

THE INFLUENCE OF HIGH IODINE INTAKE ON CHOSEN BLOOD PARAMETERS OF SHEEP

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Abstract

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The objective of the study was to evaluate the influence of high iodine intake in ewes on haematological and biochemical parameters of the blood of ewes and their lambs. Twelve pregnant ewes of the Sumava sheep breed and their newborn lambs were included in the experiment. Control group (A) consisted of 6 ewes with 7 lambs and experimental group (B) comprised 6 ewes with 6 lambs. The feed ration was enriched with calcium iodate by addition of 3 and 5 mg/kg in group A and group B, respectively. The studied parameters in ewes and lambs were haematocrit value, red blood cell count and haemoglobin concentration in blood, concentration of urea and total proteins, and alkaline phosphatase activity in blood plasma. No differences were found out in haematocrit value, red blood cell count, concentration of haemoglobin and total proteins between groups of ewes A and B with their lambs. Urea concentration and alkaline phosphatase activity were higher in ewes of group B and their lambs during the entire experimental period. An increase in the values of urea and alkaline phosphatase in the group of ewes and lambs with higher iodine intake indicates a potential risk of high iodine intake associated with changes in the thyroid activity in ewes and their lambs.

Keywords: calcium iodate, ewes, lambs, urea, alkaline phosphatase, total protein

INTRODUCTION

Iodine is an indispensable component of thyroidal hormones through which it is involved in a number of biological functions of the organism (Miller, 2006; Ares *et al.*, 2008). The thyroid hormones participate in the maintenance of protein and energetic metabolism homeostasis, they influence growth, thermoregulation, reproduction, etc. (Huszenica *et al.*, 2002). The low concentration of iodine in domestic feeds of Central and Eastern Europe causes a long-term iodine deficiency in farm animals and consumers of their products. In the last decades such deficiency has been successfully solved by administration of iodine-containing additives (Schöne and Rajendram, 2009). In accordance with NRC (2001) the recommended iodine content in a feed ration for sheep and cows is only 0.5 mg/kg.

Nevertheless, according to the EU standard (2005) the maximum level of iodine supplementation in ruminants amounts to 5 mg/kg of 88% dietary dry matter (DM). Both iodine deficiency and surplus have an undesirable influence on the growth and development of animals, mainly of the young (Bürgi, 2010). An excessive amount of iodine in farm animals may cause iodism that is manifested by anorexia, lacrimation, breathing and reproduction disorders, hyperthermia, hypoglycaemia, and a decrease in milk production (Huszenica *et al.*, 2002), decrease in feed intake leading to a subsequent drop in weight gains (Herzig and Suchý, 1996), lower concentration of haemoglobin in blood and iron in liver (Kirschmann, 1996). Consequences of excessive iodine in the human population are hyperthyroidism, hypothyroidism,

thyroid enlargement (Namba *et al.*, 1993; Sang *et al.*, 2012) and autoimmune thyroiditis (Miller, 2006; Bürgi, 2010). In hyperthyreosis increased calcium concentration in blood is determined (Brunová, 2008). In hypothyreosis an increased level of urea, creatinine and total cholesterol is found out in blood (Vlček, 2010). According to Límanová *et al.* (2008), not only hypothyreosis but also hyperthyreosis may be accompanied by anaemia. Excessive iodine contributes to a reduction in immunity functions (Boland *et al.*, 2005; Venturi and Venturi, 2009).

The objective of the study was to evaluate the influence of high nutritional intake of iodine in ewes (above the upper limit of the permitted EU standard, 2005, of 5 mg/kg of 88% dietary dry matter) on some haematological and biochemical blood parameters of ewes and their lambs.

MATERIALS AND METHODS

Twelve pregnant ewes of the Sumava sheep breed weighing 53–60 kg and their newborn lambs ($n = 13$) were included in the experiment. Environmental conditions before and during experimentation were similar. Before the experiment the ewes were divided into two groups. Group A (control) comprised 6 ewes and their 7 lambs (3 females and 4 males) and group B (experimental) consisted of 6 ewes and their 6 lambs (4 females and 2 males). Feed of group A was supplemented with calcium iodate at 3 mg iodine per kg of dietary dry matter (DM). The diet of Group B was enriched with calcium iodate at 5 mg iodine per kg of DM. Lambs were fed only maternal milk. The formulation of the daily feed ration of ewes in the 2-month period before the experiment was identical in the groups. In the experimental period (from last 1–2 months of gestation to 120–140 days after parturition) the feed ration differed only in iodine content of the mineral supplement. The content of iodine in the basal diet of ewes was 0.10 mg/kg of dietary DM. Water was supplied *ad libitum* to all sheep. The formulation of feed ration during the experiment is shown in Tab. I.

Blood samples were taken from ewes 30 and 60 days before the beginning of the experiment (0th–1st month of gravidity), 30–50 days *ante partum* and on day 1, 10, 30 and 60 *post partum* (days 180–240 of experiment). Blood samples were taken from lambs on day 1, 3, 10, 30 and 60 day of age. Blood samples were collected from ewes and lambs between 07.00 and 09.00 from the *vena jugularis* into heparin tubes for assessment of urea, alkaline phosphatase (ALP) and total proteins (TP) from blood plasma. Whole blood (taken into heparin tubes) was used for evaluation of haematocrit, red blood cell count and haemoglobin concentration. To evaluate haematocrit (l/l), and to determine red blood cell count (T/l) and haemoglobin (g/l) an Alvet 2000 analyser of the Dialab, spol. s r. o. company was used. An Ellipse biochemical analyser of the Dialab, spol. s r. o. company was used to determine urea concentration (mmol/l), activity of alkaline

phosphatase ($\mu\text{kat/l}$) and total protein concentration (g/l) in blood plasma.

The experiment was conducted in accordance with principles of the Animal Cruelty Commission at the Agricultural Faculty of the University of South Bohemia in České Budějovice.

I: The formulation of daily feed ration in the experimental period of control and experimental groups of ewes

Dietary component	Value
meadow hay	1 500 g
oat groats	270 g
lucerne granules	240 g
mineral supplement ^b	9 g

^bsupplied per kg dry matter of diet: 3 mg of I in Group A, 5 mg of I in Group B, 20.0 mg of Co, 6 000 mg of Zn, 30.0 mg of Se, 1 000 mg of Cu, 5 100 mg of Mn, 900 000 I.U. of vit. A, 110 000 I.U. of vit. D3, 1 500 mg of vit. E

Statistical analysis

Data were analysed by the Statistica 6.0 Cz software. Tukey's tests were done at a 95% significance level. Results are expressed as mean values and standard deviations (SD).

RESULTS AND DISCUSSION

Haematological and biochemical parameters of blood in farm animals are different in relation to gender, age, nutrition, genetics (breed), climatic conditions, stress, gravidity period, rearing conditions and physical load (Balıkcı *et al.*, 2007; Tripathi *et al.*, 2008). The present study was conducted to investigate a potential influence of high iodine intake in ewes on haematological and biochemical parameters of the blood of ewes and their newborn lambs.

Before the experiment (Tab. II) there was no significant difference between groups of ewes A and B in the values of the studied parameters that were consistent with literature data.

There was no significant difference between groups of ewes A and B in haematocrit value, red blood cell (RBC) count and haemoglobin concentration in blood during the experiment before parturition (Tab. III). The highest haematocrit value was measured on day 10 *post partum* in ewes of both groups and the lowest value was found out on day 30 in ewes of group A and on day 60 *post partum* in ewes of group B. The haematocrit values of our experimental ewes were lower than those reported by Antunović *et al.* (2011b) in Tsigai ewes, by Polijčák-Milas *et al.* (2009) in red deer and Herselman (2011) in sheep. No significant differences in haematocrit value and RBC count were found out between ewes of group A and B during the postpartal period. RBC count in the blood of ewes of group A and B was pronouncedly lower during the entire experiment than that reported by Antunović *et al.* (2011b) in Tsigai ewes but it was higher than in Soliman *et al.*

(2012) in lactating ewes and comparable with data of Mahmoud *et al.* (1999) in sheep. Haemoglobin concentration in the blood of ewes of group B was significantly ($P < 0.01$) higher on day 1 and 10 *post partum* in comparison with ewes of group A. According to Jain (1993) and Azab and Abdel-Maksoud (1999) RBC count and haemoglobin concentration are decreasing during gravidity and they remain low within several weeks after parturition. In our experiment there was a decrease before parturition, then the values fluctuated. On day 10 *post partum*, especially haemoglobin concentration increased insignificantly in ewes of group B while it decreased ($P < 0.05$) on day 60. In this study, the levels of haemoglobin in the blood of ewes were higher during the entire experiment than the values reported by Antunović *et al.* (2011b) in Tsigai ewes and Kiran *et al.* (2012) in sheep. Lower haemoglobin concentration was also measured by Trávníček *et al.* (2001) and Soliman *et al.* (2012) in lactating ewes. Rajendran *et al.* (2001) studied the influence of iodine at an amount of 0.407 mg/kg of dietary dry matter on haemoglobin concentration in goats; these authors did not find out any significant changes in its concentration. The highest values of haemoglobin were measured in both groups on day 10 *post partum* while they were markedly higher at higher iodine intake. This finding excludes the negative impact of high iodine intake on haemoglobin concentration in blood as reported by Kirschmann (1996). Plasma urea concentration was significantly higher ($P < 0.01$) in ewes of group B *ante* and *post partum* compared to ewes of group A while its highest concentration during the entire experimental period was measured on day 10 in ewes of group B. The finding of increased uraemia at highly excessive iodine intake indicates potential risks of the kidney activity at high iodine intake, which is consistent with data of Vlček (2010). On the contrary Taghipour *et al.* (2010), Antunović *et al.* (2011a, b) and Deghnouche *et al.* (2013) reported the lower urea concentration in lactating ewes. During the entire experiment alkaline phosphatase (ALP) activity was higher ($P < 0.01$) in B ewes than in A ewes and reached its maximum on day 60 *post*

partum. Compared to data of Ouanes *et al.* (2011) and Gwaze *et al.* (2012) our results of ewes were higher. Rajendran *et al.* (2001) did not reveal any significant influence of supplementation of 0.407 mg iodine per kg of dietary dry matter on ALP concentration in goats. As the increased activity of ALP is associated with changes in the thyroid activity (Brunová, 2008), it is necessary to pay greater attention to functional risks of excessive iodine intake. Total protein concentration in blood plasma was not significantly different between group A and B during the entire experiment. This finding was contradictory to Trávníček *et al.* (2001), who found out a lower concentration of total protein in ewes supplemented with iodine and selenium. Rajendran *et al.* (2001) concluded in their experiment with goats that the level of total protein was not influenced by supplementation of 0.407 mg iodine per kg dry matter. Compared to data of Taghipour *et al.* (2010) and Soliman *et al.* (2012), total protein concentration after parturition was similar in the studied ewes of both groups. However Antunović *et al.* (2011a) and Bonev *et al.* (2012) reported a higher concentration of total proteins in lactating ewes.

The study of the blood parameters of lambs born to ewes with high iodine intake did not show a significant difference between the groups A and B (during days of sampling) in the level of their haematocrit in blood after birth (Fig. 1). Haematocrit increased significantly in both groups only on day 30 after birth. Comparable values of haematocrit in newborn lambs were reported by Bórnez *et al.* (2009) and Lipecka *et al.* (2010). According to Sarwar *et al.* (2011) a decrease in haematocrit value can be expected in subsequent months.

RBC count (Fig. 2) in lambs of both groups after birth was at a lower level until day 10 if compared with day 30 and 60, when it increased significantly ($P < 0.05$) in both groups of lambs and when group B of lambs had an insignificantly higher RBC count during the entire experiment. Similar results like in our experimental lambs were reported by Faixová *et al.* (2007). On the other hand Soliman *et al.* (2012) and Ocal *et al.* (2013) reported a higher RBC count in lambs compared to the presented results.

II: Haematological and biochemical parameters in the blood of ewes before the beginning of experiment – without iodine supplementation

Variable	Before experiment*		Reference range	Authors
	A	B		
Haematocrit (l/l), blood	0.38 ± 0.02	0.35 ± 0.06	0.3–0.5	(Doubek <i>et al.</i> , 2007)
RBC (T/l), blood	9.4 ± 0.4	9.2 ± 1.2	9–15	(Kramer, 2000)
Haemoglobin (g/l), blood	133.0 ± 8.4	127.5 ± 23.5	90–150	(Kramer, 2000)
Urea (mmol/l), blood plasma	7.0 ± 0.4	6.9 ± 0.3	2.9–7.1	(Kaneko <i>et al.</i> , 1997)
ALP (µkat/l), blood plasma	1.24 ± 0.15	1.45 ± 0.26	1.1–6.5	(Doubek <i>et al.</i> , 2007)
TP (g/l), blood plasma	71.4 ± 3.2	68.9 ± 5.7	60–79	(Kaneko <i>et al.</i> , 1997)

* mean from 2 consumptions

RBC – red blood cell

ALP – alkaline phosphatase

TP – total protein

Data expressed as mean ± SD (group A, n = 12, group B, n = 12)

III: Haematological and biochemical parameters in the blood of ewes at iodine supplementation

Variable	Days after parturition									
	Before parturition		1		10		30		60	
	A	B	A	B	A	B	A	B	A	B
Haematocrit ^{1*}	0.35±0.02	0.35±0.06	0.37±0.03	0.34±0.04	0.38±0.01	0.39±0.01	0.32±0.03	0.37±0.01	0.35±0.01	0.33±0.03
RBC ^{2*}	6.8±0.6	7.5±1.2	7.2±0.5	6.8±0.7	7.4±0.7	7.8±0.4	7.2±0.5	7.5±0.5	7.1±0.6	6.7±0.6
Haemoglobin ^{3*}	120.3±9.5	126.5±23.5	129.3±5.7 ^a	142.8±9.1 ^b	151.3±6.2 ^c	177.5±7.8 ^d	157.8±11.3	151.3±11.1	139.0±9.5	135.5±19.5 ^e
Urea ^{4**}	7.6±0.6 ^f	8.7±1.1 ^g	7.4±1.7 ^h	8.9±1.9 ^{ch}	8.6±1.3 ⁱ	9.8±2.1 ^j	6.1±1.3 ^k	7.8±1.6 ^l	5.7±1.3 ^m	7.0±1.5 ⁿ
ALP ^{5**}	0.97±0.12 ^o	1.36±0.22 ^p	0.92±0.11 ^q	1.62±0.18 ^r	1.28±0.19 ^s	2.09±0.15 ^t	1.52±0.22 ^u	2.75±0.24 ^v	1.48±0.11 ^w	2.81±0.36 ^x
TP ^{6**}	68.4±2.3	69.6±0.7	63.5±3.9	65.5±1.2	70.0±4.9	70.5±3.3	67.9±2.9	62.4±2.4	65.1±4.6	69.1±5.4

ab, cd, ef, gh, ij, kl, mn; op, qr, st, uv, wx; P < 0.01

cd, ef, gh, ij, mn; P < 0.05

¹(l/l), ²(T/l), ³(g/l), ⁴(mmol/l), ⁵(μkat/l), ⁶blood, ^{**}blood plasma

RBC – red blood cell, ALP – alkaline phosphatase, TP – total protein

A – control group supplementation 3 mg I/kg DM of diet

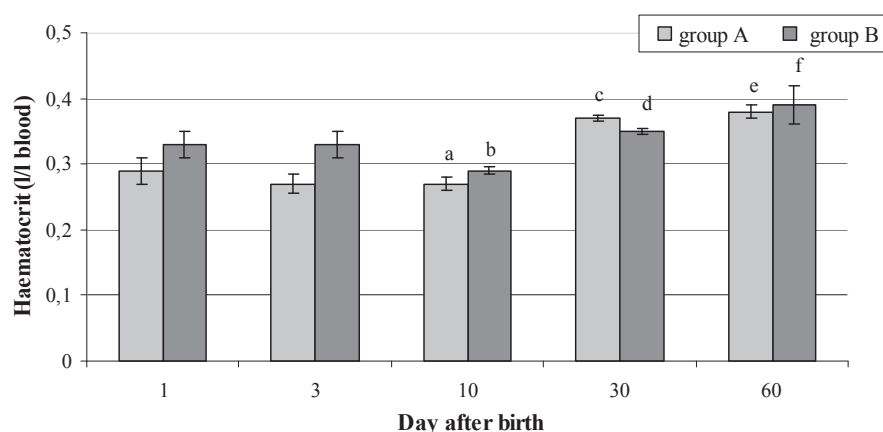
B – experimental group supplementation 5 mg I/kg DM of diet

Data expressed as mean ± SD (group A, n = 6, group B, n = 6)

Haemoglobin concentration (Fig. 3) was significantly higher ($P < 0.05$) only on day 1 after birth. A significant increase in haemoglobin concentration ($P < 0.01$ – in comparison with day 10) was measured in both groups only on day 30 and 60 after birth. A similar postnatal concentration of haemoglobin in lambs connected with increasing age was also found out by Egbe-Nwiyi *et al.* (2000). A lower haemoglobin concentration in lambs was reported by Nour El-Din *et al.* (2009), Soliman *et al.* (2012) and Ocal *et al.* (2013). Urea concentration (Fig. 4) in the blood plasma of lambs of group B after birth was higher during the entire experiment compared to lambs of group A. This difference was statistically significant ($P < 0.01$) only on day 1 after birth, when the highest level of 11.40 mmol/l over the entire period of observation was reached in group B, which was followed by a significant decrease in both groups of lambs. Compared to Keçeci (2003) and Ouanes *et al.* (2001), urea concentration in lambs is higher in the present paper. On the contrary, Bórmes *et al.* (2009) found out a lower urea concentration in lambs. This difference can be explained by the relation of urea concentration in milk to the intake of nitrogenous compounds in ewes. Alkaline phosphatase activity (Fig. 5) was higher in group B of lambs than in group A of lambs but it was statistically significant ($P < 0.05$) only until day 3 after birth. It gradually decreased in both groups from day 1 to day 60 to the value of 6.60 μkat/l ($P < 0.05$) on day 60 after birth in group A. The ALP activity of our lambs was higher than the values reported by Faixová *et al.* (2007), Ouanes *et al.* (2011) in lambs of mothers without increased iodine supplementation. Total protein concentration (Fig. 6) was significantly higher in group A of lambs from day 1 to day 10 after birth, and from day 30 after birth it decreased to the values measured in group B. Nevertheless, an insignificant decrease in the concentration subsequently occurred in lambs of group A. The statistically significant transitorily higher level of total protein in lambs of ewes receiving 3.1 mg iodine/kg of dietary dry matter was comparable until day 10 after birth with data of Eryavuz *et al.* (2003) and Ouanes *et al.* (2011).

CONCLUSION

The present study did not demonstrate a significant influence of iodine supplementation on haematocrit level, RBC count, haemoglobin and total protein concentration in ewes and their newborn lambs. During the entire experiment urea concentration and alkaline phosphatase activity were significantly higher in ewes of the experimental group (with the intake of 5.1 mg iodine per kg of dietary dry matter) than in ewes of the control group (with the iodine intake of 3.1 mg iodine per kg of dietary dry matter). Changes in the values of urea and alkaline phosphatase in lambs were similar, but they were not significant for the entire postnatal period. Changes in urea concentration were consistent with the results (significantly higher values of TSH in



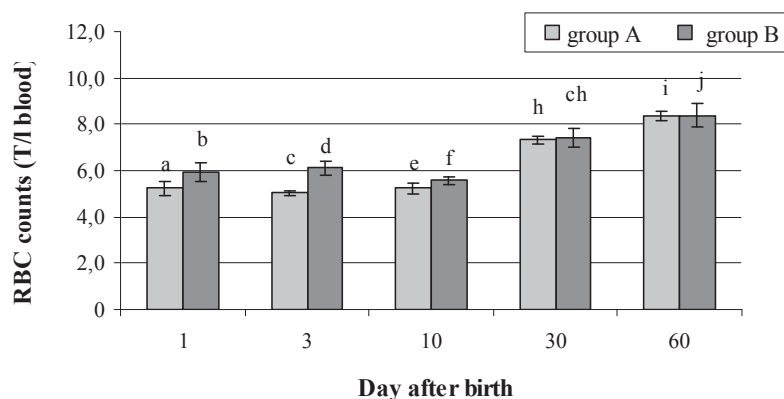
1: Haematocrit values in the blood of lambs after birth

a,b,c,d,e,f p < 0.05

Data expressed as mean \pm SD (control group A, n = 7, experimental group B, n = 6)

A – lambs from group A – supplementation of their mothers 3 mg I/kg DM of diet

B – lambs from group B – supplementation of their mothers 5 mg I/kg DM of diet



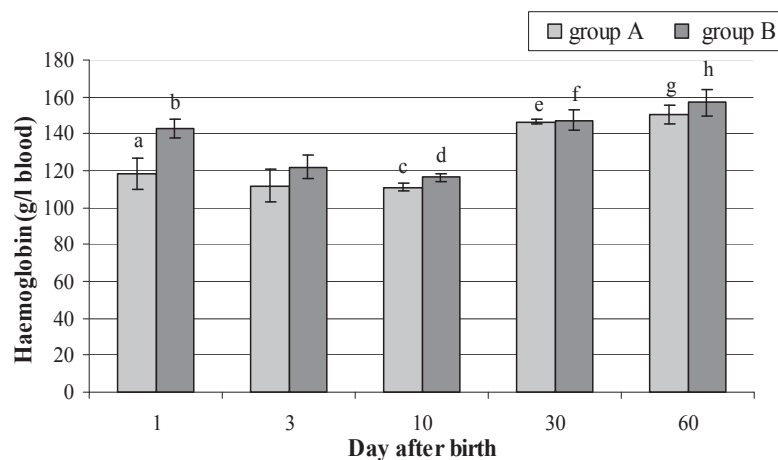
2: Red blood cell (RBC) counts in the blood of lambs after birth

a,b,c,d,e,f,h,i,j p < 0.05

Data expressed as mean \pm SD (control group A, n = 7, experimental group B, n = 6)

A – lambs from group A – supplementation of their mothers 3 mg I/kg DM of diet

B – lambs from group B – supplementation of their mothers 5 mg I/kg DM of diet



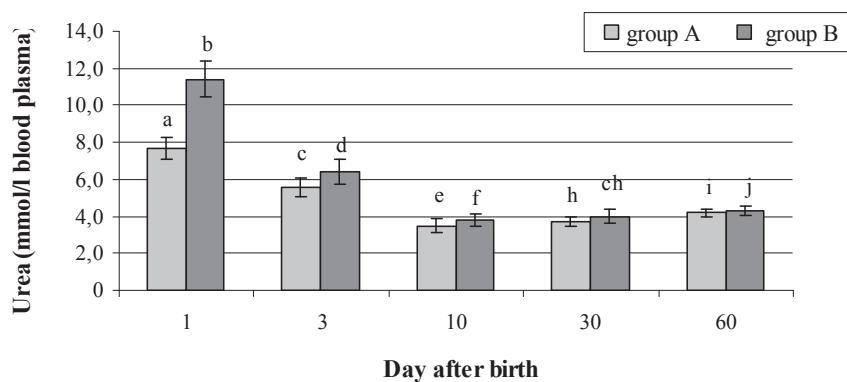
3: Haemoglobin concentrations in blood of lambs after birth

a,b p < 0.05; c,d,e,f,g,h p < 0.01

Data expressed as mean \pm SD (control group A, n = 7, experimental group B, n = 6)

A – lambs from group A – supplementation of their mothers 3 mg I/kg DM of diet

B – lambs from group B – supplementation of their mothers 5 mg I/kg DM of diet



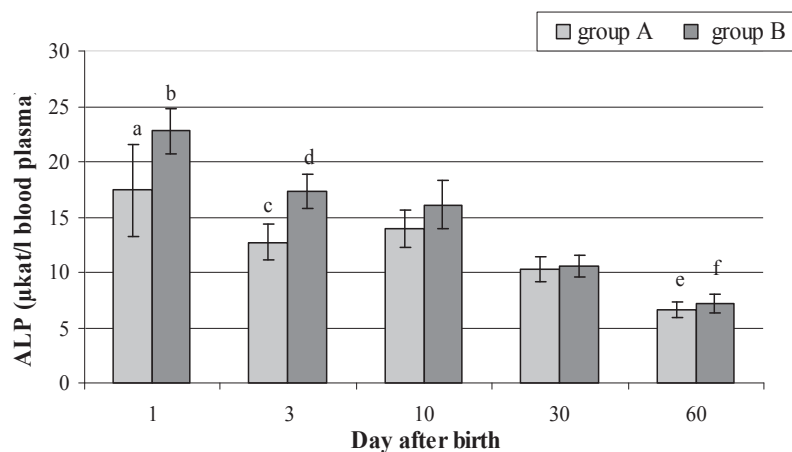
4: Urea concentrations in the blood plasma of lambs after birth

ab;cd,ef,h,eh,i,j P<0.05; bc,d,ef,h,eh,i,j P<0.01

Data expressed as mean \pm SD (control group A, n = 7, experimental group B, n = 6)

A – lambs from group A – supplementation of their mothers 3 mg I/kg DM of diet

B – lambs from group B – supplementation of their mothers 5 mg I/kg DM of diet



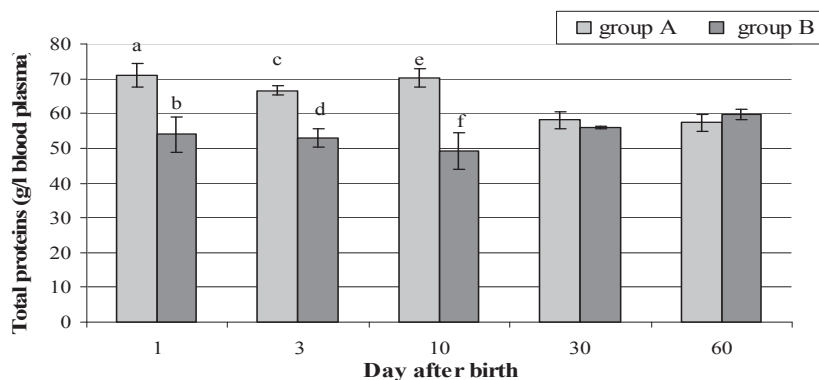
5: Alkaline phosphatase (ALP) concentrations in the blood plasma of lambs after birth

ab;cd,ef;cd,ef P<0.05

Data expressed as mean \pm SD (control group A, n = 7, experimental group B, n = 6)

A – lambs from group A – supplementation of their mothers 3 mg I/kg DM of diet

B – lambs from group B – supplementation of their mothers 5 mg I/kg DM of diet



6: Total protein concentrations in the blood plasma of lambs after birth

ab;cd;ef P<0.01

Data expressed as mean \pm SE (control group A, n = 7, experimental group B, n = 6)

A – lambs from group A – supplementation of their mothers 3 mg I/kg DM of diet

B – lambs from group B – supplementation of their mothers 5 mg I/kg DM of diet

lambs of mothers with higher iodine intake). The increased activity of alkaline phosphatase in ewes and lambs (in the group with higher iodine intake) is associated with a risk of a change in the thyroid activity.

Higher urea concentration and higher alkaline phosphatase activity in ewes and lambs with iodine intake above the upper limit of the permitted standard (EU standard, 2005) may indicate a potential risk of changes in the thyroid activity.

SUMMARY

Iodine is an essential element for the formation of thyroxine and triiodothyronine controlling nutrient metabolism. The objective of the study was to evaluate the influence of high nutritional intake of iodine in ewes (above the upper limit of the permitted EU standard, 2005, of 5 mg/kg of 88% of dietary dry matter) on some haematological and biochemical parameters of the blood of ewes and their lambs. Gravid ewes of the Sumava sheep breed were divided into two groups. Experimental group B comprised 6 ewes and 6 newborn lambs (4 females and 2 males). Control group A consisted of 6 ewes and 7 newborn lambs (3 females and 4 males). A kilogram of dietary dry matter contained 3.1 mg iodine in ewes of group A while ewes of group B were supplemented with 5.1 mg iodine as calcium iodate. Lambs were fed only maternal milk. Water was supplied *ad libitum* to all sheep. Blood samples were taken from ewes 30 and 60 days before the beginning of experiment, 30–50 days *ante partum* and on day 1, 10, 30 and 60 *post partum* and in lambs on day 1, 3, 10, 30 and 60 days of age. The influence of iodine was studied by means of changes in some blood parameters (haematocrit level, red blood cell count and haemoglobin concentration in blood, urea concentration, alkaline phosphatase activity and total protein concentration in blood plasma). There were no differences in haematocrit level, red blood cell count, haemoglobin and total protein concentration between the groups of ewes A and B and their lambs during the entire experimental period, i.e. these parameters were not influenced by iodine supplementation. Urea concentration and alkaline phosphatase activity in the blood plasma were higher in ewes of group B and their lambs during the entire experimental period. An increase in the values of urea and alkaline phosphatase in the group of ewes and lambs with higher iodine supplementation indicates a potential risk of high iodine intake associated with changes in the thyroid activity in ewes and their lambs.

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