Highlights

- Rapeseed cake warm pressed and toasted reduced iodine in milk compared to soybean meal.
- Linear reduction in milk iodine concentration with increasing rapeseed cake in the diet.
- Low glucosinolate concentration and no glucosinolate metabolites detected in the feed.

Heat-treated rapeseed expeller press cake with extremely low glucosinolate content reduce transfer of iodine to cow milk.

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Abstract

The main objective of this study was to investigate the effect of increasing dietary levels of heat treated low glucosinolate rapeseed expeller press cake (RSC) on the transfer of iodine from feed to cow milk. Eight cows of the Norwegian red cattle breed were split in two 4 x 4 Latin squares, using 4 treatments and 4 periods of 14 days each. The 4 different treatments were 1) Control, 0.0 kg RSC/day, 2) RSC-Low, 0.6 kg RSC/day, 3) RSC-Medium, 1.4 kg RSC/day and 4) RSC-High, 2.0 kg RSC/day. Irrespective of a planned constant dietary iodine content, the analysed concentration of iodine ranged from 1.4 mg/kg DM in the RSC-High diet to 1.9 mg /kg DM in the Control diet. From day 11 to 14 in each period, samples were collected and the total iodine concentrations in feed, milk and plasma were determined by inductively coupled plasma mass spectrometry. The iodohormones, triiodothyronine (T_3) and thyroxin (T_4) in plasma were determined by fluoroimmunoassy. No differences ($P>0.05$) in total iodine as well as the T_3 and T_4 plasma concentrations were observed between the four treatments, even though the plasma iodine reflected the somewhat varying dietary iodine. Feed intake, milk production and milk composition was not affected by the different treatments ($P>0.05$). Although the levels of glucosinolates were low and no glucosinolate metabolites (e.g., goitrin and indole acetonitrile) were found in the RSC, an increasing offer decreased the milk iodine concentration from 0.35 mg/kg in the Control to 0.25 mg/kg with RSC-Low, to 0.15 mg/kg with RSC-Medium and to 0.10 mg/kg with RSC-High treatments. The iodine transfer, i.e. the output of iodine via milk related to the iodine intake, amounted to 25, 19, 13 and 10% in the Control and the 3 groups with increasing dietary RSC level. This study indicates that milk iodine transfer is severely inhibited at considerably lower levels of glucosinolates in RSC than previously anticipated.

41 **Key words:** iodine diet-milk transfer; dairy cows; rapeseed cake; glucosinolate

1. Introduction

In Norway, iodine has been added to dairy cows' diets for decades (Breirem and Homb, 1958) and milk and milk products are the primary iodine source, covering 50% to 70% of the daily recommended intake for adult Norwegians (Dahl et al., 2004, Trøan et al., 2015). An analysis of iodine in milk from different regions in Norway has shown that the iodine concentration in winter milk has been reduced from 231 ± 34 µg/kg in 2000 (Dahl et al., 2003) to 122 ± 40 µg/kg in 2008 (Haug et al., 2012). In this period, the use of rapeseed products in dairy feed increased from almost zero in year 2000 to more than 5% of the diet in 2008 (Felleskjøpet, 2012). In addition, there was a shift from the use of solvent extracted rapeseed meal (RSM) to mechanically pressed heat-treated rapeseed expeller cake (RSC) (Felleskjøpet Fôrutvikling, Trondheim, Norway, pers. comm.). The observed reduction in iodine concentration in milk aligns with these changes.

According to Papas et al. (1979) and Laarveld et al. (1981), rapeseed products reduce iodine concentration in cow milk, and the presence of glucosinolates (GSL) in rapeseed is put forward as the most likely explanation. During processing and digestion, GSL break down to biological active isothiocyanates (ICT), thiocyanates (SCN), nitriles and 5-vinyl-1,3-oxazolidine-2-thione (goitrin) (Oginsky et al., 1965, Fenwick and Heaney, 1983). The majority of previous studies on iodine transfer to milk have focused on thiocyanate as the main iodine antagonist (Papas et al., 1978, 1979, Laarveld et al., 1981, Hermansen et al., 1995). Thiocyanate ion competes with iodide uptake via the sodium iodide symporter (NIS), reducing the transfer of iodine into the mammary gland and the milk (Levy et al., 1997, Spitzweg et al., 1998).

Rapeseed cultivars containing less than 18 mmol GSL/kg (equal to 30 mmol/kg fat-free matter) are defined as low GSL varieties (Newkirk, 2009). This is in contrast to high GSL rapeseed varieties with more than 100 mmol GSL/kg (Tripathi and Mishra, 2007). The introduction of low GSL varieties reduced the attention on lowered iodine concentration in milk when feeding rapeseed products. The reducing effect of rapeseed products on iodine concentration in milk have been studied with high (Papas et al., 1978, Laarveld et al., 1981) and low (Franke et al., 2009a) GSL RSM and with high (Hermansen et al., 1995) and low (Hermansen et al., 1995, Vesely et al. 2009, Koch et al. 2012) GSL RSC. In all studies, a clear reduction on milk iodine concentration by use of rapeseed products was observed. No studies, however, have investigated the effect of heat-treated RSC with GSL concentrations down to 1 mmol/kg. Thus, the objective of the present work was to investigate the influence of heat-treated RSC with extremely low GSL concentration on the iodine transfer to cow's milk. It was hypothesized that the use of RSC, even heat-treated and with this low level of GSL, reduces the transfer of iodine from feed to milk and that this reduction will depend on the amount of RSC included in the diet.

2. Materials and methods

2.1. Animals, design, feeding and experimental diets

The trial was conducted at the Animal Production Experiment Centre, Norwegian University of Life Sciences (NMBU), Ås, Norway. Eight lactating cows of the Norwegian Red cattle breed housed in tie-stalls were used. The cows featured 89 ± 24 days in milk (DIM), a body weight of 615 ± 42 kg and a daily milk yield of 35.8 ± 3.8 kg at the start of the experiment. The experiment was carried out as two separate 4 × 4 Latin squares with four treatments, cows and periods. In the

first square, all four cows were in second lactation, whereas in the second square, the four cows were in third, fourth, fifth and sixth lactation, respectively. In each period, the first 10 days were used to adapt to diet changes, whereas sampling took place from day 11 to 14.

The diets consisted of 10 kg concentrate (as is) and grass silage fed *ad libitum* to give approximately 10% refusals. The concentrate was offered in four equal meals at 06.00, 11.00, 15.00 and 18.00 hours. Fresh silage was offered at the same time. Feed refusals were removed daily before the 11.00 hour feeding. Intake of silage and concentrate was monitored three successive days in each period (day 11 to 13).

The experimental feeds were two concentrate mixtures produced at Namdal Kornsilo og Mølle A/S (Overhalla, Norway). The ingredient lists of the two mixtures are presented in Table 1. The Control mixture had no rapeseed or rapeseed products; instead, extracted soybean meal (SBM) and lignosulphonate treated SBM (SoyPass) were used as the main protein sources. In the RSC-High mixture, SBM and SoyPass were replaced with RSC. The RSC had a crude protein content of 363 g/kg DM and was a commercial product (Avena Nordic Grain Oy, Espoo, Finland) produced from a blend of rapeseed varieties (*Brassica napus* and *B. rapa*) obtained around Europe. The RSC was heat-treated in two steps. First at a temperature of 90 °C during the pressing of oil, and thereafter by steam toasting at 105 °C for 40 min and drying to a moisture content of 10–12% (Avena Nordic Grain Oy, Espoo, Finland). Iodine in the form of calcium iodate anhydrous ($Ca(IO_3)_2$) was added via the trace element premix to give a concentration of 4 mg I/kg DM in the finished concentrates (Table 1). The two concentrate mixtures were used to

compose four experimental diets designed to give 0.0 (Control), 0.6 (RSC-Low, 70% Control and 30% RSC-High), 1.4 (RSC-Medium, 30% Control and 70% RSC-High) and 2.0 (RSC-High) kg RSC/day (Table 1).

2.2. Feed sample collection, preparation and analysis

Silage was sampled from day 11 to 13 and pooled within each period to yield four samples. Three separate samples of each concentrate mixture were taken from the feedbags before starting the experiment. These samples and the silage samples were freeze-dried. After ambient air stabilization, the freeze-dried samples were ground on a cutting mill (Retsch SM 100; Retsch GmbH, Haan, Germany). A 1.0 mm screen size was used to prepare samples for analysis of DM, ash, Kjeldahl-N, fat, ash free neutral detergent fibre (aNDFom), GSL and GSL metabolites, whereas the 0.5 mm screen size was used to prepare samples for the analysis of starch. For the analysis of iodine, samples were ground with dry ice using a centrifugal mill (Retsch ZM 100; Retsch GmbH, Haan, Germany) and a 0.2 mm screen.

The dry matter in ambient air-stabilized freeze-dried samples was determined after drying at 103 °C until reaching constant weight. The ash content was determined gravimetrically after pyrolysis at 550 °C for 4 hours. Nitrogen was determined as Kjeldahl-N according to the Association of Official Analytical Chemist's method 2001.11 (AOAC, 2002), with the modification of adding 15 mL concentrated H_2SO_4. Crude protein was calculated as Kjeldahl-N x 6.25. Crude fat was determined with an Accelerated Solvent Extractor (ASE200; Dionex, Sunnyvale, CA). The concentrations of aNDFom and starch were determined according to Mertens (2002) and

McCleary et al. (1994), respectively. Residual carbohydrates (Residual CHO) were calculated as DM minus ash, protein, fat, aNDFom and starch. Additionally, a fresh silage sample for each period was analysed for fermentation products and pH at Eurofins (Moss, Norway) as described by Randby et al. (2010).

2.2.1 Analysis of glucosinolate and glucosinolate metabolites in concentrates and rapeseed cake

The RSC used and the two concentrate mixtures (Control and RSC-High) were analysed for GSL and volatile and non-volatile GSL metabolites by the Natural Resources Institute Finland (Jokioinen, Finland). Extraction, purification and desulphation of GSL were performed according to the ISO 9167:1-1992 method (ISO, 1992). Glucosinolates were determined by High Performance Liquid Chromatography (HPLC) with a diode array detector (DAD) (Palo Alto, CA, USA) (HPLC-DAD) using wavelengths of 229 nm and 260 nm. Analytical column was a Sunfire C18 (250 mm*3.0 mm, 5 µm, Waters, Milford, MA, USA). The samples were analysed at 35 °C with an acetonitrile water gradient as follows: 0–1 min 5%, 1–20 min 5–45%, 20–25 min 45%, 25–26 min 45–5% and 26–40 min hold at 5%. Sinigrin was used as a reference standard. The non-volatile, as well as indole acetonitrile and goitrin, were analysed using the same method and instrument, except that methanol was used instead of acetonitrile with a gradient of 0–1 min 5%, 1–20 min 5–100%, 20–35 min hold at 100%, 35–36 min 5% and 36–50 min hold at 5%. Goitrin was detected and quantified at the wavelength of 240 nm and indole acetonitrile at 280 nm. The pre-formed volatile GSL metabolites such as ITC and cyanides were analysed by gas chromatography equipped with a mass selective detector (GC-MS) as described by Peñas *et al.*

(2012). The limit of detection for intact glucosinolates and indole acetonitrile in HPLC-DAD analysis was 0.01 mmol/kg and for goitrin, 0.008 mmol/kg.

2.3. Blood and milk sample collection, preparation and analysis

Blood samples were drawn from the *Vena jugularis* using sodium heparin tubes (BD Vacutainer NH 170 I.U., Belliver Industrial Estate, Plymouth, UK) at 09.00 hours on day 14 in each period. After four hours at room temperature, samples were centrifuged at 3000 g for 15 min and plasma was transferred to TT-tubes and stored at −20 °C until being analysed for total iodine and thyroid hormones. Triiodothyronine (T_3) and thyroxin (T_4) in plasma were analysed at the Hormone Laboratory of Oslo University Hospital (Oslo, Norway) using competitive fluoroimmunoassy (FIA) according to the operating procedures for the applied DELFIA kit (PerkinElmer Life Sciences, Wallac Oy, Turku, Finland).

Milk yield was monitored daily at 06.30 and 15.30 hours using the Tru-Test Milk Meter (Tru-Test Distributors Ltd., New Zealand). At day 11, 13 and 14 in each period, the milk volume collected with the Tru-Test Milk Meter (approximately 2% of yield) was transferred into morning and evening flasks. The morning and evening samples were stored separately at 4 °C, whereupon they were re-heated to 39 °C and pooled within day. From the pooled sample, two aliquots were prepared. One sample was frozen (−20 ºC) for iodine analysis, and one was preserved with one tablet of 2-Bromo-2-nitropane-1, 3 diol for analysis of fat, protein, lactose and urea using a Milkoscan 6000 infrared milk analyser (Foss-Electric, Hillerød, Denmark) at TINE Distriktslaboratoriet Brumunddal (Norway).

173

174 2.4 Iodine analysis in feed, milk and plasma samples

175 The iodine concentration in the silage and concentrate samples were measured by inductively
176 coupled plasma (ICP)-MS according to Fecher et al. (1998), with some modifications. Briefly,
177 0.2–0.3 g of freeze-dried silage, or concentrate sample, was weighed into a 50 mL tube with 4.5
178 mL of MilliQ water. Then, 1 mL 25% tetramethylammonium hydroxide solution and 0.5 mL ^{129}I
179 (concentration 100 µg ^{129}I/L) were added to the sample, whereupon it was mixed and left at 90
180 °C for 3 hours with hourly mixing. After cooling, the sample was diluted with MilliQ water to 50
181 mL. From there, 10 mL was transferred to a new tube and centrifuged at 5000 g for 30 min prior
182 to ICP-MS measurements.

183

184 The defrosted milk sample was heated and homogenized in an ultrasound bath at 39 °C for 10–15
185 min whereupon 0.25 mL whole milk was transferred to a new test tube. For plasma, the sample
186 was thawed and 0.5 mL transferred to a new test tube. Thereafter, the milk and plasma samples
187 were dissolved in 0.5 mL 50% (vol/vol) mixed amines solution (CFA-C reagent, Spectrasol,
188 Warwick, NY, USA prepared in saturated EDTA solution); then 0.1 mL ^{129}I (concentration 100
189 µg ^{129}I/L) was added and the sample diluted to 10 mL prior to analysis of iodine concentration
190 according to the method of Nobrega et al. (1997).

191

192 The concentrations of iodine (*m/z* 127) in feed, plasma and milk samples were measured using an
193 Agilent 8800 QQQ ICP-MS (Agilent Technologies, USA) with nebulizer gas of 1.01 L/min, RF

194 power 1550 W, O_2 gas of 0.3 mL/min and He gas of 5 mL/min. Calibration standards of 40 µg
195 I/L and calibration blanks were matrix matched to the samples. The ^{129}I (Reifenhauser and
196 Heumann, 1990) was used as internal standard. The calculated LOD (3x standard deviation of the
197 blanks) was based on five blank samples. The limit of quantification (LOQ) (10 x standard
198 deviation of the blanks) was based on the same blank samples, taking the weight of measured
199 samples into account. A minimum of five parallels of each sample were used to measure the
200 precision of the instrument. The accuracy of the method was based on certified reference
201 materials (CRM) on milk and hay and an inter laboratory comparison sample on mixed feed
202 (IAG, 2004). The instrumental LOD were 0.011 mg I/kg for the feed and 0.030 µg I/L for the
203 milk and plasma samples. The LOQ was 0.038 mg I/kg for the feed and 0.100 µg I/L for the milk
204 and plasma samples. The milk, plasma and silage samples had a good precision with coefficient
205 of variation (CV) below 3%. For the concentrate samples, the precision was poorer with CV of
206 17%.

207

208 2.5. Calculations and data analysis

209 Feed intake (DMI) was calculated as the difference between feed offered and refusals (there were
210 no refusals of concentrate). For silage and silage refusals, oven dried DM corrected for volatiles
211 according to Åkerlind et al. (2011) was used to calculate the DM intake, whereas oven dried DM
212 determined at 103 ºC was used to calculate the DM intake of concentrate. The concentration of
213 main nutrients, iodine and GSL in the RSC-Low and RSC-Medium treatments was calculated
214 based on analyses of the Control and RSC-High treatments and their respective proportions
215 within the treatments. Likewise, the daily intake of iodine and GSL, was calculated by

multiplying analysed total iodine (mg/kg DM), and GSL (mmol/kg DM) of the Control and the RSC-High concentrate by their respective proportions within the treatments. For iodine, the contribution from the silage was added. Energy corrected milk (ECM) was calculated according to Sjaunja et al. (1990). The iodine transfer from feed to milk (IT) given as a percentage was calculated using the following equation:

$$IT = \frac{daily\ milk\ yield\ (kg) \times milk\ iodine\ concentration\ (\frac{mg}{kg})}{daily\ feed\ intake\ (kg) \times feed\ iodine\ concentration (\frac{mg}{kg})} \times 100 \quad (1)$$

The data were analysed using ANOVA with the MIXED procedure of SAS version 9.4 (SAS Insitute, Inc., Cary, North Carolina, USA). In the model, μ was the overall mean, α_i the random effect of cow (1–8), β_j the fixed effect of treatment (1–4), $\delta_{k(l)}$ the fixed effect of period k (1–4) within square l, τ_l the fixed effect of square (1–2) and ε_{ijkl} the random experimental error. To find the iodine concentration in milk, the excretion of iodine in milk and transfer of iodine from feed to milk, day was added as a repeated measurement in the model. All results are presented as least square means (LSmeans) with their standard errors (SEM) unless otherwise stated. Differences between treatments, in addition to linear, quadratic and cubic effects, were tested using the CONTRAST statement of the MIXED procedure. Significance level was $P<0.05$ unless stated otherwise. The REG procedure with influence statement was used to detect outliers in the dataset. Two observations of iodine in milk from one cow were detected as possible outliers with Cook`s distance of more than 0.20 and studentized residuals of 4.0 and 4.3. The observations from this cow did not affect the results of the MIXED procedure analyses, however, and thus were not deleted.

3. Results

3.1 Composition of the feed

Starch and aNDFom concentration differed between the two experimental concentrate mixtures (P<0.05), whereas there were only minor differences in the concentration of protein and fat (Table 1). The concentration of fermentation products in the silage (n = 4) was 27.1 ± 2.69 g/kg DM lactic acid, 6.1 ± 0.95 g/kg DM acetic acid, < 1 g/kg DM of butyric acid, 9.6 ± 1.02 g/kg DM formic acid, 2.4 ± 0.49 g/kg DM propionic acid, 49.8 ± 9.89 g/kg N NH_3-N and 9.5 ± 1.17 g/kg DM ethanol. Whereas the pH in the silage was 4.4 ± 0.08. Against the planning, the average concentration of iodine was significantly different between the two concentrate mixtures (P<0.001) (Table 1). The average concentration was 4.0 in the Control concentrate and 3.0 mg I/kg DM in the RSC-High concentrate (Table 1). Analyses of iodine in five parallels of each of the three samples of the Control concentrate varied from 2.7 to 6.0 mg/kg DM, whereas in the RSC-High concentrate, they varied from 2.3 to 4.5 mg/kg DM.

The concentration of all GSL was below the LOD in the Control concentrate (Table 2). In the RSC, analysed total GSL was 1.07 mmol/kg DM. In the RSC-High concentrate, analysed GSL was 0.36 mmol/kg DM, which is higher than theoretical the GSL concentration of 0.21 mmol/kg based on 20% inclusion of RSC. Regarding GSL, the highest concentration was observed for progoitrin both in the RSC and RSC-High concentrate (Table 2). No ITC, cyanides or goitrin in the RSC or in the concentrates were detected.

3.2. Intake of iodine, glucosinolates and feed, milk yield and milk composition

There were significant differences ($P<0.05$) in iodine intake between the different treatments. The intake varied from 39 ± 0.4 mg I/day in the Control group to 30 ± 0.5 mg I/day in the RSC-High group (Table 3). No detectable intake of GSL was observed in the Control group, whereas the intake of GSL was 3.19 mmol/day in the RSC-High group (Table 3). No significant differences ($P>0.05$) in DMI, milk yield, ECM or milk composition between the treatments were observed (Table 3).

3.3. Iodine in plasma, T_3 and T_4 in plasma and iodine in milk

The plasma iodine concentration showed a significant ($P<0.05$) linear decrease with increasing RSC intake, but no difference between treatments (Table 4). There were no differences between treatments with respect to either T_3 or T_4 (Table 4). Significant differences ($P<0.001$) in milk iodine concentrations, milk iodine secretion and iodine transfer to milk were observed among all dietary treatments (Table 4). The linear relationship of increasing RSC intake was significant for all three variables, whereas the quadratic relationship was significant only for iodine concentration in milk and secretion of iodine in milk (Table 4). For iodine transfer from feed to milk, the quadratic relationship was close to significant ($P=0.059$) (Table 4). No cubic effects were found. The Control treatment was significantly ($P<0.001$) different from the three RSC diets. In addition, there were differences ($P<0.05$) between treatment RSC-Low and both RSC-Medium and RSC-High (Table 4).

4. Discussion

281 The level of GSL in the rapeseed product used in our study was only 1.1 mmol/kg (Table 2),
282 which is considerably lower than the 18 mmol GSL/kg considered as the upper limit for "double-
283 zero" rapeseed varieties (Newkirk, 2009). In agreement with Franke et al. (2009a) and
284 Hermansen et al. (1995), using an RSM with 3.5 mmol GSL/kg DM and an RSC with 4.5 mmol
285 GSL/kg, respectively, our study confirms that rapeseed products reduce milk iodine transfer even
286 when varieties low in GSL are used.

287

288 The Control concentrate exhibited 1 mg/kg DM higher iodine concentration than the RSC-High
289 concentrate (Table 1), resulting in decreasing iodine intake with increasing intake of RSC (Table
290 3). Franke et al. (2009a) demonstrated that there is a linear relationship with increasing iodine
291 intake and iodine concentration in milk. Assuming such a linear relationship, the reduction in
292 milk iodine concentration should have been from 0.35 mg/kg in the Control group to 0.32, 0.29
293 and 0.27 mg I/kg in the RSC-Low, RSC-Medium and RSC-High groups, respectively. The
294 observed reduction in milk iodine concentration (Table 4), however, was considerably higher
295 than expected from reduced iodine intake. Moreover, although the iodine intake was reduced up
296 to a quarter and iodine intake ranged between 39 and 30 mg/day, a constant rate of iodine transfer
297 to milk in this interval can be assumed. Voigt and Kiefer (2007) reported a constant iodine
298 transfer coefficient to milk when iodine intake increased from 10 up to 500 mg per cow and day.
299 Thus, the main reduction effect on iodine transfer from 25% in the Control up to 10% in the
300 RSC-High group must be attributed to the different intake levels of RSC and not to the minor
301 differences of iodine intake.

302

303 Iodine transfer from feed to milk decreased linearly (P<0.05) with increasing RSC intake, and
304 thus GSL intake (Table 4). Weiss et al. (2015) showed a linear decrease in milk iodine
305 concentration when GSL intake increased from zero to 31 mmol/day by feeding canola meal to
306 dairy cows. Likewise, Franke et al (2009a) found a clear decrease in milk iodine concentration
307 between a rapeseed free diet and a diet providing 11.0-13.7 mmol GSL/day. Whereas, Hermansen
308 et al. (1995) reported 60% reduced iodine in milk independent of a GSL intake range from 10 to
309 42 mmol/day from RSC or RSM compared to a Control with SBM. What is common to all these
310 studies, however, are that they had GSL intakes considerably higher than the 3.2 mmol/day
311 applied in the present study (Table 3).
312
313 In agreement with Schöne et al. (1994), no GSL metabolites in the RSC, or the dry concentrates,
314 were detected, probably due to the volatilization of the metabolites (Schöne et al., 1994). Schöne
315 et al. (1997) claimed that there were two sources of thiocyanate in milk and blood, first minor
316 amounts originated from GSL degradation in the digestive tract of the dairy cows (Oginsky et al.,
317 1965) and second from detoxification of cyanide from nitrile originating from GSL degradation.
318 Thus, it is possible that GSL metabolites are present in higher concentrations than GSL analyses
319 of the feed indicate.
320
321 In contrast to our results (Table 4), Koch et al. (2012) and Franke et al. (2009b) showed that the
322 total iodine concentration increased in blood serum in cows fed RSC and RSM, probably due to
323 the inhibition of the iodine transfer into thyroid and mammary glands (Cavalieri, 1997). In the
324 present study, the difference in daily iodine intake between the Control and the RSC-High
325 treatments was approximately 9 mg (Table 3). When the iodine intake changes, the iodide

326 concentration in blood also changes, but the T_3 and T_4 hormone levels are shown to be unaffected
327 at an iodine intake from 3 to 120 mg/day (Franke et al., 2009b). The results from this study
328 confirm these findings (Table 4), showing that the hormone concentrations were independent of
329 the level of RSC in the diet.
330
331 **5. Conclusion**
332 In spite of the fact that the RSC used was heat-treated and contained only 1.1 mmol GSL/kg,
333 increasing the intake of RSC from 0.6 to 1.4 and then to 2 kg/day linearly reduced the iodine
334 transfer from feed to milk, while the plasma thyroid hormone concentration was unaffected. The
335 results suggest that RSC inhibits milk iodine transfer at considerably lower GSL levels than
336 demonstrated in earlier studies. To ensure a stable iodine concentration in milk, not only the
337 iodine concentration in the feed but also the intake of RSC of the dairy cow should be considered.
338
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344
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445

446 **Table 1.** Ingredient composition (g/kg) of experimental concentrate mixtures and analysed concentration of main nutrients (g/kg dry
447 matter (DM)) and iodine (mg/kg DM) in concentrates (n = 3, LSmeans ± SEM) and silage (n = 4, means ± SD).

	Control[1]	RSC-Low[2]	RSC-Medium[2]	RSC-High[1]	SEM[6]	P	Silage
Ingredient composition;							
Soybean meal	110	77	33	0			
SoyPass	50	35	15	0			
Rapeseed expeller press cake	0	60	140	200			
Barley	474	463	447	436			
Oats	200	200	200	200			
Wheat bran	70	70	70	70			
Molasses	60	60	60	60			
Dry fat (gigant)	10	9	9	8			
Vitamin premix[3]	0.5	0.5	0.5	0.5			
Limestone meal	9.6	9.6	9.6	9.6			
Monocalcium phosphate	3.0	3.0	3.0	3.0			
Magnesium oxide	4.6	4.6	4.6	4.6			
Feed salt	7.4	7.4	7.4	7.4			
Trace element premix[4]	1.0	1.0	1.0	1.0			
Crina Ruminant	0.05	0.05	0.05	0.05			
Biotine 2%	0.10	0.10	0.10	0.10			
Main nutrients;							
Dry matter (g/kg)	873	873	873	873	4.2	0.95	268 ±10

Crude ash	63	65	67	68	1.5	0.08	90 ± 6.5
Crude protein	169	170	172	173	3.5	0.47	127 ± 5
Crude fat	32	34	37	39	2.0	0.07	31 ± 4
Starch	396[a]	385	370	359[b]	3.5	<0.01	-
aNDFom[5]	170[a]	180	194	204[b]	4.1	<0.01	492 ± 23
Residual CHO[5]	171	167	162	158	12.7	0.52	260 ± 25
Iodine	4.0[a]	3.7	3.3	3.0[b]	0.18	<0.01	0.28 ± 0.04

448 [1] Control = concentrate with soybean meal and SoyPass, RSC-High = concentrate with 20% (wt/wt) heat treated rapeseed expeller press cake
449 (RSC).
450 [2] RSC-Low and RSC-Medium are calculated based on proportions of Control and RSC-High. RSC-Low = 70% of Control and 30% of RSC-
451 High, RSC-Medium = 30% of Control and 70% of RSC-High.
452 [3] Vitamin premix: Providing per kg feed: Vitamin A 5700 IU, Vitamin E 40 mg, Vitamin D3 2300 IU.
453 [4] Trace element premix. Providing per kg feed: 20 mg Mg (Magnesium oxide), 0.25 mg Co (Cobalt carbonate), 65 mg Zn (Zink sulphate), 15 mg
454 Cu (Copper(II) sulphate), 0.32 mg Se (Sodium selenite) and 3.5 mg I (Calcium iodate anhydrous ($Ca(IO_3)_2$)).
455 [5] aNDFom = ash corrected Neutral detergent fiber analyzed after pretreatment with heat stable amylase, Residual CHO (Rest fraction of
456 carbohydrates) = Dry matter – (ash + protein + fat + aNDFom + starch).
457 [6] SEM = Standard error of LSmeans
458 [a-b] LSmeans ± standard error of LSmeans (SEM[4]) within a row with different superscripts differ between only Control and RSC-High ($P < 0.05$).

Table 2. Total glucosinolate (GSL) and GSL profile in the rapeseed expeller press cake (RSC) and the concentrates (mmol/kg DM) (n = 3, means ± SD)

Glucosinolates;	RSC	Control[1]	RSC-Low[2]	RSC-Medium[2]	RSC-High[1]
Progoitrin	0.43 (± 0.00)	<0.01	0.05	0.11	0.16 (± 0.07)
Glucoalyssin	0.02 (± 0.01)	<0.01	<0.01	<0.01	<0.01
Gluconapin	0.26 (± 0.00)	<0.01	0.03	0.08	0.11 (± 0.03)
4-OH-glucobrassicin	0.02 (± 0.00)	<0.01	<0.01	<0.01	<0.01
Glucobrassicanapin	0.09 (± 0.02)	<0.01	<0.01	<0.01	<0.01
Glucobrassicin	<0.01	<0.01	<0.01	<0.01	<0.01
Unknown GSL	0.21 (± 0.00)	<0.01	0.03	0.07	0.10 (± 0.01)
Total GSL	1.07 (± 0.01)	< 0.01	0.11	0.25	0.36 ± (0.03)

[1] Control = concentrate with soybean meal and SoyPass, RSC-High = concentrate with 20% (wt/wt) heat treated rapeseed expeller press cake (RSC).

[2] RSC-Low and RSC-Medium are calculated based on proportions of Control and RSC-High. RSC-Low = 70% of Control and 30% of RSC-High, RSC-Medium = 30% of Control and 70% of RSC-High.

Limit of detection of individual GSL is 0.01 mmol/kg.

Table 3. Effect of treatment on daily intake of feed, iodine and glucosinolate (GSL) (n = 8), production of milk and energy corrected milk (ECM) and concentration of fat, protein, lactose and urea in milk (n = 8)

						Effects		
	Control[1]	RSC-Low[2]	RSC-Medium[2]	RSC-High[1]	SEM[3]	Linear	Quadratic	Cubic
Grass silage (kg DM)	12.0	12.0	12.2	12.2	0.24	0.34	0.85	0.54
Concentrate (kg DM)	8.8	8.8	8.8	8.8	-	-	-	-
Total (kg DM)	20.8	20.8	21.0	21.0	0.24	0.34	0.85	0.54
Iodine (mg)	39[a]	36[b]	33[c]	30[d]	0.10	<0.01	0.25	<0.01
GSL (mmol)	<0.01	0.96	2.23	3.19	-	-	-	-
Daily production								
Milk (kg)	26.5	27.3	26.2	27.4	1.28	0.59	0.77	0.16
ECM (kg)	27.6	28.6	26.4	28.3	1.24	0.99	0.54	0.04
Composition								
Fat (g/kg)	42.4	42.3	39.7	41.4	1.26	0.25	0.41	0.17
Protein (g/kg)	34.4	34.9	34.3	34.8	0.61	0.79	0.95	0.25
Lactose (g/kg)	47.3	47.7	47.6	47.8	0.36	0.40	0.84	0.59
Urea (mmol/L)	3.11	3.08	2.90	2.83	0.19	0.17	0.91	0.74

[1] Control = concentrate with soybean meal and SoyPass, RSC-High = concentrate with 20% (wt/wt) rapeseed expeller press cake (RSC).
[2] RSC-Low and RSC-Medium are calculated based on proportions of Control and RSC-High. RSC-Low = 70% of Control and 30% of RSC-High, RSC-Medium = 30% of Control and 70% of RSC-High.
[3] SEM = standard error of LSmeans
[a-d] LSmeans within a row with different superscripts differ between treatments (P<0.05).

Table 4. Iodine concentration in milk, daily secretion of iodine in milk, iodine transfer from feed to milk (n = 24) and concentration of iodine and thyroid hormone in plasma (n = 8)

						Effects		
	Control[1]	RSC-Low[2]	RSC-Medium[2]	RSC-High[1]	SEM[3]	Linear	Quadratic	Cubic
Iodine in milk (mg/kg)	0.35[a]	0.25[b]	0.15[c]	0.10[d]	0.031	<0.01	<0.0	0.15
Daily secretion of iodine in milk (mg/day)	9.6[a]	6.9[b]	4.1[c]	2.9[d]	0.88	<0.01	0.01	0.23
Iodine transfer from feed to milk (%)	25[a]	19[b]	13[c]	10[d]	2.5	<0.01	0.06	0.17
Iodine in plasma (µg/kg)	104	103	99	93	4.0	0.02	0.41	0.88
T_3 in plasma (nmol/L)	2.0	2.0	2.0	2.0	0.09	0.85	0.66	0.48
T_4 in plasma (nmol/L)	64	67	67	68	3.3	0.06	0.48	0.72

[1] Control = concentrate with soybean meal and SoyPass, RSC-High = concentrate with 20% (wt/wt) rapeseed expeller press cake (RSC).
[2] RSC-Low and RSC-Medium are calculated based on proportions of Control and RSC-High. RSC-Low = 70% of Control and 30% of RSC-High, RSC-Medium = 30% of Control and 70% of RSC-High.
[3] SEM = standard error of LSmeans
[a-d] LSmeans within a row with different superscripts differ between treatments (P<0.05).