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THE EFFECT OF IODINE AND STRUMIGENS LONG-TERM FOODBORNE INTAKE ON HISTOMETRICAL PARAMETERS OF THYROID GLAND IN GIMMERS

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Abstract

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In 2010 and 2011 two experiments on gimmers of Šumava mountain sheep were performed. The first experiment was carried out on 12 animals (experimental period was 11 months); control group was fed with 3 mg I*kg^{-1} in dry matter per day and experimental group was fed with 5 mg I*kg^{-1} in dry matter per day. The second experiment lasted 10 months and it was carried out on 12 animals. Feed ration for control group contained 10 mg I*kg DM^{-1}. Feed ration for experimental group contained 10 mg I*kg DM^{-1} too; moreover it included rapeseed meal and 1 g of sodium nitrate. The aim of the first experiment was focused on impact of high iodine intake on structure of thyroid gland. The aim of the second experiment was to discover effect of strumigens during high iodine intake on structure of thyroid gland. The animals were slaughtered after the experiment and there was executed the dissection of thyroid gland. The samples of thyroid gland were processed during classic paraffin method and dyed with haematoxylin and eosin. For finding of histometrical parameters was used program Leica IM 500 Version 4.0. The length, the width and the area of follicles were measured. The follicles were divided into three groups after this procedure (by the length); in each group were measured 20 thyreocytes. In group with intake 5 mg I*kg DM^{-1} (experiment from year 2010): higher weight of thyroid ($p < 0.01$), significant higher ($p < 0.05$) proportional representation of large and medium follicles, significantly ($p < 0.05$) higher average size of follicles, demonstrably lower ($p < 0.05$) height of epithelium were found. There were not found any differences in monitored parameters between the groups from experiment which was carried out during the year 2011. After comparing results from the both experiments significantly higher percentage representation of large follicles a significantly lower representation of small follicles, distinctly higher average size and higher height of epithelium in all size categories, in groups with iodine intake 10 mg I*kg DM^{-1}was found (from second experiment – year 2011).

follicle, epithelium, thyreocyte, rapeseed meal, nitrates, morphometry

Thyroidal hormones crucial affect the whole metabolism of organism. Iodine intake from the environment is the critical factor, which influences function of thyroid gland. The thyroid gland in sheep is in region of the first tracheal ring, thyroid is formed from two lobes, which are connected by *istmus*. Normal thyroid weight is approximately 3–7 grams in adult sheep (Kratochvíl, 1998). The long-term insufficient or superfluous iodine intake may be reflected on function and structure of thyroid

gland. The long-term lack of iodine caused: decrease of production thyroidal hormones -thyroxine and triiodothyronine, growing of epithelial cells, reduction of follicles size and growing of gland weight (Kratochvíl, 1998). The long-term high iodine intake caused hyper function of thyroid gland at the young age (later to hypo function, due to reduced production of TSH – thyroid stimulating hormone). There was found relationship with development of autoimmune disease of thyroid gland in human (Burgi, 2010; Guan *et al*., 2009; Dal Maso *et al*., 2009; Knobel and Medeiros-Neto, 2007). Thyroidal hormones influence production (milk yield, intensity of growth, growth wool) and reproduction of animals (Pugh and Baird, 2012). The impact of iodine excess on thyroid structure may be reflected in reduce of height of thyreocytes, an increase of follicles size due to accumulation of colloid. The weight of thyroid is usually lower, in some cases the weight of thyroid gland grows due to accumulation of colloid (colloid goitre) (Deborah and Stabenfeldt, 2007; Zachary and McGavin, 2011).

The aim of this work was focused on influence of long term foodborne high intake of iodine and strumigens on structure and others parameters of thyroid gland.

MATERIAL AND METHODS

In 2010 and 2011 two experiments on barren gimmers of Šumava mountain sheep bread were performed. Animals from both experiments were come from the same breed and were bred in the same conditions. The both experiment group were fed with experimental feeding mixture and *ad libitum* hay. Animals were (12 barren gimmers) burdened with high iodine foodborne intake during 11 months in year 2010. The control group (group A) received 3 mg I*kg DM^{-1} and experimental group (group B) received 5 mg I*kg DM^{-1}. Average body weight was 74–88 kg and average age was 24 months. The second experiment (in year 2011) was carried out on 12 animals and experimental time was 10 months. The control group (group C) received feeding mixture 10 mg I*kg DM^{-1}, the experimental group (group D) was fed with mixture with the same iodine concentration, moreover the experimental feeding mixture rape seed meal and sodium nitrate (1g per head and day). The aim of this experiment was focused on the impact of strumigens (glukosinolates and sodium nitrate) on structure of thyroid gland by high iodine intake.

The animals were slaughtered in slaughterhouse after experiment, the thyroid glands were dissected, weighted and samples for histological examination were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 5 µm thick slices and stained with haematoxylin and eosin.

Histometrical measurement was carried in program for image analysis Leica IM 500 Version 4.0., (in combination with camera Leica DC 320 and microscope Leica DM 2500). The length, the width and the area of 60 follicles were measured in three visual fields (from different parts of slice). Measured follicles were divided into three categories (Jelínek *et al*., 2003): large (175.1–615.0 µm), medium (80.1–175.0 µm), small (15.0–80.0 µm). The proportion of these categories was found. Dates were processed in program STATISTICA 7.0. (StatSoft, Inc.), Anova and regression and correlation analysis were used.

RESULTS

There are mentioned the values of thyroid weight and relative thyroid weight (Tab. I). The lowest thyroid weight was achieved in group A. The differences to group B were significant. Between groups C and D were not observed distinct variations.

In Fig. 1 percentage representation size categories of follicles in particular groups of animals are illustrated. It was found statistically important differences ($p < 0.01$) between groups A and B in all size categories of follicles. Groups C and D were not different in these parameters. After comparing all the groups the differences in percentage representation of large and small follicles were found.

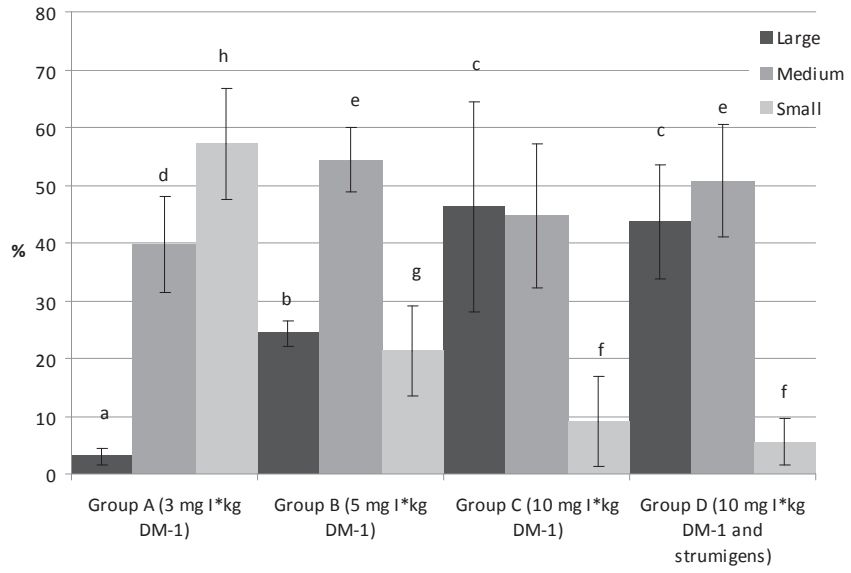
The lowest average follicle sizes were found in group A; the differences to group B were found in all monitored parameters (the length, the width, the area of follicles). Differences between groups C and D were not apparent. Only average areas of follicles were lower in group D, the difference to group C was not significant. When we drew a comparison dates between both experiments, sizes of follicles were significantly bigger by group C and D (compared to groups A and B).

Differences ($p < 0.05$) in high of follicular epithelium were found between groups A and B in category of large follicles and in total average size

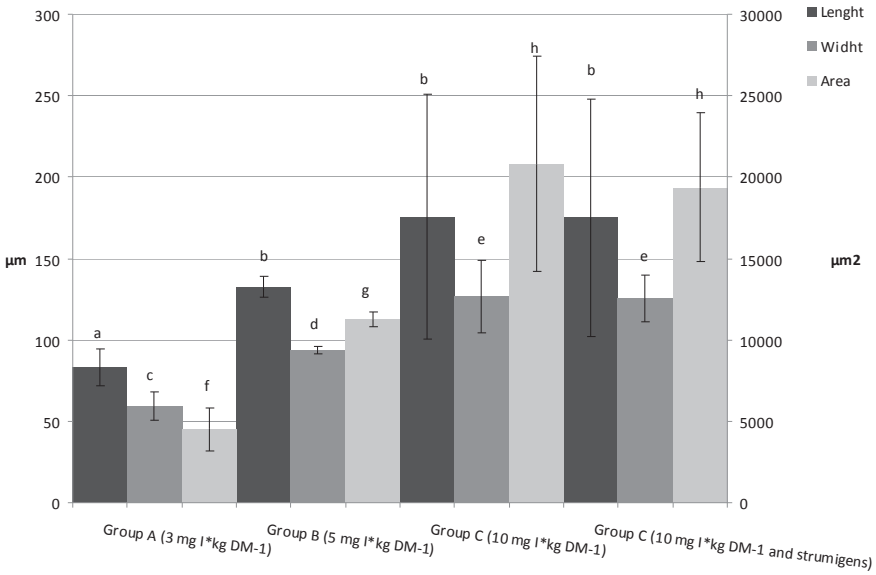
I: *Average thyroid weight and relative thyroid weight in barren gimmers*

Group	n	Iodine intake (mg I*kg DM^{-1})	Thyroid weight (g)	Relative thyroid weight (%)
A	6	3	3.74 ± 0.37^{a}	0.0050 ± 0.0005
B	6	5	5.10 ± 1.12^{b}	0.0057 ± 0.0009
C	6	10	4.89 ± 0.79	0.0108 ± 0.0018
D	6	10*	4.60 ± 1.10	0.0110 ± 0.0026

*experimental feeding mixture was contained rape seed meal and 1 g sodium nitrate per head and day
$^{a;b}$ $p < 0.01$



1: *Percentage representation of follicle size categories*
a:b; a:c; b:c; h:g; h:f; g:f $p < 0.01$, d:e $p < 0.05$



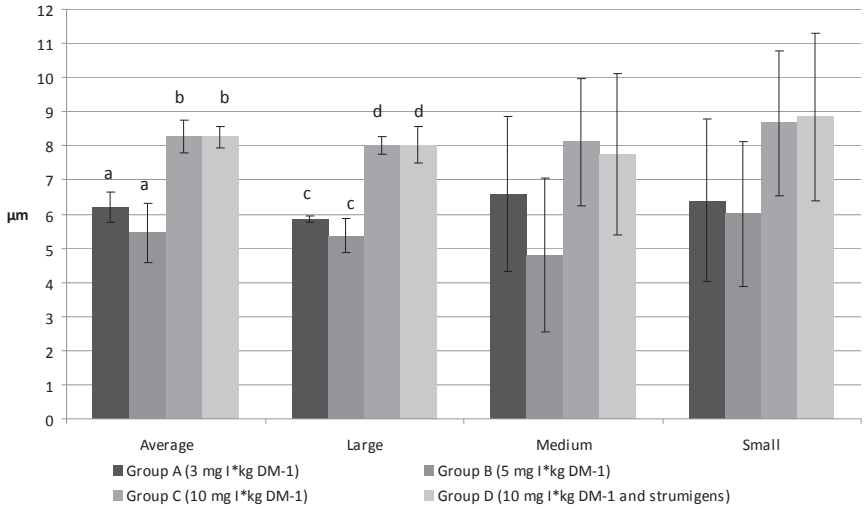
2: *Average length, width and area of follicles*
a:b; c:d; c:e; d:e; f:g; f:h; g:h $p < 0.01$

of follicles. The difference was not significant in medium and small follicles. The high of epithelium in groups C and D was not differed in any size category of follicles.

In groups from the second experiment (year 2011, group C and D) significantly higher epithelium in all size categories of follicles was found (in comparison with groups from experiment from 2010 – groups A and B).

DISCUSSION

Thyroid weight (Tab. I) in all groups corresponded with values, which was published by Kratochvíl (1998). In group D, which was fed with experimental feed mixture with strumigens, was not found any manifestation of goitre or others anomalies, which described Kursa *et al.* (2000). Thyroid weight, in this cause, was not affected by strumigens, as state Trávníček *et al.* (2001). Significantly higher percentages representation of small follicles (Fig. 1) and concurrently bigger height of epithelium (Fig. 3) in group A (compared to group B) is according to Jelínek *et al.* (2003) the sign of higher metabolic activity. The author documented this fact using increase proliferative activity of thyrocytes in small follicles in cattle. Information about sizes of thyroidal follicles in barren gimmers is presented



3: *Height of thyrocytes in particular size categories of follicles*
a:b; c:d $p < 0.01$

mainly in elder publications. Salem *et al.* (1986) stated average size of follicles 55–70 µm and maximal size 160 µm. This fact approximately responds with our data from group A (Fig. 2). Richter (1989) considered as normal size of follicles 25–230 µm. Kratochvíl (1998) stated for gimmers with normal histological structure size of follicles on average 103.42–145.06 µm. Differences were not found (Fig. 3) in height of epithelium in comparison with others authors in any groups. The height of epithelium is, by Krabačová (2003), very responsive indicator of thyroid activity. The author stated, that height of thyrocytes by sheep with normal histological structure is in average 5.23–7.06 µm. Kratochvíl (1998) stated values about 8.5–9 µm. Distinctly higher size of epithelial cells in groups C and D (in comparison with group A and B) were effected on high dose of iodine (res. iodide). Ganong (2005) stated inhibitive impact on organification of iodide and thus on synthesis of hormones (Wolff-Chaikoff effect).

CONCLUSIONS

Group B had higher thyroid weight ($p < 0.01$) in comparison with group A. In comparison with literary data any difference was not found. Group B had strongly higher percentage representation of large and medium follicles, on the other hand the count of small follicles was significantly lower ($p < 0.05$) in comparison with group A. Group B had significantly higher size of follicles and lower average height of thyrocytes and height of thyrocytes in category of large follicles ($p < 0.05$). This fact according to the same authors is the sign of lower thyroid function. Strumigenous effect was not occurred at group D. Significant difference was not found in monitored parameters between groups C and D. Animals from experiment from 2011 had high number of large follicles and distinctly lower number of small follicles, higher average size of follicles, in comparison of the both experiments.

SUMMARY

The aims of two experiments were focused on the effect of different high iodine dosage and strumigens on structural parameters of thyroid gland in barren gimmers of Šumava mountain sheep. Control group in the first experiment (experimental time was 11 months) was fed with 3 mg I*kg DM^{-1} and experimental group was fed with 5 mg I*kg DM^{-1}. Both groups in the second experiment were fed with 10 mg I*kg DM^{-1}, but feeding mixture for experimental group contained strumigens (rape seed meal and 1g of sodium nitrate per head and day). The animals were slaughtered after ending of the experiment and dissection of the thyroid gland was found. The samples of the thyroid were processed by classic paraffin histological method and dyeing by haematoxylin and eosine was used. Histometrical parameters (the length, the width and the area of follicles and the height of epithelial cells) were determined to using program for image analysis. Differences between groups from the first experiment (the iodine intake 3 and 5 mg I*kg DM^{-1}) were found in thyroid weight, percentage representation of size follicles categories, average follicle size and average height of epithelium and height of epithelium in large follicles. Between groups from the second experiment any distinct difference in monitored parameters was not found. Significant differences were found in percentage representation of large and small follicles, average size of follicles and in height of epithelial cells, when we compared data from both experiments.

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