Long term study on the effects of iodine sources and levels without and with rapeseed cake in the diet on the performance and the iodine transfer into body tissues and eggs of laying hens of two breeds

Gerhard Flachowsky*, Ingrid Halle*, Anna S. Schultz*, Hubertus Wagner**, and Sven Dänicke*

Abstract

The objective of the study was to investigate the influence of two iodine species and five amounts on performance, iodine concentration of body samples and eggs of hens of two breeds fed with diets without or with 10 % rapeseed cake (RSC). 432 laying hens (23 weeks old) were allocated to 18 groups with 24 birds (12 hens of the Lohmann Selected Leghorn; LSL; 12 Lohmann Brown Hens; LB) each. The hens were fed for 168 days with a standard feed diet (0.35 mg I/kg) supplemented with 0/0.25/0.5/2.5/5.0 mg I/kg feed from Potassium iodide (KI) or from Calcium iodate $(Ca(IO_3)_2$. Blood samples from 12 hens (6 LSL/6 LB) and eggs were taken on the days 0/4/8/15/29/85/164; these hens were slaughtered and samples were taken. Iodine supplementation did not significantly influence feed intake, egg weight and laying performance of hens. The highest iodine concentration was measured in the thyroid gland. Eggs showed the highest iodine concentration in groups supplemented with 5 mg I/kg, about 91 % of iodine was deposited in the yolk and only 9 % in the egg albumin. Hens fed with 10 % RSC had 24 % less I in the eggs compared with diets without RSC. I from KI effected 20 % more I in the eggs compared with $Ca(IO_3)_2$. LSL-hens showed about 20 % lower I-concentration in eggs than LB-hens. All measured samples of hens fed with 10 % RSC contained lower iodine concentrations than samples from non-RSC-hens.

Keywords: *Iodine, laying hens, eggs, rapeseed cake, feed intake, laying performance, I-content in body samples and eggs, I-carry over, cover of human need*

Zusammenfassung

Langzeit-Studie zum Einfluss von Jodquellen und -mengen ohne und mit Rapskuchen im Futter auf Leistung und Jodtransfer in Körperproben und Eier von Legehennen aus zwei Herkünften

In einer Langzeit-Fütterungsstudie sollte der Einfluss unterschiedlicher Jodquellen und -dosierungen mit und ohne 10 % Rapskuchen (RSC) in der Futtermischung auf Legeleistung und Konzentration an Jod in Geweben und in Eiern untersucht werden. 432 Legehennen (23 Wochen) wurden auf 18 Gruppen mit je 24 Tieren (12 Lohmann Leghorn; LSL; und 12 Lohmann Brown Hennen; LB) aufgeteilt. 168 Tage erhielten die Hennen eine Standard-Ration, die mit 0, 0,25, 0,5, 2,5 und 5,0 mg I/kg aus Kalium Jodid (KI) oder Calcium Jodat $(Ca(ICO_3)_2$ supplementiert wurde. Der Jodgehalt der Basalmischung betrug 0,35 mg/kg. Blutproben von 12 Hennen (je 6 LSL und LB-Hennen) jeder Behandlung und Eier wurden nach 0, 4, 8, 15, 29, 85 und 164 Versuchstagen gewonnen. Diese Hennen wurden geschlachtet, Proben entnommen und der Jodgehalt analysiert. Die Jod-Ergänzung des Futters hatte keinen signifikanten Einfluss auf Futteraufnahme, Eimasse und Legeleistung, aber sie erhöhte die Jod-Konzentration in allen untersuchten Körperproben und in den Eiern. Die höchsten Jodkonzentrationen in Eiern wurden bei 5 mg I/kg Futter ermittelt. Der RSC-Einsatz hatte im Mittel 24 % weniger I im Ei zur Folge. KI und LB-Hennen wiesen höhere I-Konzentrationen in den Eiern auf als $Ca(ICO_3)_2$ und LSL-Hennen.

Schlüsselwörter: *Jod, Legehennen, Eier, Rapskuchen, Futteraufnahme, Legeleistung, I-Gehalt in Körperproben und Eiern, I-carry over, Bedarfsdeckung Mensch*

* Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Institute of Animal Nutrition, Bundesallee 37, 38116 Braunschweig, Germany

** Max-Rubner-Institute, Analysis Division, E.-C.-Baumann-Straße 20, 95326 Kulmbach, Germany

Contact: Ingrid.Halle@fli.de

1 Introduction

Iodine (I) is an essential trace element for humans and animals that was discovered as a novel element about 200 years ago. Two centuries of iodine research showed many interesting results, but it opened also some new questions in animal and human nutrition (EFSA, 2005; 2014; Küpper et al., 2011). Iodine is part of the thyroid hormones triiodothyronine (T3) and thyroxine (T4) and therefore, it is highly important for key processes in the body (e.g. Decuypere et al., 2005; Schöne and Rajendram, 2009; Zimmermann, 2012).

Iodine deficiency still remains a major issue of public health in many countries, including some European countries (e.g. Zimmermann and Andersson, 2012). In 2001 the WHO estimated that about 800 million people suffer from iodine undersupply (<100 µg I/L urine; WHO et al. 2001; WHO 2004; EFSA, 2014), but altogether there is a decrease in the number of iodine-deficient countries (from 54 to 30; 2003 to 2012; Zimmermann and Andersson, 2012). People in ten countries showed excessive iodine intake (Pearce et al., 2013).

Extensive efforts to improve iodine supply for humans, for example by supplementing iodine in table salt by the food industry and in households (e.g. Andersson et al., 2010; van der Haar et al., 2011; Zimmermann and Andersson, 2011; EFSA, 2013b) or enriching foods of animal origin by iodine supplements in feed extending animal requirements (e.g. Schöne and Rajendram, 2009; Flachowsky et al., 2014) contributed to a better iodine supply.

In some countries 40 % and more of the total human iodine intake derives from milk and milk products (e.g. Dahl et al., 2003; Johner et al., 2012; Villanueva, 2016). Besides milk, eggs of laying hens are also characterized by a high transfer of feed-iodine into the animal product, especially in the egg yolk (e.g. Röttger et al., 2012; Slupczynska et al., 2014) and may contribute up to 10 % and more of the total iodine intake (e.g. Sager, 2011) of the human consumer. Hester (2017) considered a high innovation potential of eggs for improvement the I-supply of people.

It has to be mentioned, that there is a small range between human iodine requirements (130 to 200 µg I per day for adults; EFSA, 2014) and the upper level (UL; 500 to 600 µg I per day for adults; DACH, 2000; SCF, 2002), so that the maximum level is only about three times higher than humans demand.

Thus, on the one hand there is a certain risk of deficiency, but on the other hand a risk of overdosing, especially if people consume high amounts of iodine-fortified food of animal origin such as milk and eggs. Therefore, dose-response studies with animals, characterized by a high carry-over of iodine from feed into their food products, were required by the EFSA (2005).

The most important objectives of such studies can be summarized as follows:

1. A better evaluation of the contribution to human supply by animal food-products.
2. Knowledge of further iodine inputs and the effects of antagonists on the iodine content of food of animal origin.
3. Influence of further factors, such as animal species, categories and breeds, animal keeping, hygiene measurements etc. on iodine-content of food of animal origin.

Because of preventive consumer protection, the EFSA recommended in 2005 a reduction of the iodine-upper level in feed of dairy cows and laying hens from 10 to 5 mg I per kg feed with 88 % dry matter (DM). Based on these statements feeding experiments with dairy cows (Franke, 2009; Flachowsky et al., 2014), laying hens (Schultz, 2012) and meat producing animals (Berk et al., 2008; Meyer et al., 2008; Röttger et al., 2011) were carried out at the Friedrich-Loeffler-Institute, Institute of Animal Nutrition in Braunschweig (Germany). The objective of the present study with laying hens was to investigate the transfer of iodine from feed into body-tissues and eggs in a long-term feeding study with two laying-hen breeds under consideration of two iodine-sources, five iodine-levels and antagonists (rape seed cake) of iodine-transfer present in the feed.

2 Materials and methods

2.1 Experimental design

Due to the conclusions by the EFSA (2005), a long-term feeding study with 432 laying hens with feed iodine supplementation up to 5 mg I/kg was conducted. The following factors were considered in the study:

- Two laying hen breeds (Lohmann Selected Light (LSL): hens with white feathers and white eggs and Lohmann Brown (LB) with brown feathers and brown eggs, 216 hens each
- Two iodine sources (KI and $Ca(IO_3)_2$)
- Five iodine supplementing levels of both sources (0, 0.25, 0.5, 2.5, 5.0 mg I/kg feed)
- One diet each without or with 10 % rapeseed cake (RSC) with 13.8 mmol glucosinolate/kg.

The experiment started when the hens were 23 weeks of age and continued six laying months (one laying month = 28 d) which amounts to 168 days. The laying hens were allocated to 18 groups (24 hens per group) that obtained diets with different iodine supplementations of the two iodine sources. The groups were arranged with 50 % LSL and 50 % LB hens (12 hens each per group). The mean initial body weight of the LSL-hens amounted to 1365.2 ± 130.1 g, that of LB-hens to 1636.5 ± 163.2 g per hen. For the allocation of individual feed intake and egg production, the hens were kept separately in battery pens, with 48 x 46 cm per section. Nine groups were supplied with 10 % RSC (see Table 1; RSC with 91.6 % dry matter). RSC was included in the isoenergetic and isonitrogenic calculated diets in order to observe the influence of glucosinolates (Fenwick and Curtis, 1980; Schöne et al., 1993; Tripathi and Mishra, 2007) on feed intake, animal health, poultry performance, mass and iodine concentration of thyroid gland and iodine transfer from feed into eggs. Feed and water were provided for *ad libitum* consumption. Leftovers of feed were weighed weekly to calculate the feed intake.

2.2 Feeding of laying hens

Table 1 shows the composition of basal diets (according to GfE,1999). 216 hens of both breeds (groups 1 to 9) consumed the basal diet without RSC, the hens of the groups 10 to 18 (216 hens of both breeds) were fed by a mixture with 10 % RSC. Each single hen cage (24 cages per group) was equipped with a feeding trough and a nipple drinker. From week 23 on, the weekly light duration increased by 30 or 15 minutes weekly to 16 hours of light at the 34^{th} week of age. The temperature in the trial room was between 18 and 21 °C.

Table 1

Ingredient composition (g/kg), analysed and calculated nutrients (g/kg) and energy content (MJ ME/kg) of the basal diets

Ingredients	Without RSC	With RSC
Maize	642.1	623.9
Soybean meal	216.0	144.0
RSC [1)]	0.0	100.0
Soybean oil	26.0	16.0
L-Lysine HCl	-	0.4
DL-Methionine	0.9	0.7
Major elements [2)]	105.0	105.0
Trace elements and vitamins [3)]	10.0	10.0
Analysed and calculated nutrients (g/kg) and energy		
Dry matter	890.0 [4)]	892.5 [4)]
Crude protein	152.0 [4)]	150.9 [4)]
Crude fibre	28.0 [4)]	33.0 [4)]
Lysine	8.5 [5)]	8.5 [5)]
Methionine plus cysteine	6.9 [5)]	6.9 [5)]
ME, MJ/kg	11.0 [5)]	11.0 [5)]

[1)] rapeseed cake (RSC), Glucosinolate 13.8 µmol/g dry matter;
[2)] per kg diet: 23g $Ca_2H_2(PO_4)_2$, 80g $CaCO_3$, 3g NaCl
[3)] per kg diet: 3 mg vitamin A, 0.625 mg cholecalciferol, 20 mg a-tocopherol; 2.5 mg thiamine, 7 mg riboflavin, 4 mg pyridoxine, 20 µg cyanocobalamine, 4 mg menadione, 40 mg nicotinic acid, 10 mg Ca-pantothenic acid, 0.6 mg folic acid, 25 µg biotin, 400 mg choline chloride, 40 mg Fe, 10 mg Cu, 100 mg Mn, 80 mg Zn, 0,25 mg Se, 0.55 mg Co, 125 mg butylated hydroxytoluene, 4 mg canthaxanthin
[4)] analysed
[5)] calculated

The following parameters were investigated during the study:

- Feed intake per hen
- Laying performance per hen
- Initial and final body weight of hens
- Saturation of egg-iodine concentration
- In weeks 4, 12 and 24 all eggs laid over three consecutive days were collected and separated in yolk, albumin and shell and the weights of fractions was measured
- Iodine concentration of eggs (after 0, 4, 8, 15, 85 and 164 days), breast and thigh meat, serum and liver after six laying month of iodine application
- Iodine concentration and mass of the thyroid gland.

2.3 Taking samples and chemical analysis

The laying performance was examined by recording the egg mass, the laying intensity, and the Feed Conversion Ratio (FCR; kg feed intake per kg egg mass production). To obtain a representative sample for the egg mass, eight eggs per hen and month were weighed.

Eggs for the iodine determination were obtained from 12 hens per group (6 LSL and 6 LB each), throughout the experiment in increasing time intervals (days 0, 4, 8, 15, 29, 85, and 164). The eggs were always taken from the same individuals ("defined hens"). The iodine content per egg refers to the mass of the respective egg in which the iodine content was measured. The "defined hens" were slaughtered after six laying months (168 days) of iodine application.

2.4 Feed and iodine analyses

Feed analyses were performed for the feed mixtures from each experimental group. Dry matter, the proximates; (crude fibre, crude ash, crude protein, ether extract), starch, sucrose, NDF, ADF and calcium were analysed according to the guidelines of the "Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten" (VDLUFA, 1976-2017). The metabolisable energy (N-corrected; AME_N) of the mixtures was calculated with the energy estimation equation of the World`s Poultry Science Association (Vogt, 1986). The glucosinolates of the rapeseed cake were determined with the official method of the European Commission (EC, 1990; Rothe et al., 2004).

2.4.1 Iodine in feed

The iodine content of feed samples (mg/kg fresh matter (FM)) was measured using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS; Fecher et al., 1998; Shelor and Dasgupta, 2011; for details see Wagner, 2007 and Röttger et al., 2011). The measured solutions were prepared in an alkaline medium in order to avoid vaporisation of iodine. Feed samples (5 g) were boiled for 30 min with an ammonia solution (0.59 mol/L). After cooling, samples were filled with ammonia solution up to 1 000 mL, and then filtered with a fluted filter. Prior to the use, filters were flooded twice with boiling distilled deionised water and then dried in an oven to remove I and to avoid contamination. After filtration with a syringe filter (Ministart 0.45 mm), 10 mL of the filtrate were taken for measurements.

2.4.2 Iodine in body samples and eggs

Blood serum was diluted 1:10 with 0.11 mol/L Tetramethylammonium hydroxide solution (TMAH, Alfa Aesar GmbH Co KG Karlsruhe, 25 % w/w aq.solu., Electronic Grade, 99.9 %) before measuring. Other organs (with the exception of the thyroid gland) and egg samples were homogenised with a mixer (ESGE-ZauberStab[R]) prior to digestion. After mechanical homogenisation, meat, liver or eggs were digested chemically with TMAH (Fecher et al., 1998) and a 1 g sample heated for 3 h at 90 °C in a gastight Erlenmeyer flask, with

5 mL distilled deionised water and 1 ml TMAH. After cooling, the solution was filled up to 25 mL with distilled deionised water. 10 mL of the solution were taken for measurement using ICP-MS, 0.5 mL tellurium oxide solution (100 µg/L; tellurium (IV) oxide, Alfa Aesar GmbH & Co KG Karlsruhe, 99.9 %) as the internal standard. The masses of 127 for iodine and 125 for tellurium were used. During calculation of iodine concentration, the standard addition method was applied during calibration.

2.5 Statistical analysis

Data were analysed with a three-way ANOVA using the following fixed linear model: $y_{ijfgk} = \mu + C_i + D_j + E_f + H_g + (CDEH)_{ijfg} + e_{ijfgk}$, with y_{ijfgk} = *ijfgk-th* observation, μ = general mean, C_i =effect of *i-th* iodine source, D_j = effect of *j-th* feed iodine supplementation, E_f = effect of *f-th* RSC feed component, H_g = effect of *g-th* hen breed, $(CDEH)_{ijfg}$ effect of *i-th* interaction among these above factors and e_{ijfgk} = effect of random error connected with *ijfgk-th* observation. Multiple comparisons of means were carried out using the Student-Newman-Keuls Test (P ≤ 0.05). The statistical analyses were performed by the SAS (2002-2012, Version 9.4).

3 Results and discussion

3.1 Composition of diets

The differences in the crude nutrient content of all experimental diets were relatively small and could be neglected (not shown). The intended increase of the feed iodine concentration was achieved in the experiment (not shown) and all indicated values of daily iodine intake are based on the analysed feed iodine concentration.

The iodine concentration of the water was low (1.7 ± 0.2 µg l/L). At an average daily water uptake of about 180 ml per hen (Leeson and Summers, 2001), this results in a negligible daily iodine intake by water of approximately 0.3 µg and therefore was excluded from further calculations.

3.2 Effects on laying hen performance

During the experiment no impacts on animal health were observed. Two hens (0.46 %) died during the 168 experimental days (one hen of group 7; one hen of group 10).

Table 2 shows the means of zootechnical data over the whole study period for all groups. LB-hens had a significantly higher daily feed intake (P < 0.001) than LSL-hens, and thus a higher body weight at the end of the study (final body weight: LB: 1.91 kg/hen, LSL: 1.55 kg/hen).

Over the whole study, laying intensity was high (96.7 %) and it was significantly higher in LSL-hens (97.3) compared to LB-hens (96.1 %). LB-hens produced heavier eggs (59.3 g) than LSL-hens (58.2 g/egg), but the egg mass per hen and day was similar (56.6 and 57.0 g for LSL and LB-hen per day). Because of the significant lower feed intake and the small increased daily egg mass production, LSL-hens had a significantly lower FCR compared with LB-hens (Table 2).

In agreement with studies by Halle and Schöne (2013), RSC application also influenced the feed intake and the egg weight significantly (Table 2). Hens fed with a mixture without RSC consumed 4.7 g more feed per day and produced 1.8 g more egg mass daily. The FCR was decreased significantly, but – as the impact of iodine supplementation – this also has no biological relevance since the difference was minor (0 % RSC: 2.01 vs. 10 % RSC: 1.99 kg/kg; P > 0.05).

The iodine supplementation did not significantly influence laying intensity and egg weight, but showed some effects on feed intake and feed to egg mass ratio (Table 2). The significant impact of feed iodine supplementation on FCR was due to the small standard deviation, but has no biological relevance.

The effect of iodine supplementation on performance was assessed by examination of the groups without RSC component (groups 1 to 9). The daily feed intake, laying intensity and egg mass did not differ significantly between the groups. In none of the parameters significant differences of the groups 2 to 9 compared to the control group 1 in the respective month were found. The egg weight increased in all groups, from 50.9 ± 5.5 g in the first month up to 60.5 ± 5.9 g in the sixth laying month, while the daily feed intake (114.4 ± 12.2 g) did not vary significantly. Thus, the FCR decreased in all groups with the duration of the experiment from 2.2 ± 0.3 in the beginning to 1.9 ± 0.1 in the sixth laying month.

In conclusion, partially significant effects of the applied feed iodine supplementations were detected, but these differences were so small that a biological relevance cannot be deduced. This conclusion is supported by similar previous studies as those by Kaufmann et al. (1998) and Yalcin et al. (2004), when they supplied hens with equal iodine levels.

KI and $Ca(IO_3)_2$ showed no significant impact on measured parameters (Table 2). This result agrees with similar studies, dealing with the effect of feed iodine supplementation on performance in poultry (e.g. Hixson and Rosner, 1957; Groppel, et al., 1991; Kaufmann et al., 1998).

3.3 Weight of egg fractions during experiment

In agreement with published results (Ternes et al., 1994; Houpalathi et al., 2007), the egg weight in the present study increased with duration of laying period (Table 3). It was accompanied with an absolute and relative increase in the yolk fraction. The absolute albumen fraction increased also, but the relative fraction was nearly constant. Similar results are reported by Szentirmai et al. (2013), who fractionated eggs of Tetra Brown and Tetra White layers between the age of 20 and 72 weeks. The egg weight increased from 48.3 (Brown layers) and 42.4 (White layers) to >65 g (both genotypes) and the egg yolk ratio changed from 20.1/20.4 % (Brown/White) to >25 % (both genotypes) after 36 weeks of age until the end of their study. In contrast to the present study (Table 3), white egg layers had an about 2 % higher yolk ratio than brown layers. An increase of egg yolk ratio over the duration of the laying period was also reported by Hartmann et al. (2000) in hens and by Applegate et al. (1998)

Table 2

The main effects of dietary treatments [hen breed (LSL – Lohmann Selected Leghorn, LB – Lohmann Brown), rapeseed cake (RSC) in the feed, iodine sources (Iodide/Iodate) and supplementation level] on feed intake and laying performance of hens (12 hens per group; laying period: 168 days)

Group	Hen breed	RSC [1] (%)	Iodide (mg/kg FM)	Iodate (mg/kg FM)	Feed intake g/hen/day	Laying rate (%)	Egg weight (g/egg)	Feed to egg mass ratio (kg/kg)	Final body weight (g/hen)
1	LSL	0	0	0	114.3	97.4	60.3	1.96	1680
2		0	0,25	0	113.3	97.7	59.6	1.94	1608
3		0	0.50	0	111.5	96.9	58.6	1.97	1598
4		0	2.5	0	111.7	97.0	59.2	1.98	1593
5		0	5.0	0	112.2	96.9.	59.2	1.96	1561
6		0	0	0.25	114.4	96.6	58.6	2.02	1610
7		0	0	0.50	112.0	97.6	59.0	1.96	1623
8		0	0	2.5	109.8	97.1	58.5	1.94	1565
9		0	0	5.0	111.3	98.4	58.1	1.95	1619
10		10	0	0	107.8	97.2	58.1	1.94	1565
11		10	0.25	0	106.9	97.2	56.6	1.97	1552
12		10	0.50	0	107.8	97.3	57.7	1.93	1485
13		10	2.5	0	106.0	97.6	57.0	1.95	1446
14		10	5.0	0	104.3	98.1	57.1	1.88	1510
15		10	0	0.25	110.2	97.6	58.1	1.96	1526
16		10	0	0.50	105.1	97.4	57.5	1.90	1461
17		10	0	2.5	101.4	96.8	56.3	1.90	1493
18		10	0	5.0	107.6	97.0	57.4	1.96	1477
1	LB	0	0	0	117.1	93.7	60.0	2.12	1993
2		0	0.25	0	112.9	93.4	58.9	2.11	1962
3		0	0.50	0	118.2	96.2	60.8	2.03	1949
4		0	2.5	0	121.5	94.4	62.1	2.07	1937
5		0	5.0	0	115.8	97.0	59.3	2.01	1856
6		0	0	0.25	116.7	96.9	59.0	2.05	1862
7		0	0	0.50	119.3	97.2	62.0	1.99	1961
8		0	0	2.5	118.0	96.4	59.4	2.08	2030
9		0	0	5.0	114.9	95.5	59.8	2.04	1964
10		10	0	0	111.1	97.5	57.1	2.02	1831
11		10	0.25	0	114.6	96.3	58.0	2.06	1927
12		10	0.50	0	110.3	95.8	57.6	2.02	1852
13		10	2.5	0	113.3	96.2	59.4	2.04	1791
14		10	5.0	0	111.6	97.2	58.2	2.02	1961
15		10	0	0.25	118.9	97.0	59.8	2.09	1924
16		10	0	0.50	110.3	95.0	58.0	2.06	1807
17		10	0	2.5	115.0	95.8	60.7	2.00	1921
18		10	0	5.0	117.5	97.8	58.6	2.08	1880
Standard error					9.4	6.3	6.1	0.21	170
Anova, P-Values									
Hen breed					<0.001	<0.001	<0.001	<0.001	<0.001
RSC					<0.001	>0.05	<0.001	>0.05	<0.001
Iodide					>0.05	>0.05	>0.05	<0.05	>0.05
Iodate					<0.001	>0.05	>0.05	<0.05	>0.05

Table 3

Effect of hen breed (LSL – Lohmann Selected Leghorn; LB – Lohmann Brown) on egg fractions at 4th, 12th and 24th laying weeks (g per egg; % of total egg)

Week of laying	Hen breed	Number of eggs analyzed	Egg weight (g)	Shell (g)	Yolk (g)	Albumen (g)	Shell (%)	Yolk (%)	Albumen (%)
4	LSL	670	54.6 ^{e}	7.1 ^{b}	14.6 ^{e}	32.9 ^{e}	13.0 ^{a}	26.7 ^{e}	60.2 ^{d}
	LB	685	55.9 ^{d}	7.0 ^{c}	14.2 ^{f}	34.6 ^{d}	12.6 ^{b}	25.5 ^{f}	61.9 ^{a}
12	LSL	703	59.6 ^{c}	7.0 ^{c}	16.8 ^{c}	35.8 ^{c}	11.7 ^{d}	28.2 ^{b}	60.1 ^{d}
	LB	696	60.5 ^{b}	7.2 ^{a}	16.5 ^{d}	36.8 ^{b}	12.0 ^{c}	27.3 ^{d}	60.7 ^{c}
24	LSL	682	60.7 ^{b}	6.4 ^{e}	17.7 ^{a}	36.6 ^{b}	10.6 ^{e}	29.2 ^{a}	60.2 ^{d}
	LB	668	61.4 ^{a}	6.5 ^{d}	17.1 ^{b}	37.8 ^{a}	10.6 ^{e}	27.9 ^{c}	61.5 ^{b}
	Standard error	-	4.3	0.6	1.6	3.1	0.8	2.1	2.2

a,b, c, d within weeks of laying means with different letters in a column show significant differences between breeds

in ducks. Such changes may have influence on the nutrient content and the nutritive value of eggs in long term studies investigating iodine content of eggs (see Table 5).

3.4. Tissue iodine concentration

Table 4 shows the iodine concentration of muscle samples as well as liver and serum iodine concentration at the end of the study (168 days).

3.4.1 Blood serum

Blood acts as transport medium of iodine and thyroid hormones T_3, T_4, and inactive rT_3. Due to this function, the iodine concentration in blood is relatively high. In the present experiment, the iodine concentration of blood serum increased significantly with increasing iodine supplementation of the feed (Table 4). It was significantly reduced from 70.4 to 41.2 µg/l through the RSC component.

The results of studies comparing the effect of different iodine sources were inhomogeneous (Franke et al., 2009). Groppel et al. (1991) applied feed enriched with up to 100 mg I/kg as KI or KIO_3 to broilers and found no significant difference in the resulting iodine concentrations of the blood plasma, thyroid gland, muscle, heart, liver and kidney. Except the findings concerning blood samples, this agrees with the results of the present study. Usually, differences in iodine deposition in tissues were only reported, when organically bound iodine was compared with anorganic compounds (Rys et al., 1997). In the present study significant effects of the iodine source on the blood serum iodine concentration were detected (Table 4), which was also found in comparable studies (Röttger et al., 2011). However, until now, no homogenous picture could be given for the dependency of iodine concentration of blood serum on iodine source. Thus, further parameters like recent iodine uptake seem to play a role in this case.

3.4.2 Breast and thigh meat

Compared to other investigated tissues the iodine concentrations of meat (Table 4) were very low. In broilers (Röttger et al., 2011) the meat iodine concentrations increased significantly when the feed was enriched with iodine, while in laying hens no significant increase was measured during an experimental period of four weeks (Röttger et al., 2012). Thus, the iodine equilibrium of meat seems to be maintained even at long-term application of feed, supplemented with up to 5 mg I/kg. Meyer et al. (2008) measured the iodine concentrations of different muscles in growing bulls, such as *Musculus longissimus dorsi* and *Musculus glutaeus medius* and found different iodine concentrations. In laying hens the mean carry-over factor was higher in thigh meat (0.02) than in breast meat (0.01). Meyer et al. (2008) explained this effect with the metabolic rate of the muscles, and mentioned that also Downer et al. (1981) found higher iodine concentrations in metabolically more active muscles. In broilers this difference in carry-over factor was only found in groups provided with $Ca(IO_3)_2$ (Röttger et al., 2011). These findings give evidence that metabolic rate seems to be the only one impact factor of muscle iodine concentration, and may also be a reason for differences between hens and broilers. In addition, other impact factors as mentioned above may be also important (e.g. age, laying performance, growing, feeding regimes).

3.4.3 Liver

The higher iodine concentrations measured in liver tissue compared to muscle tissue (Table 4) results from its physiological function. The liver is an important location for the dehalogenation of T_4 to T_3 and rT_3 (Visser et al., 1988). In the liver a lower iodine concentration was detected in laying hens than in broilers (Röttger et al., 2011). Hence, besides the iodine supplementation, the amount of iodine accumulation in the liver seems to depend on further factors. Due to its function at thyroxine dehalogenation, the iodine status may be closely related to the metabolic rate, as indicated by the lower iodine level of livers of laying hens with less locomotion. Another reason could be related to the egg production of laying hens. Yolk-specific components such as precursor-molecules (e.g. vitellogenin) and triglycerides are synthesised within the liver and then transported to the ovary (Schneider et al., 1998). It is possible, that iodine in form of

Table 4

The main effects of dietary treatments [hen breed (LSL – Lohmann Selected Leghorn, LB – Lohmann Brown), rapeseed cake (RSC) in the feed, iodine sources (Iodide/Iodate) and supplementation level] on tissue iodine concentrations of laying hens after 168 feeding days

Group	Hen breed	RSC (%)	Iodide (mg/kg FM)	Iodate (mg/kg FM)	Breast meat (µg I/kg FM)	Thigh meat (µg I/kg FM)	Liver (µg I/kg FM)	Serum (µg I/L)
1		0	0	0	4.7	2.0	10.5	16.2
2		0	0.25	0	4.2	2.9	7.7	15.4
3		0	0.50	0	7.4	4.4	10.2	25.2
4		0	2.5	0	17.4	18.5	37.8	123.3
5		0	5.0	0	40.1	40.2	50.2	172.5
6		0	0	0.25	8.6	10.1	19.6	47.6
7		0	0	0.50	19.1	7.2	19.8	26.2
8		0	0	2.5	11.8	15.1	34.7	65.4
9		0	0	2.5	15.8	32.9	59.0	102.7
10	LSL	10	0	0	1.5	3.6	21.7	18.1
11		10	0.25	0	0.6	7.6	18.3	14.4
12		10	0.50	0	1.7	12.5	20.0	17.6
13		10	2.5	0	13.3	35.5	13.3	57.8
14		10	5.0	0	22.4	39.1	18.8	74.2
15		10	0	0.25	3.3	4.6	5.2	20.2
16		10	0	0.50	19.1	10.9	16.8	19.6
17		10	0	2.5	23.3	14.5	39.9	55.1
18		10	0	5.0	22.9	22.4	87.8	81.0
1		0	0	0	4.3	2.8	18.5	18.9
2		0	0.25	0	4.6	3.8	7.2	17.7
3		0	0.50	0	5.8	5.9	17.0	29.4
4		0	2.5	0	13.2	20.7	31.9	121.6
5		0	5.0	0	22.7	36.3	59.9	222.6
6		0	0	0.25	6.2	10.8	21.6	54.9
7		0	0	0.50	6.4	8.6	25.0	36.1
8		0	0	2.5	13.9	14.6	40.7	74.0
9		0	0	5.0	14.2	22.4	46.7	95.7
10	LB	10	0	0	0.9	7.6	38.6	14.7
11		10	0.25	0	0.4	6.1	24.3	18.8
12		10	0.50	0	0.5	13.7	19.8	18.4
13		10	2.5	0	8.7	15.7	12.1	40.4
14		10	5.0	0	15.2	20.9	18.7	93.2
15		10	0	0.25	11.7	5.9	6.0	19.6
16		10	0	0.50	20.0	15.2	19.5	22.6
17		10	0	2.5	18.0	21.5	28.5	54.3
18		10	0	5.0	21.0	23.6	21.5	102.3
Standard error					11.6	13.7	26.2	21.6
Overall mean					11.8	15.0	26.4	55.8
Anova, P-values								
Hen breed					>0.05	>0.05	>0.05	>0.05
RSC					>0.05	>0.05	>0.05	<0.001
Iodide					<0.001	<0.001	<0.05	<0.05
Iodate					<0.001	<0.001	<0.001	<0.001

thyroid hormones leaves the liver and enter the oocytes by this pathway. Further investigations are necessary, to find out which mechanism takes place, for example if thyroid hormones enter the oocyte within apolipoprotein particles or if receptor-mediated endocytosis takes place (McNabb and Wilson, 1997). This could be another reason – besides the active transport within the ovary – for the high iodine concentration in yolk, and a lower iodine concentration of liver of laying hens.

3.4.4 Mass and iodine concentration of thyroid gland

The iodine concentration of the thyroid glands increased significantly with increasing feed iodine supplementations. The iodine concentration in thyroids of birds fed with 10 % RSC was significantly lower than that of birds provided feed without RSC (2691 vs. 4654 µg I/g, $p < 0.0001$).

In groups fed diets without RSC there was no significant difference between thyroid masses after the different feed iodine supplementations, what indicates that iodine application up to 5 mg I/kg diet had no negative effect on thyroid mass.

The thyroid mass was significantly increased by 10 % dietary RSC (0.27 ± 0.05 g vs. 0.17 ± 0.03 g, $p < 0.0001$). The lower iodine concentration and the increased mass of the thyroid glands of groups provided with 10 % RSC reflect the competitive inhibition of the Sodium-Iodine-Symporter and the goitrogenic effect of the rapeseed glucosinolates (Schöne et al., 1993). It had been expected, that the iodine supplementation would balance this effect, and that hens fed with 10 % RSC and increased iodine supplementations would have normal thyroid masses. Instead, thyroid masses of hens supplied with 10 % RSC, increased with increasing iodine supply. This shows that iodine supply cannot balance the negative effect of 10 % RSC with a glucosinolate content of 13.8 mmol/kg RSC. Moreover, the effects of RSC and increased iodine supplementations seem to add up, which could have led to iodine induced hyperthyroidism (Stanbury et al., 1998).

Also Schöne et al. (1993) found an increase in thyroid gland mass of broilers, which may have occurred due to iodine deficiency, since the animals were fed diets containing rapeseed meal. In conclusion, long-term application of feed supplemented with 10 % RSC had a goitrogenic effect, which was enhanced by feed supplementation with iodine, indicated by significantly higher thyroid gland masses in these groups. In the RSC-groups the supplementation with 5 mg I/kg feed did not elevate the iodine concentrations in the thyroid glands up to the level of the control group (0 % RSC without iodine supplementation).

3.5 Iodine in eggs

Hens excrete a considerable amount of ingested iodine with the eggs (see Table 5). This is the most important difference in iodine storage between growing animals such as broilers, growing pigs and ruminants (Berk et al., 2008; Franke et al., 2009; Meyer et al., 2008; Röttger et al., 2011) on the one hand and laying hens and lactating mammals (Franke et al., 2009; Flachowsky et al., 2014) on the other hand.

All the experimental factors such as iodine source and level, rapeseed cake, hen breed and duration of experiment have had significant influence on iodine concentration of eggs. Increasing feed iodine supplementations caused a significant increase in the iodine concentration in the eggs (Table 5). Active iodine transport into growing oocytes of bird ovaries, which is hormonally controlled, was proved by Newcomer (1982) and Newcomer et al. (1984). The Sodium-Iodine-Symporter has been detected in ovaries of different mammals, for example in humans (Spitzweg et al., 1998) and mice (Perron et al., 2001), as well in amphibian species (*Xenopus laevis and Rana catesbeiana*, Carr et al., 2008). This Symporter has not yet been proven in the chicken ovary, but the high iodine concentrations in the yolk give strong evidence for an active transport of iodine into the yolk.

Duration of experiment has had a significant influence of iodine concentration in eggs. It increased from 138 (initial value) to 202 (4^{th} day) and to 448 to 480 µg/kg total egg until day 29 (Table 5). There was a further increase of iodine concentration in eggs up to 539 (85^{th} day) and 643 µg I/kg total egg (164^{th} day). Similar results are reported by Slupczynska et al. (2014). The mean value increased in their study from 596 after three month of feeding up to 744 µg/kg total egg after five month of feeding. The diets were supplemented with 1, 3 and 5 mg I/kg mixed feed. The increase of iodine in total eggs during experimentation may be caused by saturation of all hen organs with iodine and the increase of proportion of egg yolk during laying period (see Table 3). This also explains the higher iodine proportion measured in the yolk, compared to albumen (KI: 93 % in yolk, 7 % in albumen; $Ca(IO_3)_2$: 87 % in yolk, 13 % in albumen). Similar values are also reported by Richter (1995); Kaufmann et al. (1998); Stibilj and Holcman (2002) and Röttger et al. (2012). It is assumed that iodine enters the egg albumen 18 to 20 hours before laying via diffusion during its formation within the tuba (magnum). Increasing of feed iodine supplementation has also been investigated by other authors (e.g. Richter, 1995; Lewis, 2004; Röttger et al., 2012; Saki et al., 2012; Slupczynska et al., 2014). They found a significant increase of the iodine content of eggs (Table 5).

There are significant differences between both I-sources. The mean value of the I-content of eggs was much higher for KI (472 µg/kg) compared with $Ca(IO_3)_2$ (389 µg/kg total egg). One reason for lower iodine content in eggs could be the lower intestinal uptake of iodate, since this has to be converted into iodine (Lewis, 2004). Slupczynska et al. (2014) compared KI with KIO_3 and found in agreement with present data (see Table 5) after three months of supplementation of 3 mg I/kg feed 671 µg for KI and 522 µg for KIO_3, and after five months treatment 816 µg for KI and 698 µg/ kg eggs for KIO_3.

Apart from the iodine supply RSC has also had a significant influence on the iodine content of eggs (see Table 5). The mean of the I-content of eggs was much higher in diets without RSC (490 µg/kg) compared with 10 % RSC in mixed feed (372 µg/kg total egg). The supplementation reduced the iodine concentration of eggs on average by 24 %. Similar

Table 5

The main effects of dietary treatments [hen breed (LSL – Lohmann Selected Leghorn, LB – Lohmann Brown), rapeseed cake (RSC) in the feed, iodine sources (Iodide/Iodate) and supplementation level] on feed intake and laying performance of hens on Iodine concentration of eggs (µg/kg edible eggs) depending on day of experimentation

Group	Hen breed	RSC (%)	Iodide (mg/kg FM)	Iodate (mg/kg FM)	Day 4	Day 8	Day 15	Day 29	Day 85	Day 164
1	LSL	0	0	0	104	106	105	84	112	172
2		0	0.25	0	131	175	167	142	215	264
3		0	0.50	0	123	174	212	217	275	391
4		0	2.5	0	199	539	542	536	614	759
5		0	5.0	0	460	1147	1348	1433	1319	1603
6		0	0	0.25	127	156	148	163	200	374
7		0	0	0.50	130	177	204	196	267	433
8		0	0	2.5	219	557	610	626	675	920
9		0	0	5.0	299	904	1184	1138	1300	1186
10		10	0	0	129	98	80	71	100	113
11		10	0.25	0	141	135	186	104	126	195
12		10	0.50	0	98	121	160	123	167	206
13		10	2.5	0	221	575	590	515	589	603
14		10	5.0	0	247	907	914	949	1034	1066
15		10	0	0.25	100	105	101	102	149	167
16		10	0	0.50	109	157	124	119	148	182
17		10	0	2.5	198	538	479	479	429	654
18		10	0	5.0	269	891	855	927	796	996
1	LB	0	0	0	135	117	138	116	150	220
2		0	0.25	0	227	229	188	168	294	323
3		0	0.50	0	152	192	245	254	338	494
4		0	2.5	0	287	732	599	764	833	1021
5		0	5.0	0	434	1315	1514	1570	1729	1858
6		0	0	0.25	148	182	164	167	240	439
7		0	0	0.50	167	236	241	295	296	510
8		0	0	2.5	268	725	651	700	843	1157
9		0	0	5.0	469	1217	1342	1310	1384	1754
10		10	0	0	129	107	82	100	122	135
11		10	0.25	0	148	138	124	169	177	225
12		10	0.50	0	145	226	218	213	253	262
13		10	2.5	0	209	536	569	648	669	675
14		10	5.0	0	350	925	1034	1140	1236	1302
15		10	0	0.25	119	185	130	150	271	200
16		10	0	0.50	137	191	180	195	213	263
17		10	0	2.5	184	507	490	503	532	661
18		10	0	5.0	283	1057	1047	1151	1246	1425
Standard error					62.3	120.9	120.0	130.2	116.0	147.0
Overall mean					202	448	467	480	537	643
Anova, P-values										
Hen breed					<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
RSC					<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Iodide					<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Iodate					<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

results are reported by Goh and Clandinin (1977); Schöne et al. (2013) and Slupczynska et al. (2014). Slupczynska et al. (2014) compared diets without with diets containing 10 % rapeseed meal in feed of Hy-Line Brown Pullets. Rapeseed meal reduced the I-content from 671 to 522 µg after three months and from 814 to 698 µg/kg egg after five months of feeding of diets with 3 mg I/kg.

Also hen breeds (LSL and LB) have had influence on the iodine content of eggs (see Table 5). LB hens accumulated higher amounts of iodine in all phases of laying period and for all dosage levels in their eggs than LSL-hens hens. An influence of hen breed on iodine concentration in eggs can be seen in Table 5 and it is also described by other authors (e.g. Rys et al., 1997). The all over average without RSC for LSL-hens was 442 and for LB-hens 539 µg I/kg egg mass; the values with 10 % RSC were 336 and 404 µg I/ kg resp. 76 and 75 % of values without RSC.

3.6 Carry over of iodine from feed into eggs

Carry over describes the transition of one substance (e.g. iodine) from one compartment (e.g. feed) into another one (e.g. eggs) in the food chain (Schenkel, 2012). The iodine intake per kg egg mass is calculated based on iodine content of feed and the feed to egg mass ratio. There is a clear tendency of carry over depending on the level of iodine supplementation of feed (Table 6). The higher the I-supplementation, the lower is the carry over from feed into eggs (decrease from 21.5 to 12.5 %). The iodine sources have only a small influence on the carry over into eggs (18.0 for KI and 16.0 % for $Ca(IO_3)_2$; Table 6). The average of I-carry over for LSL-hens is 15.3; that of LB hens was calculated as 18.6 %. Large differences were calculated between groups without RSC in the diets (19.6 %) on one side and with 10 % RSC in the diets (14.3 %) on the other side (see Table 6). The carry over values given above and in Table 6 are more or less in agreement with previous literature reports (e.g. Romijn and Velthuizen, 1955: 21 %; Richter, 1995: 12.5 to 18.6 %; Unak et al., 2003: 15 %; Röttger et al., 2012: 9.7 to 14.1 %).

Table 6
Carry over values of Iodine from feed into eggs (% of Iodine intake found in eggs)

Influencing factors	Carry over (%)
Iodine supplementation (mg/kg FM)	
0.25	21.5
0.5	18.1
2.5	12.8
5	12.5
Iodine source	
KI	18.0
$Ca(IO_3)_2$	16.0
Rapeseed cake in diet	
Without	19.6
+ 10 % RSC	14.3
Hen breed	
Lohmann Selected Leghorn (LSL)	15.3
Lohmann Brown (LB)	18.6

3.7 Contribution of eggs to I-intake of humans

The iodine content of eggs depends on many influencing factors as shown and discussed above. Table 7 summarizes the influence of iodine content in feed as one of the most important factors for the iodine content in eggs. It also shows the impact of the number of daily consumed eggs on the iodine intake and the contribution to meet the iodine requirements of consumers (150 µg/d) according to EFSA (2014). One egg per day of hens supplemented with 1 mg I per kg feed may cover the iodine requirements of adults up to about 10 %, but one egg per day of hens supplemented with 3 or 5 mg I per kg feed covers about 25 or 40 % of daily human demand (Table 7). Travnicek et al. (2006) concluded that one egg per day from large hen flocks with I-supplementation would cover 7 to 14 % of human requirements. Sager (2011) analysed Austrian eggs and found 31 µg I per egg, what is adequate to about 20 % of iodine requirements of adults. Villanueva (2016) calculated about 14 µg iodine intake per day from eggs for Catalan people.

Table 7
Model calculation of the contribution of different numbers of daily consumed eggs (½, 1, 2, 3 eggs) to the daily iodine intake of consumers, depending on iodine supplementation of the hen feed (1, 3, 5 mg I/kg feed FM); shown as total amount of iodine contributed by eggs and percentage shares of the daily iodine demand EFSA, 2014; calculation by Schultz, 2012)

Iodine concentration of the hen feed [mg/kg FM]	1	3	5	1	3	5
Number of daily consumed eggs	Total amount of iodine contributed by eggs [µg]			% of daily demand (150 µg/d)		
½	7.4	18.6	29.8	4.9	12.4	19.9
1	14.8	37.2	59.6	9.9	24.8	39.7
2	29.5	74.3	119.1	19.7	49.5	79.4
3	44.3	111.5	178.7	29.5	74.3	119.1

4 Conclusions

Iodine source and level of iodine supplementation up to 5 mg/kg mixed feed did not significantly influence feed intake, laying performance and feed conversion ratio of hens. Heavier hens consumed more feed, laid heavier eggs, had a lower laying rate and needed more feed per egg mass than hens lighter in weight. Egg weight and yolk ratio increased with duration of laying. Such changes may have influence on the nutritive value of eggs. Rapeseed cake reduces the feed intake and the egg weight, but not the laying rate and the feed to egg mass ratio.

Iodine supplementation significantly increased the iodine-concentrations in all analysed body samples and in eggs. The highest iodine concentration was measured in the thyroid gland. Eggs showed the highest iodine concentration in groups supplemented with 5 mg I/kg, about 91 % of iodine was deposited in the yolk and only 9 % in the egg albumin. All measured tissue samples of hens fed with 10 % RSC contained lower iodine concentrations than samples from non-RSC-hens. Its supplementation reduced the iodine concentration of eggs on average by 24 %. Laying hen breeds and iodine sources did also influence the I-concentration in body samples and eggs, but to a smaller degree. Under consideration of data mentioned above, eggs may substantially contribute to meet human requirements for iodine.

A further reduction of the presently authorised maximum iodine concentration in complete feed for laying hens from 5 to 3 mg/kg feed with 88 % DM as recommended by the EFSA (2013a) and the EU (2015) would not have any practical consequences on iodine content of eggs, because of already lower iodine concentration in complete feed for laying hens (0.5 to 1.5 mg I/kg; Grünewald et al., 2006) under German field conditions.

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