Baňoch et al. 2011; Dušová et al. 2012).

The aim of this study was to show the effect of long-term excessive iodine in feed on histometric and functional indicators in ewes and gimmers.

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Materials and Methods

Animals and experimental design

The study was conducted on ewes (n = 12) and gimmers, (n = 12) of Sumava mountain sheep; each group was subdivided into two groups (group A and B) of six animals. Both groups A were administered 3 mg iodine per kg dry matter DM feed dose; groups B were supplemented with 5 mg iodine per kg DM (Table 1). The dose of 5 mg per kg of 88% dry matter (DM) corresponds to the higher limit permitted in the norm (Commission Regulation of the EC No. 14/59/2005). The diet was composed of meadow hay (1 500 g), oat groats (270 g) lucerne granules (240 g) and mineral supplement (9 g). Iodine in mineral feed supplement was added in the form of calcium iodide (CaI_2). The ewes were at first carrying, subsequently lambing, lactating, and finally remained barren. The experimental period started in the first month of gestation. Environmental conditions (feed, housing conditions and temperatures) before and during experimentation were similar. The experiment was ended after 11 months when all the animals were slaughtered and a sample of their thyroid gland was taken for histometric examination and determination of iodine content. Prior to the slaughter, blood samples for determination of thyroidal hormones and the TSH in blood serum were collected. Ewes and gimmers were conducted under protocols approved by the Faculty of Agriculture, University of South Bohemia in České Budějovice and National Committees (Protocol No.2/08).

Table 1. Characteristics of experimental groups of sheep.

Group	Age (months)	Body weight (kg)	Iodine in feed (mg I/kg DM)
Ewes A (n = 6)	76 ± 4	53.2 ± 4.5	3
Ewes B (n = 6)	75 ± 3	56.3 ± 4.8	5
Gimmers A (n = 6)	29 ± 2	59.2 ± 4.6	3
Gimmers B (n = 6)	28 ± 2	58.4 ± 5.4	5

DM - dry matter

Methods

The whole thyroid gland was dissected and weighed. Samples for histological examination and samples for determining the iodine content were collected from the central part of each thyroid gland lobe. Samples were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 5 µm thick slices and stained with haematoxylin and eosin. The measurements were conducted using Leica IM 500 Version 4.0 visual analysis program and Leica DC 320 camera in combination with Leica DM 2500 microscope. The size of 60 follicles (their length and width) and the height of 20 follicular cells inside each were determined from three fields of vision in different parts of the histological section. The examined follicles were classified into three categories by length (Jelínek et al. 2003): large (175.1–615.0 µm), medium sized (80.1–175.0 µm) and small (15.0–80.0 µm) follicles. The number of positive nuclei in thyrocytes in ten different fields of vision from different parts of the section was estimated as the indicator of proliferative activity of the thyroid follicular cells. Immunohistochemical reaction PCNA (proliferation cell nuclear antigen) was performed on every sample of the thyroid gland. Histological sections were deparaffined and the activity of endogenous peroxidase was blocked by 3% peroxide (10 min) and nonspecific binding sites were blocked (1% bovine albumin for 5 min). Monoclonal mouse anti-proliferation cell antigen/clon PC10 from Dako for PCNA detection was used. For visualization the detection system Dako EnVision™ was used.

Serum concentration of triiodthyronine (T3), tyroxine (T4), and their free fraction (FT3, FT4) were measured by radioimmunoassay (IMMUNOTECH a. s., Czech Republic); concentrations of thyroid-stimulating hormone (TSH) were determined by ELISA using commercially available kit (ELISA development, s.r.o., Czech Republic). The concentration of iodine in thyroid gland was determined by modified colorimetric methods according to Bednář et al. (1964).

The obtained results were evaluated by STATISTICA 7.0. (StatSoft, Inc.) with the help of ANOVA, regression and correlation analyses ($P < 0.05$).

Results

Histometric characteristics of the thyroid gland

Thyroid glands of sheep from group B supplemented with 5 mg iodine/kg DM showed higher thyroid weight, larger follicles, higher percentage of large follicles and lower follicular cells compared to group A supplemented with 3 mg iodine/kg DM (Table 2). When comparing the thyroid gland indicators of the two groups with the same iodine supplementation, the results showed that the thyroid glands of ewes had higher weight, higher iodine content, larger follicles, lower follicular cells, and

Table 2. Thyroid gland indicators in sheep (ewes and gimmers) supplemented with iodine.

Indicator	Ewes		Gimmers	
	A	B	A	B
Weight (g)	7.5 ± 4.2[a]	9.67 ± 0.47[b]	3.74 ± 0.37[ab]	5.10 ± 1.12[ab]
Iodine (mg/kg)	1159.4 ± 382.2	1252.4 ± 400.0	396.1 ± 154.0	501.4 ± 170.6
Large follicles (%)	19.7 ± 15.2[c]	34.9 ± 11.9[d]	3.1 ± 1.4[cda]	24.4 ± 2.2[da]
Medium follicles (%)	51.0 ± 16.5[d]	42.6 ± 9.6[a]	39.7 ± 8.3[cd]	54.4 ± 5.6[ac]
Small follicles (%)	36.4 ± 12.1[c]	23.1 ± 8.9[a]	57.2 ± 9.6[ca]	21.3 ± 7.7[c]
Length of follicles (μm)	132.9 ± 28.4[c]	146.8 ± 24.7[d]	83.0 ± 11.1[ca]	132.6 ± 6.2[da]
Width of follicles (μm)	88.9 ± 21.3[c]	94.5 ± 23.1[b]	59.2 ± 8.5[abc]	93.7 ± 2.3[a]
Height of epithelium (μm)	5.7 ± 0.20[a]	4.91 ± 0.54[c]	6.2 ± 0.4[ad]	5.4 ± 0.1[cd]
Count of PCNA positive cells	3.2 ± 1.28[ac]	4.8 ± 1.49[bc]	16.3 ± 6.5[abc]	6.3 ± 1.7[a]

PCNA - proliferation cell nuclear antigen. Data are expressed as mean ± SD. [a,b] $P < 0.01$. [c,d] $P < 0.05$

Table 3. Correlation between selected indicators in sheep (ewes and gimmers) supplemented with iodine.

Indicator		Correlation coefficient
Average epithelium height	Average follicle size	-0.60
Count of PCNA positive cells	Percentage of large follicle	-0.47
Count of PCNA positive cells	Percentage of small follicle	0.45
Percentage of large follicle	Thyroid weight	0.73
Iodine content in the thyroid gland	Thyroid weight	-0.35

PCNA - proliferation cell nuclear antigen, n = 24, $P < 0.05$

decreased proliferating activity (Table 2). Correlations between some indicators are shown in Table 3.

Thyroid follicles of ewes contained homogenous colloid mostly without resorptive vacuoles and the epithelial cells were mostly low cuboidal. In one thyroid gland specimen from ewes of group B, a small accumulation of lymphocytes without any trace of tissue alteration was found. Follicles of the gimmers contained mostly homogenous colloid with individual resorptive vacuoles and the epithelial cells were mostly cuboidal low to cuboidal. For all gimmers and several ewes, cysts of different sizes with cornified or uncornified squamous epithelium were present in different parts of the thyroid.

Biochemical properties of the thyroid gland

The mean content of T4 and T3 and their free fractions (FT4 and FT3) as well as the content of TSH was always higher or the same for gimmers compared to ewes with the same iodine supplementation (Table 4). The most significant difference ($P < 0.01$) was found in the content of TSH between ewes and gimmers of group A and between ewes and gimmers of group B. The maximum concentration of TSH was measured for gimmers A and gimmers B.

Discussion

The mean weight of thyroid gland in ewes of both groups in our study was higher than the reported weight of animals with normal histological structure (Kratochvíl 1998). We

Table 4. The concentration of thyroidal hormones and thyroid-stimulating hormone in blood serum of sheep (ewes and gimmers) supplemented with iodine.

Indicator	Ewes		Gimmers	
	A	B	A	B
T4 (nmol/l)	60.42 ± 12.89^{af}	63.08 ± 11.03^{ec}	84.16 ± 8.08^{ac}	82.80 ± 11.38^{ef}
T3 (nmol/l)	2.55 ± 0.37^{a}	2.14 ± 0.34^{c}	2.50 ± 0.03^{ac}	2.37 ± 0.23
FT4 (pmol/l)	13.67 ± 1.81^{a}	13.12 ± 2.04^{cd}	17.26 ± 1.17^{ac}	16.50 ± 0.99^{d}
FT3 (pmol/l)	3.29 ± 0.35^{a}	2.91 ± 0.46^{cd}	3.98 ± 0.38^{ad}	4.66 ± 0.74^{c}
TSH (ng/ml)	0.91 ± 0.21^{ac}	0.97 ± 0.22^{db}	2.78 ± 1.01^{ad}	2.14 ± 1.47^{cb}

Data are expressed as mean ± SD. $^{a,b,\,c,d}$ $P < 0.01$; ^{e,f} $P < 0.05$

found higher iodine content in the gland of both groups of ewes compared to the two groups of gimmers. It can be connected to the longer accumulation time in the gland due to the age of the animals. They were exposed earlier to standard iodine doses or were burdened metabolically with pregnancy or nursing.

The histological picture of glands of both groups of ewes (especially for ewes of group B) in our study corresponds to lowered activity of the gland (Derycke et al. 1999; Jelínek et al. 2003). Furthermore, all indicators of lower activity were more pronounced in group B. When comparing histological pictures of ewes and gimmers with the same iodine supplementation, ewes showed signs of lower secretory activity of the thyroid according to McGavin et al. (2001).

When comparing ewes and gimmers, smaller mean size of follicles and generally higher follicular cells were demonstrated in ewes. According to Shan et al. (2009), the height of the follicular cells is an objective indicator of the thyroid gland activity. Lower proliferative activity of thyrocytes (presented in ewes by the level of PCNA) is according to Di Fulvio et al. (2000) a sign of lower activity of the thyroid gland. This fact was confirmed by correlation between count of PCNA positive cells and percentage of large or small follicles and other correlations.

When comparing the concentrations of T4, T3 FT3 and FT4 in both groups of ewes in our study, the concentration of free forms of hormones are lower according to Nazifi et al. (2008), Badiei et al. (2010) or Eshratkhah et al. (2011). Concentrations of hormones found in both groups of ewes corresponded to lowered activity of the thyroid gland as demonstrated by histological findings, too. Average values of TSH approached 1 ng/ml for both ewe groups in our study. In humans, this concentration would indicate euthyroid state with possible first signs of dysfunctions but so far without any clinical impact (Obregon et al. 2005). Concentrations of T3 and T4 in gimmers were similar to values mentioned in literature. Contents of FT3 and FT4 were in ewes lower that those given by for example Nazifi et al. (2008) or Badiei et al. (2010). Individual maximum values that over-exceeded 3.5 ng/ml in both groups of gimmers would imply latent hypothyroidism in humans. Higher values of TSH and simultaneous lower or normal concentrations of FT3 and FT4 would diagnostically indicate hypothyroidism or subclinical hypothyroidism (Ślebodziński 1994; Racek et al. 2006).

The presented data show the effects of the highest permitted iodine dose on histometrical and biochemical properties of the thyroid gland. The results demonstrate a decrease of thyroid function in animals with higher iodine intake. According to Ruffin et al. (2012), lower thyroid function negatively influences production and reproduction in sheep. For this reason they recommend optimization of iodine dosage in sheep at value 0.5–0.8 mg I per kg dry matter (NRC, 2007).

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