Iodine status in ewes with the intake of iodine enriched alga *Chlorella*

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ABSTRACT: The effect of increased intake of iodine at different selenium intake was studied in three groups of lambing ewes consisting of five animals and in their lambs for 76 days (from day 4 to day 80 *post partum*). Iodine in blood plasma, milk and urine was determined by a modified method according to Sandell-Kolthoff. Mineral supplement contained iodine and selenium in the form of I- or Se-enriched alga *Chlorella*. The content of iodine and selenium per 1 kg DM of experimental diet was as follows: group G1 0.7 mg I and 0.2 mg Se, group G2 0.7 mg I and 0.4 mg Se, group G3 1.3 mg I and 0.4 mg Se. The increased intake of iodine was not accompanied by an iodine increase in blood plasma until day 60 of lactation in connection with its high excretion into milk. The highest iodine content in milk was recorded on day 20 to 30 of lactation while there was a drop on day 60 of lactation. Iodine content in the blood plasma of lambs reflected iodine content in the milk of their mothers. The highest content of iodine in milk, blood plasma and urine was in the group with its highest intake (G3). Lower urinary output of iodine and higher iodine output in milk in lambing ewes of group G2 compared to group G1 document the higher retention and utilization of iodine in ewes with a higher supply of selenium. The average content of iodine in milk in group G1, G2 and G3 was as follows: 724.2 ± 485.3; 885.9 ± 460.6; and 1 126 ± 262.5 μg/l.

Keywords: organically bound iodine; organically bound selenium; milk; blood plasma; urine; ewes; lambs

General supplementation of iodine in humans and farm animals, mainly in inland areas of all continents, requires experimental research on its supplementary sources. The majority of the papers dealing with iodine supplementation in farm animals accentuate health and hygienic aspects connected with a subsequent content of iodine in foods from animal sources (Herzig et al., 1999; Kursa et al., 2005; Trávníček and Kursa, 2001). E.g. in dairy cows the alimentary intake of iodine should not reach the level when its content in milk exceeds 500 μg/l (Berg et al., 1988, Kursa et al., 2005). Anke et al. (1994a), Kaufmann et al. (1998) and others consider iodine excretion through milk in the range of 80 to 200 or 250 μg/l to be the evidence of optimum saturation of cows with iodine even in the status of higher requirements and concurrently. The required daily iodine intake in humans may be covered by its milk content 200 μg iodine per litre (Stránský and Ryšavá, 1997). A chronic surplus of iodine is considered as undesirable (Anke, 2007) and is associated not only with the increased occurrence of thyrotoxicosis (Swanson et al., 1990) and chronic thyroiditis but also with hypothyroidism

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(Baker, 2004) and restriction of iodine transport (Anke et al., 1994b). Selenium is indispensable for iodine metabolism. In the form of selenoenzymes – deiodases it transforms thyroxine into active triidothyronine, while the effect of Se surplus on the activity of selenoenzymes is not negligible (Bratter, 1996).

In gravid animals the daily intake of iodine should amount to 0.2 mg/kg feed DM, in lactating individuals to 0.3–0.8 mg/kg feed DM (Anke, 2004). In conditions of the Czech Republic the standardised need of iodine is 0.3 for gravid ewes and 0.4 mg I/kg feed DM for lactating ewes (Sommer et al., 2004).

Mineral supplements for farm animals contain iodine in an inorganic form (KI, KIO_3, $CaIO_3$) most frequently. Greater attention is also paid to its organic compounds, e.g. ethylenediamine dihydroiodide (EDDI), iodine on an oil base – iodinated fatty acid esters (Herzig et al., 2000), and to the iodine-enriched alga *Chlorella* (Kotrbáček et al., 2004). Organic compounds of iodine are characterized by stable biological activity in animals. According to Swanson et al. (1990) the utilization of iodine from EDDI is comparable with inorganic forms of iodine but its retention in tissues of lactating cows is higher. In experiments with feeder pigs the utilization of iodine from KI amounted to 93.6% and from EDDI to 92.6% (Herzig et al., 2000). A positive effect of supplementing an organic form of selenium (Se-enriched alga *Chlorella*) on its content in milk and on glutathione peroxidase (GSH-Px) activity was proved in sheep (Trávníček et al., 2008).

Higher tolerance to superfluous intake of iodine and/or to intoxication was observed in organic compounds of iodine (EDDI) compared to inorganic compounds – calcium iodate, potassium iodate (Miller and Swanson, 1973). According to Pauliková et al. (2002) sheep are more resistant to iodine surplus compared to cattle.

The content of iodine in milk and urine is a reliable indicator of iodine saturation. Milk and urinary output of iodine correlates with its content in the blood plasma and with its intake (Anke, 2004). Almost 90% of the iodine surplus in non-lactating individuals is excreted with urine (Lamberg, 1993). The concentration of 70–100 μg/l is considered as the limit value of iodine content in ewe's milk (Underwood and Suttle, 2001) while the respective limit value for urine is 100–150 μg/l (Ferri et al., 2003). With the intake of 0.35–0.43 mg iodine per 1 kg feed DM the content of iodine in the blood serum of sheep was 40.0 ± 7.7 μg/l (Anke, 2004).

The importance of iodine supplementation in operational conditions was demonstrated by the results of a monitoring conducted in Southern Bohemia in 1998–1999. In sheep flocks with supplementary intake of iodine from mineral licks the average iodine content in ewe's milk was 243.3 ± 87.2 μg I/l while in flocks without supplementary intake it was only 47.9 ± 87.2 μg I/l (Trávníček and Kursa, 2001). After a single injection application of 1 ml of the product Lipiodol containing 480 mg I in the form of iodinated fatty acid esters the iodine content in ewe's milk increased four times compared to the control group and amounted to 2 393 μg I/l on average, and the iodine content in urine was higher than 300 μg I/l for three months (Ferri et al., 2003).

The objective of the present paper was to evaluate the effect of an increased supply of organic form of iodine to lambing ewes at different intake of organically bound selenium on iodine content in blood plasma, milk and urine of lambing ewes and in the blood plasma of their lambs. Iodine and selenium were administered in iodine-enriched algae of the genus *Chlorella*.

MATERIAL AND METHODS

Fifteen pregnant ewes of the Šumava sheep breed were included in an experiment. The ewes were divided into three groups, each comprising five animals: G1, G2 and G3. Female sheep were stabled in groups with 1.6 m^2 of area and 380 mm of feeding trough falling on 1 sheep. The formulation of feed ration in the period before the experiment (period of ewe gravidity and the first 3 days of lactation) was identical for all groups. In the experimental period (days of lactation 4 to 80) the feed ration for the particular groups differed only in iodine and selenium content in the mineral supplement. The formulation of feed ration in the experimental period: 1 500 g meadow hay, 240 g lucerne granules, 270 g oat groats, 9 g mineral supplement. The mineral supplement contained per 1 kg DM 109.4 mg iodine and 27.0 mg selenium for group G1, 109.4 mg iodine and 54.3 mg selenium for group G2, and 211.1 mg iodine and 54.3 mg selenium for group G3. The mineral supplement contained iodine and selenium in the form of I- and Se-enriched alga *Chlorella*, respectively. The content of iodine and selenium per 1 kg DM of experimental diet was as follows: group G1 0.7 mg

Table 1. The average composition of daily feeding ration per ewe with different intake of iodine and selenium

Group	Before experiment			Experiment						
	G1,G2,G3			G1,G2, G3	G1		G2		G3	
Component of feed ration	DM* (kg)	I (mg)	Se (mg)	DM* (kg)	I (mg)	Se (mg)	I (mg)	Se (mg)	I (mg)	Se (mg)
Bulky and grain feed	1.3	0.2	0.1	1.7	0.3	0.1	0.3	0.1	0.3	0.1
Mineral mixture				0.009	1.0	0.2	1.0	0.5	1.9	0.5
Total content		0.2	0.1		1.3	0.3	1.2	0,6	2.2	0.6
Content (mg/kg DM*)		0.15	0.08		0.7	0.2	0.7	0.4	1.3	0.4

*DM – dry matter

Table 2. Live weight of ewes and their lambs and reproductive parameters of ewes

Group (number of ewes = 5)	G1	G2	G3
Order of lactation	4	4	4
Mean weight of ewes at the beginning of experiment – day 1 *post partum* (kg)	54.5	56.0	53.0
Number of lambs born per ewe	1	1	1
Net natality	1	1	1
Lamb sex – males	4	3	3
Lamb sex – females	1	2	2
Mean birth weight of lambs	4.7 ± 0.4	4.5 ± 0.7	4.6 ± 0.5
Mean weight of lambs on experimental day 60 (kg)	15.4 ± 1.8	15.2 ± 2.1	15.7 ± 1.5
Net natality	1	1	1

I and 0.2 mg Se, group G2 0.7 mg I and 0.4 mg Se, group G3 1.3 mg I and 0.4 mg Se. Table 1 shows the total iodine (and selenium) intake in the bulky and concentrate component of feed ration and mineral supplement in the experimental groups. Table 2 lists the basic characteristics for female sheep and their lambs.

The technology of controlled culture in solar bioreactors of Microbiological Institute of the Academy of Sciences of the CR in Třeboň according to Doucha's and Livansky's patent (1999) was used for the production of algal biomass.

Iodine content in lambing ewes was determined in blood plasma, milk and urine, in lambs it was measured in blood plasma. The milk was extracted by draining the teats, blood was taken from the *v. jugularis* and urine by catheterizing the bladder. Iodine in blood plasma, milk and urine was deter-

Table 3. Average iodine content in the blood plasma of ewes (μg/l)

Group	Before experiment			Experiment		
	n	$\bar{x}$	SD	*n*	$\bar{x}$	SD
G1	5	81.9	5.6	25	89.6[a]	38.9
G2	5	85.4	12.5	25	108.0	72.1
G3	5	83.1	9.6	25	123.9[b]	79.9

[a,b]$P < 0.05$

mined on the basis of alkaline ashing by a spectrophotometric method according to Sandell-Kolthoff (Bednář et al., 1964). The principle of the determination is a reduction of Ce^{4+} to Ce^{3+} in the presence of As^{3+} due to the catalytic effect of iodine. Dry mineralisation takes place in the alkaline environment at 600°C. The above-described method was used to determine total iodine (inorganically and organically bound iodine to proteins). Blood samples were taken in lambing ewes on the day of parturition (within 24 hours *post partum*), on day 10, 30, 60 and 80 *post partum*; milk was sampled on day of lactation 1–3, 10, 20–30 and 60. Milk was extracted from several sheep repeatedly between the 20th and 30th day so that the number of samples analyzed in the experimental period differed from the presumed number of 15 (G1 group shows 16 samples, G2 shows 18 and G3 shows 19).

Urine samples were taken in ewes on day 60 *post partum*. Blood samples in lambs were taken on the day of birth and on day 3, 10, 30 and 60 of age. The feeding of experimental diet started on day 4 *post partum* and lasted for 76 days. Neither infectious nor metabolic disease occurred in female sheep and in their lambs during the experiment. The colostrum intake related problems arose in one of the G2 group lambs when the experiment began, so this lamb was excluded from the subsequent monitoring process. The actual status in a number of analyzed samples of the lamb blood plasma in the experimental period is shown in Table 6.

Statistical processing of data included the calculation of mean values (x), standard deviations (SD), minimum and maximum values and medians; statistical significance was determined by ANOVA – Tukey's test.

RESULTS AND DISCUSSION

Table 1 shows the content of iodine and selenium in the feed ration of lambing ewes. Through the intake of 9 g mineral mixture the content of iodine per 1 kg feed DM amounted to 0.7 mg I/kg DM in lambing ewes of group G1 and G2, which is by 75% more than recommended for lactating ewes by Sommer et al. (1994) and by 133% more than suggested in Underwood and Suttle (2001). The feed ration of group G3 ewes contained 1.3 mg I/kg DM, i.e. approximately 3.3 times more than is the recommended intake (Sommer et al., 1994). The content of Se in group G1 was 0.2 mg/kg DM and it corresponds to the recommended amount (Sommer et al., 1994; Schenkel and Flachowsky, 2000), in group G2 and G3 it was 0.4 mg/kg, i.e. twice as much as in group G1.

Iodine content in the blood plasma and colostrum of lambing ewes (Tables 3 and 4) in the period before the start of supplementation (day 1–3 *post partum*) corresponded to the values indicating a sufficient supply of iodine to the ewes (Anke, 2004; Underwood and Suttle, 2001). The supplementation of iodine (from day 4 *post partum*) was not accompanied in any group by its increase in blood plasma until day 60 of lactation (Figure 1). The average plasma concentration of iodine ranged

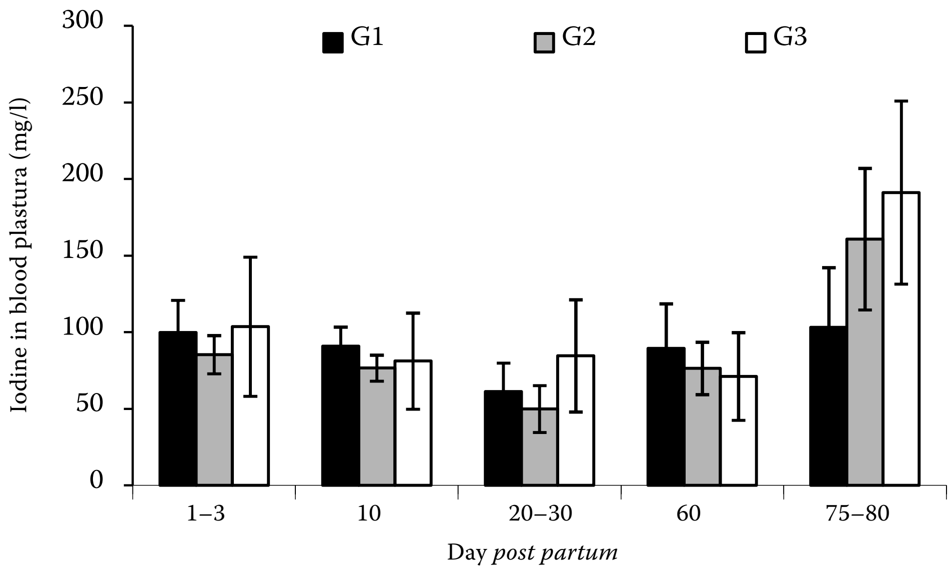

Figure 1. Iodine content in the blood plasma of lambs (μg/l)

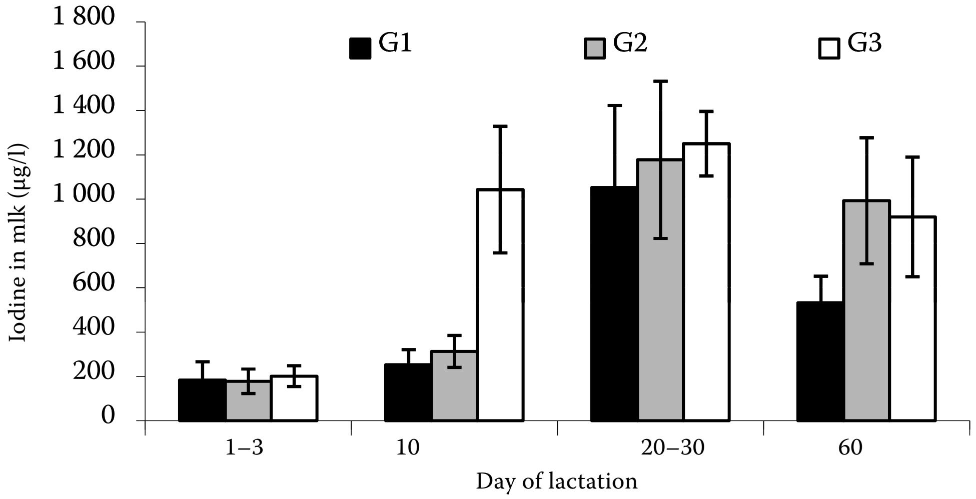

Figure 2. Iodine content in the milk of ewes

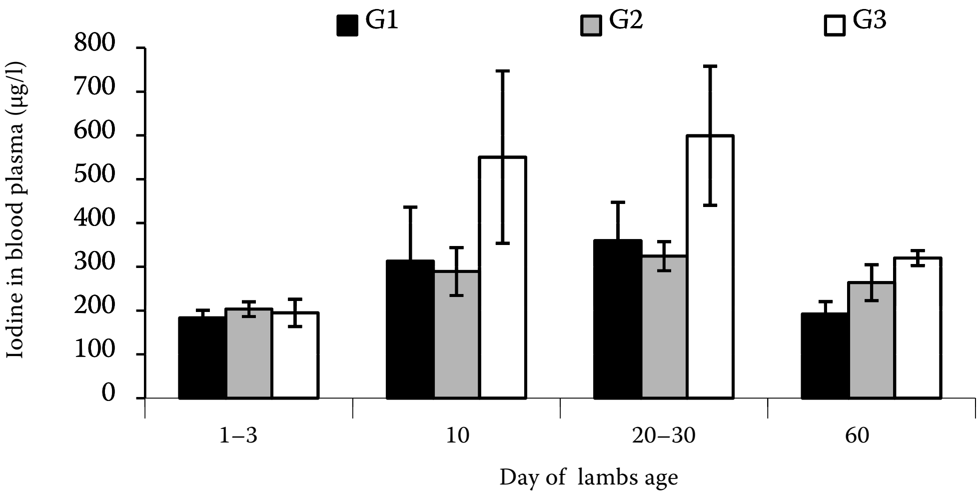

Figure 3. Iodine content in the blood plasma of lambs (μg/l)

from 49.9 ± 15.3 to 90.8 ± 12.7 μg/l in this period. Between day 20 and day 30 *post partum* a decrease in the plasma content of iodine was observed accompanied by an increase in its concentration in milk (Figure 2), which documented a large removal of iodine from blood to mammary gland and milk (Anke, 2004). In connection with decline in lactation and lower output of iodine in milk its plasma concentration increased statistically significantly and on day 80 *post partum* it reached the following

Table 4. Average iodine content in the milk of ewes (μg/l)

Group	Before experiment			Experiment		
	n	$\bar{x}$	SD	n	$\bar{x}$	SD
G1	5	183.6	102.4	16	724.2[a]	485.3
G2	5	177.8	55.4	18	885.9	460.6
G3	5	201.0	47.0	19	1 126.0[b]	262.5

[a,b]$P < 0.01$

Table 5. Average iodine content in the urine of ewes (μg/l)

Group	Day 56 of experiment		
	n	$\bar{x}$	SD
G1	5	197.7	104.9
G2	5	139.7	40.4
G3	5	278.6	126.5

values in group G1, G2 and G3: 103.2 ± 39.0, 160.8 ± 46.2 and 191.2 ± 59.8, respectively (Figure 1). The higher iodine content in the diet of group G3 contributed to an iodine increase in blood plasma in connection with its lower transfer to milk. Table 3 shows the average content of iodine in the blood plasma of lambing ewes of the particular groups before the experiment and during its supplementation. The effect of higher iodine intake in group G3 is also evident from the higher average iodine content in blood plasma in the experimental period compared to the other groups ($P < 0.01$).

Average iodine content in milk (Table 4) before the experiment ranged from 177.8 ± 55.4 μg/l (group G2) to 201.0 ± 47.0 μg/l (group G3). Differences were not statistically significant. After six days of feeding the iodine-enriched diet (day 10 of lactation) iodine content in milk increased by 38% in group G1, by 76% in group G2 and almost 5.2 times in group G3 to the value 1 042.9 ± 285.4 μg/l (Figure 2). In a subsequent period (day of lactation 20 to 30) iodine content in milk increased above 1 000 μg/l in all groups and its average content in the particular groups was as follows: G1 1 055.0 ± 470.2, G2 1 177.3 ± 354.8 and G3 1 250.3 ± 145.8 μg I/l. On day 60 of lactation there was a drop in iodine content in milk (Figure 3) in connection with the lower activity of the mammary gland in the advanced lactation stage. Iodine concentration in urine on day 60 of lactation corresponds to the high excretion of iodine by kidneys. In group G3 it reached the average value 278.6 ± 226.5 μg I/l (Table 5) and exceeded the reference values (Ferii et al., 2003) by more than 100 μg I/l. Table 4 shows the average iodine content in milk between day 10 and 60 of lactation compared to the average content in the period before the experiment. Differences are highly statistically significant. Table 4 and Figure 2 document that the supplementation of iodine in the form of I-enriched algae amounting to 0.7 mg I/kg DM already leads to an increase in iodine in ewe's milk exceeding on average 700 μg I/l while the supplementation of 1.3 mg I/kg DM results in more than 1 100 μg I/l. These concentrations of iodine in milk are considered as undesirable from the aspect of human nutrition (Anke, 2007).

The dynamics of iodine content in the blood plasma of lambs (Figure 3) was similar to the dynamics of I content in ewe's milk. The highest concentration was recorded between day 20 and 30 of lamb's age while a drop occurred on day 60. The lambs of the group with the highest level of iodine supplementation (G3) also had on average a higher content of iodine in blood plasma (Table 6). Iodine content in the blood plasma of lambs was higher than in the plasma of their mothers (3.6 times in G1, 2.6 times in G2 and 3.9 times in G3). These differences in iodine content in the plasma of lambs and their mothers indicate the importance of the mammary gland for a removal of superfluous iodine with milk in lactating animals, i.e. the role of homeorrhetic mechanisms in the regulation of iodine content in the organism. The high content of iodine in milk poses a risk of its surplus for the nursed young, especially with the high intake of iodine in already high-pregnant mothers. The average values of iodine in the plasma of lambs exceeded the reference values (Anke, 2004) 8 times (group G1) to 12 times (group G3).

The differences in iodine content in the blood plasma, milk and urine of lambing ewes and in the

Table 6. Average iodine content in the blood plasma of lambs (μg/l)

Group	Before experiment			Experiment		
	n	$\bar{x}$	SD	n	$\bar{x}$	SD
G1	5	183.6	17.3	15	324.2	167.9[a]
G2	5	203.2	16.8	12	285.3	49.8[c]
G3	5	194.0	31.0	14	484.4	160.7[b,d]

[a,b]$P < 0.05$, [c,d]$P < 0.01$

blood plasma of lambs between group G1 and G2 on the one hand and group G3 on the other are caused by the higher iodine supplementation in group G3. The differences in iodine content between group G1 and G2 with the identical supply of iodine but with different supply of selenium (G1 0.2 mg Se/kg DM and G2 0.4 mg Se/kg DM) can be explained by the participation of selenoenzymes deiodases in iodine metabolism. The deiodase enzymes act to release iodine from a molecule to iodine of a richer thyroxin hormone thus forming the active triiodinethyronine hormone (Bratter, 1996; Köhrle, 2005). Lower urinary output of iodine, higher output of iodine in milk and iodine increase in the plasma of G2 lambing ewes in the later phase of lactation document the higher retention and utilisation of iodine in the organism of lambing ewes with a higher intake of selenium.

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