Effect of various sources and levels of iodine, as well as the kind of diet, on the performance of young laying hens, iodine accumulation in eggs, egg characteristics, and morphotic and biochemical indices in blood

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ABSTRACT Young hens were fed over a period of 150 d with 2 kinds of diets including corn and soybean meal or corn, soybean, and rapeseed meal. Diets were enriched with potassium iodide (KI) or potassium iodate (KIO_3) as an I source in amounts equal to 1, 3, or 5 mg of supplemented I/kg of feed. The hen performance, egg quality, hematological and morphotic indices in blood, hepatic enzyme activity, lipid indices in blood serum as well as I accumulation in wet egg content were determined. Introduction of 00-variety rapeseed meal into the diet improved the laying rate and feed conversion ($P < 0.05$); however, better egg weight was noted by feeding the hens with a diet without rapeseed meal. Use of KI as an I source enhanced the egg weight. The increased I level in the diet had an equivocal influence on egg weight, improved the feed conversion per 1 kg of eggs, and decreased the proportion of damaged eggs. The use of corn, soybean, and rapeseed meal in hen diets significantly improved yolk color; similar results were noted after an increase in I levels in the diets after 3 mo of feeding. Hematological indices of hen blood demonstrated significantly higher red blood cells numbers and hemoglobin concentrations with the use of KI. The use of a diet containing rapeseed meal led to an enhancement of hepatic enzyme activity, especially of alkaline phosphatase ($P = 0.007$). Lipid metabolism indices were not influenced by the kind of diet or the I source or level. The accumulation of I in wet egg content was negatively influenced by the use of a diet containing rapeseed meal ($P = 0.000$). The application of KI as an I source enhanced ($P = 0.003$) the accretion of I in eggs after 5 mo of treatment. Enhanced I supply significantly increased accumulation of I in eggs ($P = 0.000$) after 3 and 5 mo of the experiment from 260 and 310 to 1,011 and 1,256 μg/kg of wet egg content, respectively.

Key words: hen, iodine, performance, egg, iodine accumulation

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INTRODUCTION

The biological necessity of I is mainly related to thyroid gland functions (Van Middlesworth, 1996), especially as a constituent of triiodothyronine and tetraiodothyronine hormones, which play an essential role in metabolism regulation, intermediary cell activity, and cellular oxidation processes (Hetzel and Maberly, 1986; Sourgens et al., 1986; McDowell, 1992; Delange, 1998; Goldhaber, 2003; Lewis, 2004). Moreover, reproduction properties (Trávníček et al., 1997), circulation and muscular systems, maturity processes of cells and tissues, functions of the brain and nervous system, and pituitary gland activity, skin, and secondary skin product formation and regeneration indirectly depend on the supply of I to an organism (Delange, 1994, 1998, 2002; Van Middlesworth, 1996; Liu et al., 2001). Thyroid hormones perform an important role in the regulation of cholesterol levels in humans and rats (Mathe and Chevallier, 1976; Ryś et al., 1997). Excess of this trace element (200–500 mg of I/kg) evokes a dangerous toxicosis in organisms and disorders in the conversion of triiodothyronine to thyroxin (Arrington et al., 1967; Marcilese et al., 1968; Bürgi et al., 2001; Goldhaber, 2003; Baker, 2004).

Plant foods used in human nutrition are generally poor in I, which leads to insufficient I intake and, in consequence, to I deficiency (Haldimann et al., 2005, Röttger et al., 2012). With the aim of increasing I levels in human diets, the application of iodized salt has been popular. However, this is not always a beneficial solution for all human groups (i.e., children, pregnant woman, or the sick; Nair et al., 1998; Szybinski et al., 2001; Brzóska et al., 2005). Iodine may be provided

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in I-enriched milk, fish, and sea products (He et al., 2000; Delange, 2002; Haldimann et al., 2005), but also through fortification of I contents in basic human food (e.g., in eggs; Groppel et al., 1991; Kaufmann et al., 1998, Yalçin et al., 2004).

Enrichment of animal origin products with I could be achieved in a nutritional way by application of various dietary I sources and I levels added into the animal diet. Compounds such as KI, NaI, $Ca(IO_3)_2$, and $Ca(IO_3)_2 \times 6H_2O$ are allowable as I sources. The NRC (1994) recommendations stated the I requirement for young and adult hens at 0.33 to 0.48 mg of I/kg, whereas the German Society for Nutrition Physiology (**GfE**, 1999) recommendations permit the level of 0.5 mg of I/kg of DM of feed. In EU Commission (2005) regulation 1459/2005 (WHO, UNICEF, ICCIDD, 2001; EFSA, 2005), the permitted I levels for laying hens were reduced from 10 to 5 mg per kg of feed (12% of humidity). Therefore, the acceptance by ethical commissions of studies in which greater I dietary levels will be used may be impossible.

Absorption and utilization of I from animal diets could be negatively modified by goitrogenic substances that are present in some plant origin feeds, especially those originating from plants of the *Cruciferae* family. The use in animal feed of rapeseed products that has been popular in many countries may decrease the I binding in thyroid hormones and affect thyroid gland functions (Wheeler and Hoffman, 1950; Wemheuer and Paufler, 1993; Tripathi et al., 2001a,b).

The aim of the present study was to examine the effect of various sources and levels of bioactive mineral elements (i.e., I supplements) as well as the presence of rapeseed meal in laying hen diets on the efficacy of egg content enrichment with I. Moreover, performance and egg traits were recorded during performed studies. Because high levels of I were applied for the whole experiment, morphological and biochemical indices in blood and blood serum were assayed as indices of the health status of hens.

MATERIALS AND METHODS

Birds and Management

Sixteen-week-old Hy-Line Brown pullets (1.34 kg of BW) were located in battery pens and before the laying period were fed with a mixture containing about 145 g/kg of CP and 11.5 MJ/kg of ME.

At the age of about 18 wk, at the beginning of laying, the hens were allocated into cages (3 hens per cage) according to BW and laying at the start time. Afterward, the cages were randomly divided into 12 treatments each consisting of 6 replications (i.e., 18 hens per treatment). The free access of birds to water (nipples) was ensured. The environmental conditions in the summer to autumn period were registered; the room temperature varied between 16 and 25°C and the lighting program was set up for 14 h of light daily. The management was in compliance with European Union regulations. The Local Ethics Commission for Experiments with Animals accepted all experimental procedures.

Diets and Feeding Program

The diet composition was based on our own chemical analyses of feed components and was calculated using linear optimization. The energy concentration was calculated by the use of values given in the European Table of Energy Values for Poultry Feedstuffs (WPSA, 1989). Both prepared basal diets [i.e., corn-soybean mixture (**CSM**) and corn-soybean-rapeseed meal mixture (**CSRM**)] were isoproteic and isoenergetic and contained about 165 g/kg of CP and 11.6 MJ of ME/kg. Each diet was enriched with I using either KI or KIO_3 in amounts appropriate to the anticipated I supplement at the level of 1, 3, and 5 mg of I/kg of feed (Table 1). All the feed mixtures were prepared before the experiment. A homogenous mixture of I within the feed was obtained by preparation of premixtures of I supplements with an I free mineral-vitamin premix that was made for the purposes of the experiment. The determined concentration of I in 1 kg of basal diet consisted of 0.52 and 0.49 mg of I/kg of CSM and CSRM, respectively. The feed mixtures were given at a dosage of 110 g per hen daily.

Analytical Methods in Feed Mixtures

The chemical composition of feed components and complete mixtures was determined according to standard methods (AOAC International, 2000). The nitrogen content was determined using a Kjeltec 2300 Foss Tecator apparatus (Höganäs, Sweden) and CP content was calculated by multiplying the nitrogen content by 6.25 (AOAC, 984.13). Crude fiber content was analyzed according to the Henneberg-Stohmann method with the use of a Fibertec Tecator apparatus (Häganäs, Sweden, AOAC, 978.10). After prior wet mineralization of samples with nitric acid (HNO_3) using a MarsX apparatus (CEM Corporation, Matthews, NC), calcium and sodium content in feeds was determined by atomic absorption spectrophotometry using an AA 240 FS apparatus with SIPS 20 (Varian, Mulgrave, Australia; AOAC, 968.08). Phosphorus concentration in feeds was analyzed after prior wet mineralization with nitric acid (HNO_3) and perchloric acid ($HClO_4$) according to the ammonium vanadomolybdate method using a Specol 11 spectrophotometer (Carl Zeiss, Jena, Germany) at a wavelength of 470 nm (AOAC, 965.17). With the aim of determining amino acid composition, samples of components were hydrolyzed with 6 *M* hydrochloric acid (HCl) for 24 h at 110°C, then amino acids were separated according to the Moore and Stein (1963) method. For the determination of sulfur amino acids, the feed

Table 1. Basal composition of the experimental diets[1]

Item	Diet	
	CSM	CSRM
Ingredient, % of diet		
Corn meal	15.00	15.00
Wheat meal	28.44	24.09
Barley meal	20.00	20.00
Soybean meal, solvent extracted	20.18	13.23
Rapeseed 00 meal, solvent extracted	—	10.00
Rapeseed oil	4.28	5.71
Dicalcium phosphate	0.481	0.435
Chalk	8.931	8.851
NaCl	0.015	0.013
DL-Methionine	0.046	0.024
L-Lysine	0.128	0.148
Vitamin and mineral premix[2]	2.50	2.50
Nutritive value of mixtures		
ME, MJ/kg	11.65	11.65
CP, g/kg	165.00	162.00
Crude fiber, g/kg	36.26	28.97
Ca, g/kg	38.80	38.90
P available, g/kg	4.30	4.32
Na, g/kg	1.70	1.71
Methionine, g/kg	4.30	4.31
Lysine, g/kg	9.00	8.97
I, mg/kg	0.52	0.49
Nutritive recommendation for Hy-Line laying hens		
ME, MJ/kg	11.65	
CP, g/kg	165.00	
Crude fiber, g/kg	to 40.00	
Ca, g/kg	38.80	
P available, g/kg	4.30	
Na, g/kg	1.70	
Methionine, g/kg	4.30	
Lysine, g/kg	9.00	

[1]CSM = corn-soybean mixture; CSRM = corn-soybean-rapeseed meal mixture.

[2]Supplied per kilogram of diet: vitamin A, 12,000 IU; vitamin D_3, 3,300 IU; vitamin E, 30.0 mg; vitamin K_3, 3.0 mg; vitamin B_1, 2.20 mg; vitamin B_2, 6.60 mg; vitamin B_6, 3 mg; vitamin B_{12}, 27 μg; pantothenic acid, 10.2 mg; nicotinic acid, 30 mg; folic acid, 0.9 mg; choline, 0.315 mg; Mg, 113 mg; Zn, 72 mg; Co, 0.48 mg; Cu, 10.0 mg; Fe, 60 mg; Se, 0.30 mg; I, 0.0 mg.

samples were oxidized (0°C, 16 h) with formic acid and hydrogen peroxide (H_2O_2; 9:1, vol:vol) before HCl hydrolysis and then were separated using an AAA 400 Ingos Analyzer (Prague, Czech Republic). The I content in basal mixtures was analyzed by a spectrophotometric method based on the Sandell-Kolthoff reaction (Gąsior and Szczypuła, 2010).

Performance, Egg Traits—Data Collection

Data collection was realized for very young hens with the aim of the designation of their reaction to I supply over the period of the beginning of egg production.

The laying performance of young hens was recorded over a period of 150 d. The number of laying eggs, from 10 d after the start of laying, was registered for each cage daily and all the eggs were weighed individually. Moreover, the number of damaged eggs were recorded. Feed intake and the mortality of hens were controlled weekly for each cage-replication. On the basis of the collected data mean laying rates (%), egg weight (g), egg mass (g/head per day), feed efficiency per egg (g/g), and per kilogram of eggs (kg/kg), as well as the number of damaged eggs (%) were calculated for the whole period of the experiment.

After 3 and 5 mo of feeding with the experimental diets, 30 eggs from each treatment were randomly sampled. Collected eggs were weighed as well as the yolk, albumen, and eggshell. Eggshell thickness (mm) was measured with a micrometer screw (0.001-mm micrometer IP 54, Wilson Wolpert, Maastricht, the Netherlands). Eggshell breaking strength (kg) was measured using a ZWICK/Roell (Ulm, Germany) apparatus. Each measurement was repeated twice. Egg yolk color intensity was evaluated visually using a La Roche yolk color fan scale.

Determination of Iodine Content in Eggs

At the end of 3 and 5 mo of the experiment, 12 eggs were randomly sampled from each treatment. Their content was freeze-dried and mineralized at 580°C with $NaOH + ZnSO_4 \times 7H_2O$ supplement. Iodine concentrations in egg content were determined according to the method modified by Gąsior and Szczypuła (2010), on the basis of the speed of reaction catalyzed by I: 2

Table 2. Laying hen performance[1]

Item	Laying rate, %	Egg weight, g	Egg mass, g/hen per day	Feed efficiency		Damaged eggs, %
				Per egg, g	Per kg of eggs, kg	
Treatment						
CSM						
1 mg of I as KI	83.0	56.0	45.5	122	2.18	$5.20^{A,a}$
3 mg of I as KI	$77.5^{A,a}$	56.6	$43.8^{A,a}$	$132^{A,a}$	$2.32^{A,a}$	2.23^{b}
5 mg of I as KI	87.3^{b}	57.3^{A}	50.1^{B}	116^{b}	2.02^{B}	2.37^{b}
1 mg of I as KIO_3	83.8	55.7^{B}	46.6	121	2.17	3.58
3 mg of I as KIO_3	88.4^{B}	56.0	49.5^{b}	114^{B}	2.04^{B}	2.53
5 mg of I as KIO_3	85.5	56.3	48.1	118	2.10	2.04^{b}
CSRM						
1 mg of I as KI	86.0	56.6	48.7	118^{b}	2.08	4.02
3 mg of I as KI	85.1	56.0	47.6	119	2.13^{b}	1.69^{B}
5 mg of I as KI	88.8^{B}	55.6^{B}	49.4^{b}	114^{B}	2.04^{B}	1.50^{B}
1 mg of I as KIO_3	87.2^{b}	56.0	48.8^{b}	116^{b}	2.07^{b}	4.39
3 mg of I as KIO_3	86.1	55.0^{B}	47.3	117^{b}	2.14	2.61
5 mg of I as KIO_3	86.2	56.7	48.8^{b}	117^{b}	2.07	3.16
SEM	0.605	0.103	0.337	0.001	0.016	0.201
P-value	0.009	0.000	0.006	0.004	0.004	0.000
Kind of diet						
CSM	84.2^{a}	56.3^{a}	47.4	120^{a}	2.13	2.99
CSRM	86.6^{b}	56.0^{b}	48.4	116^{b}	2.09	2.89
I source						
KI	84.6	56.4^{a}	47.7	120	2.13	2.83
KIO_3	86.2	55.9^{b}	48.2	117	2.10	3.05
Level of I supplement, mg						
1	85.0	56.1	47.7	119	2.13^{ab}	4.29^{A}
3	84.3	55.9^{a}	47.0^{a}	121	2.16^{a}	2.26^{B}
5	87.0	56.5^{b}	49.1^{b}	116	2.06^{b}	2.27^{B}
Source of variation, *P*-value						
Kind of diet	0.035	0.038	0.093	0.026	0.067	0.778
I source	0.157	0.011	0.389	0.108	0.288	0.520
I level	0.120	0.014	0.020	0.106	0.021	0.000
Kind of diet × I source	0.112	0.131	0.200	0.082	0.145	0.026
Kind of diet × I level	0.709	0.006	0.346	0.636	0.363	0.896
I source × I level	0.011	0.125	0.031	0.010	0.027	0.212
Kind of diet × I source × I level	0.111	0.006	0.036	0.090	0.036	0.632

[a,b]Means within a column marked with different superscripts differ significantly at $P < 0.05$.

[A,B]Means within a column marked with different superscripts differ significantly at $P < 0.01$.

[1]CSM = corn-soybean mixture; CSRM = corn-soybean-rapeseed meal mixture.

$Ce^{4+} + As^{3+} \rightarrow 2\ Ce^{3+} + As^{5+}$ (Knapp et al., 1998; Zhang et al., 2005). The principle of the assessment is the reduction of Ce^{4+} to Ce^{3+} in the presence of As^{3+}, where iodide ions (I^-) act as a catalyst for this reaction.

Blood Collection and Analysis

At the end of the experiment, 6 hens were randomly selected from each treatment and designated for blood sampling. Blood samples were taken from the vena brachialis by a specialized veterinarian and divided into 2 test tubes. In whole heparinized blood samples, the health status hematological indices were determined: red blood cells (**RBC**), hemoglobin (**HGB**), hematocrit, platelets, and leukogram—white blood cells, as well as the proportions of lymphocytes, heterophiles, monocytes, basophils, and eosinophils. Moreover, the activity of hepatic enzymes was assayed in blood serum: aspartate aminotransferase (**AST**), alanine aminotransferase (**ALT**), and alkaline phosphatase (**ALP**). The same was performed for indices of lipid metabolism: triglycerides, total cholesterol, high-density lipoproteins, and low-density lipoproteins. The hematological indices were assayed by the optic and impedance method using a Cell-Dyn 3700 (Abbott Laboratories, Abbott Park, IL). In blood smears colored according to the May–Grunwald–Giemza method, leukograms were microscopically determined by the use of a Burker chamber and Natt and Harric liquid. Biochemical indices in blood serum were determined spectrophotometically by the use of an XL300, Erba apparatus, and Biosystem chemicals. All blood analyses were conducted at Vet-Lab, a certified veterinary laboratory in Wroclaw.

Statistical Evaluation of Results

All numerical data were evaluated using 1-, 2-, or 3 factorial ANOVA using Statistica version 10 computer software (Statistica, StatSoft Inc., 2012). The differences among treatments and for experimental factors were tested according to the following statistical models:

$$y_{ij} = \mu + \alpha_i + e_{ij}$$

(for differences between treatments),

$$y_{jik} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

(for kinds of diet or inclusion levels of I supplement or their levels), or

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + e_{ijkl},$$

where y_{ij}, y_{ijk}, and y_{jikl} are the variance associated with parameter α; μ is the overall mean; α_i is the treatment effect; β_j is the kind of diet, I-supplement, or their level; γ_k is an effect for the iodine level; $(\alpha\beta)_{ij}$ is the interaction effect; and e_{ij} or e_{ijk} (e_{ijkl}) is an error term.

The individual measurements or averages for replication laying rates or feed consumption were treated as experimental units, and differences between treatment averages (means) were analyzed for significance ($P < 0.05$ or $P < 0.01$) using Tukey's multiple-range test. All numerical data in tables are presented as average values and are accompanied by SEM values.

RESULTS AND DISCUSSION

Hen Performance

The productivity of young hens was acceptable. The laying rate over the 150 d of the experimental period was a mean of about 85%, with an average egg weight of about 56 g. Hen performance was affected by experimental factors (Table 2). Hens receiving 10% rapeseed meal in their diet demonstrated higher laying rates ($P = 0.035$) and better feed efficiency per egg ($P = 0.026$), but lower egg weights ($P = 0.038$) compared with those in hens receiving rations without rapeseed meal. Thus, it could be stated that incorporation of 10% rapeseed meal into the feed mixture for hens had a positive effect on hen performance. Gheisari et al. (2011), in experiments with laying hens, stated that including different levels (0, 5, 10, 15, and 20%) of rapeseed meal in the

Table 3. Effect of experimental factors on egg characteristics after 3 mo of treatment[1]

Item	Proportion of egg weight, %			Yolk color, Roche points	Shell strength, kg	Shell thickness, mm
	Yolk	Albumen	Shell			
Treatment						
CSM						
1 mg of I as KI	29.9	56.4	13.3	12.7	3.45	0.354
3 mg of I as KI	27.8	57.9	14.1	11.5^{A}	3.95	0.347
5 mg of I as KI	30.3	56.5	14.2	13.0^{BCD}	3.70	0.344
1 mg of I as KIO_3	30.6	55.5	14.3	13.2^{BCD}	3.97	0.372
3 mg of I as KIO_3	30.9	54.2	14.9^{A}	12.7	3.42	0.376
5 mg of I as KIO_3	30.1	55.2	14.7	12.2^{B}	4.07	0.380
CSRM						
1 mg of I as KI	29.5	57.1	14.0	12.5^{ABC}	3.47	0.355
3 mg of I as KI	30.4	55.5	14.2	13.2^{BCD}	3.52	0.358
5 mg of I as KI	29.4	56.1	14.5	14.0^{D}	3.35	0.372
1 mg of I as KIO_3	29.1	56.1	14.1	12.5^{ABC}	3.77	0.351
3 mg of I as KIO_3	28.0	58.0	14.3	13.0^{BCD}	3.95	0.353
5 mg of I as KIO_3	28.8	58.1	13.0^{B}	13.7^{CD}	3.85	0.349
SEM	0.236	0.298	0.110	0.113	0.068	0.003
P-value	0.096	0.102	0.011	0.000	0.304	0.266
Kind of diet						
CSM	29.9	55.8	14.2	12.6^{A}	3.76	0.362
CSRM	29.1	56.8	14.0	13.2^{B}	3.65	0.356
I source						
KI	29.5	56.6	14.1	12.8	3.57	0.355
KIO_3	29.5	56.1	14.2	12.9	3.84	0.363
Level of I supplement, mg						
1	29.7	56.2	13.9	12.7^{a}	3.67	0.357
3	29.3	56.4	14.4	$12.6^{A,a}$	3.71	0.358
5	29.6	56.5	14.1	$13.2^{B,b}$	3.74	0.362
Source of variation, *P*-value						
Kind of diet	0.095	0.094	0.251	0.000	0.418	0.398
I source	0.945	0.387	0.404	0.577	0.051	0.194
I level	0.647	0.882	0.177	0.003	0.897	0.863
Kind of diet × I source	0.012	0.005	0.002	0.100	0.291	0.004
Kind of diet × I level	0.630	0.916	0.130	0.000	0.583	0.893
I source × I level	0.758	0.498	0.040	0.024	0.252	0.878
Kind of diet × I source × I level	0.036	0.095	0.282	0.032	0.192	0.464

[a,b]Means within a column marked with different superscripts differ significantly at $P < 0.05$.

[A–D]Means within a column marked with different superscripts differ significantly at $P < 0.01$.

[1]CSM = corn-soybean mixture; CSRM = corn-soybean-rapeseed meal mixture.

Table 4. Effect of experimental factors on egg characteristics after 5 mo of treatment[1]

Item	Proportion of egg weight, %			Yolk color, Roche points	Shell strength, kg	Shell thickness, mm
	Yolk	Albumen	Shell			
Treatment						
CSM						
1 mg of I as KI	31.6	54.6	13.8^{A}	12.7	4.25	0.369
3 mg of I as KI	31.3	53.9	14.4	13.5	4.02	0.371
5 mg of I as KI	31.9	53.1	14.9	12.2	3.82	0.372
1 mg of I as KIO_3	32.9	52.0	15.2	13.0	4.17	0.379
3 mg of I as KIO_3	33.1	51.1^{a}	14.4^{A}	12.7	4.10	0.369
5 mg of I as KIO_3	32.6	52.3	15.1	12.5	3.65	0.347
CSRM						
1 mg of I as KI	32.3	52.3	15.0	13.0	4.05	0.379
3 mg of I as KI	33.9	50.7^{a}	15.3	12.7	3.70	0.347
5 mg of I as KI	29.9	55.2	15.2	13.0	3.70	0.360
1 mg of I as KIO_3	32.7	52.0	15.3	13.2	3.27	0.350
3 mg of I as KIO_3	32.1	52.1^{b}	16.0^{B}	13.5	3.65	0.367
5 mg of I as KIO_3	31.4	54.5	14.3^{A}	13.5	3.47	0.354
SEM	0.238	0.289	0.107	0.091	0.069	0.002
P-value	0.069	0.006	0.000	0.056	0.072	0.051
Kind of diet						
CSM	32.2	52.5	14.6^{A}	12.7^{a}	4.00^{A}	0.367
CSRM	32.0	52.8	15.2^{B}	13.1^{b}	3.64^{B}	0.360
I source						
KI	31.8	53.3	14.8	12.8	3.92	0.366
KIO_3	32.5	52.3	15.0	13.0	3.72	0.361
Level of I supplement, mg						
1	32.4	52.7	14.8	13.0	3.93	0.369
3	32.6	51.9^{A}	15.0	13.1	3.86	0.363
5	31.4	53.8^{B}	14.8	12.8	3.66	0.358
Source of variation, *P*-value						
Kind of diet	0.652	0.945	0.001	0.029	0.006	0.104
I source	0.126	0.052	0.117	0.214	0.112	0.240
I level	0.075	0.012	0.502	0.309	0.190	0.162
Kind of diet × I source	0.176	0.027	0.125	0.085	0.252	0.877
Kind of diet × I level	0.083	0.009	0.000	0.097	0.432	0.687
I source × I level	0.562	0.781	0.012	0.643	0.372	0.097
Kind of diet × I source × I level	0.120	0.221	0.031	0.152	0.518	0.013

[a,b]Means within a column marked with different superscripts differ significantly at $P < 0.05$.

[A,B]Means within a column marked with different superscripts differ significantly at $P < 0.01$.

[1]CSM = corn-soybean mixture; CSRM = corn-soybean-rapeseed meal mixture.

diet induced no effects on their performance, including laying rate, egg weight, and feed conversion ratio. The weight of eggs from hens receiving KI as an I source was higher ($P = 0.011$) than that observed when using KIO_3. The I source did not influence the remaining performance results. Kaufmann et al. (1998) also found similar effect of I additives on hens performance results. Opaliński et al. (2012), in experiments with Hy-Line Brown hens, stated that an I source given at the same level, 1 mg/kg, as $Ca(IO_3)_2$ or I-enriched yeast had no effect on estimated hen performance, including egg weight. Higher levels of added I significantly modified the egg weight as well as egg mass ($P = 0.014$ and $P = 0.020$, respectively), improved the feed efficiency per kilogram of eggs ($P = 0.021$), and reduced the number of damaged eggs ($P < 0.01$). Similarly, Saki et al. (2012) reported that increasing I supplementation led to higher egg weights. Contrasting results were obtained by Röttger et al. (2012) who stated that different I concentrations in feed did not affect hen performance. Better results in terms of BW gain, feed intake, and conversion were observed by He et al. (2002) during supplementation of pig diets with KI. These and our results are still in contrast to the findings of Yalçin et al. (2004) and Röttger et al. (2012); however, by enhancing I dosages to 12 and 24 mg/kg, the egg weight was less than that with a lower I supply. Neither these authors nor Richter (1995) stated any significant effect on hen performance.

It is difficult to present unequivocal conclusions concerning the application of either various levels of I supplements or types of diet due to interactions between experimental factors. In hens receiving a CSM diet, the highest laying rate was stated in groups receiving 3 mg of I/kg of a diet given as KIO_3. This was significantly higher ($P = 0.009$) than in birds on a diet with the same level of I but given as KI where the laying rates in this group of birds were the lowest. Generally, taking into consideration the tendency in hen laying rate regardless of dietary type and at I level of 1 or 3 mg/kg of diet, better results were obtained if KIO_3 rather than KI served as the I source. The opposite result was observed if the I level was at 5 mg/kg of diet; in that case a slightly higher hen laying rate was stated if the I source was KI. The interaction kind of diet × I source × I level was significant ($P = 0.006$) for egg weight and for feed efficiency ($P = 0.036$). A similar tendency was

Table 5. Hematological indices of hen blood[1]

Item	RBC, 10^{12}/L	HGB, mmol/L	HCT, L/L	PT, g/L
Treatment				
CSM				
1 mg of I as KI	2.627	7.40	0.313	53.88
3 mg of I as KI	2.738	7.75	0.325	45.73
5 mg of I as KI	2.640	7.44	0.311	54.65
1 mg of I as KIO_3	2.450	7.53	0.314	48.65
3 mg of I as KIO_3	2.345	7.30	0.303	52.95
5 mg of I as KIO_3	2.242	7.36	0.305	50.80
CSRM				
1 mg of I as KI	2.565	7.39	0.308	50.82
3 mg of I as KI	2.495	7.36	0.311	47.63
5 mg of I as KI	2.457	7.07	0.300	43.95
1 mg of I as KIO_3	2.428	7.29	0.307	53.32
3 mg of I as KIO_3	2.403	7.16	0.299	45.92
5 mg of I as KIO_3	2.395	6.96	0.286	45.00
SEM	0.033	0.090	0.004	1.016
P-value	0.169	0.972	0.943	0.311
Kind of diet				
CSM	2.517	7.46	0.31	51.11
CSRM	2.461	7.20	0.30	47.77
I source				
KI	2.587^{A}	7.40^{A}	0.311	49.44
KIO_3	2.377^{B}	7.27^{B}	0.302	49.41
Level of I supplement, mg				
1	2.518	7.40	0.310	51.67
3	2.495	7.39	0.309	48.06
5	2.433	7.21	0.300	48.60
Source of variation, *P*-value				
Kind of diet	0.441	0.554	0.400	0.100
I source	0.002	0.003	0.281	0.998
I level	0.542	0.627	0.563	0.291
Kind of diet × I source	0.083	0.077	0.987	0.759
Kind of diet × I level	0.882	0.905	0.903	0.184
I source × I level	0.840	0.814	0.819	0.625
Kind of diet × I source × I- level	0.589	0.638	0.709	0.200

[A,B]Means within a column marked with different superscripts differ significantly at $P < 0.01$.

[1]RBC = red blood cells; HGB = hemoglobin; HCT = hematocrit; PT = platelets; CSM = corn-soybean mixture; CSRM = corn-soybean-rapeseed meal mixture.

shown for feed efficiency calculated in grams per egg. In the experiment conducted by Lichovnikova and Zeman (2004), neither an increased rapeseed meal content in the diet nor I level had any effect on estimated hen performance.

Egg Quality Characteristics

Egg quality characteristics were stated after 3 and 5 mo of the experiment (Tables 3 and 4). After 3 mo of treatment, there was no significant influence as a result of the kind of diet, I source, or I level in the yolk, albumen, and shell proportions in the egg weight or in shell strength and thickness (Table 3). Statistical analysis showed significant differences in yolk color determined according to the Roche scale that were higher for yolks in eggs of hens receiving CSRM. In eggs of hens receiving for 3 mo in ration I supplements at a level of 1 or 3 mg/kg, yolk color estimated in Roche points was lower than in hens receiving 5 mg/kg of I ($P < 0.01$). After 5 mo of the experiment, a similar tendency was demonstrated for the effects of the kind of diet, I sources, and I levels on egg characteristics (Table 4). Additionally, the effect of dietary type on the ratio of the eggshell weight to whole egg weight and eggshell strength was shown. Obtained data are difficult to unambiguously interpret due to different kind of interactions between experimental factors. No impact on the eggshell index, eggshell breaking strength, or eggshell thickness as well as the egg yolk index was reported by Yalçin et al. (2004) in an experiment in which the I supplementation was increased from 3 to 24 mg/kg. Opaliński et al. (2012) found higher albumen weight in eggs by application of increasing amounts of I supplements (from 1 to 2 mg of I per kg of feed). Increasing egg weight and eggshell ratio were obtained in treatments with 10 or 15 mg/kg dietary I by Saki et al. (2012); however, the lowest value of albumen height was indicated after supply of 5 mg of I/kg.

Hematological and Morphotic Indices in Blood

Diet type and level of I supplement had no effect on the analyzed indices (Table 5). However, unexpected significant ($P = 0.002$) influences of I sources on RBC and HGB concentrations were found. Values of these indices were higher in blood of hens fed with a diet

enriched with I by the use of KI rather than a KIO_3 supplement. All interactions among experimental factors were nonsignificant. The nonsignificant influence of diet type, I source, and I level on white blood cell number and their appearance was shown (Table 6); only the percentage share of monocytes in white blood cells was higher ($P = 0.014$) in the blood of hens fed with a diet containing soybean meal as a protein source. All analyzed blood indices were in the range of recommendations acknowledged as physiological. All interactions were nonsignificant.

In analyzed publications, only a little information was found concerning the influence of I supply on hematological indices in laying hens. Lewis (2004) reported that applying I supplementation could increase HGB levels in blood. In our studies, a similar effect was found with an I source. Trávníček et al. (2000) reported that a moderate excess (15 mg/kg) of dietary I could lead to a decrease in phagocytic heterophil activity and the phagocytic index, as well as an increase in heterophils in relation to lymphocytes. Nevertheless, the reason for these results cannot be explained and discussed in the context of the available literature.

Hepatic Enzyme Activity in Blood Serum

The mean activity of AST amounted to about 205 to 315 U/L and that of ALT to about 2.7 to 5.9 U/L. The activity of ALP was in total high and varied in a wide range from about 260 to 735 U/L of blood serum (Table 7). The implementation of rapeseed meal into the diet led to a significant ($P = 0.07$) increase in ALP activity. Other experimental factors did not affect the activities of the remaining hepatic enzymes.

As revealed by Yue and Kang (1995), a high intake of I (65 and 130 mg/kg) from diets evokes elevated plasma aspartate aminotransferase and alanine aminotransferase activity in hens as well as increased activity of alkaline phosphatase. However, unclear or no changes were stated in terms of the activity of other enzymes (i.e., superoxide dismutase, glutamic-pyruvic transaminase). High levels of I in a diet could affect alkaline phosphatase activity (Yue and Kang, 1995). The AST activity determined in our investigation generally shows elevated values as (average about 205–315 U/L) compared with values acknowledged as physiologically correct (142–219 U/L, Krasnodębska-Depta, 2005), but experi-

Table 6. Leukogram of laying hen blood[1]

Item	WBC, 10^9/L	Percentage in WBC				
		Lymphocytes	Heterophils	Monocytes	Basophils	Eosinophils
Treatment						
CSM						
1 mg of I as KI	14.44	59.33	30.00	7.50	2.33	0.83
3 mg of I as KI	14.22	65.33	22.50	8.67	2.17	1.33
5 mg of I as KI	18.33	70.83	20.50	4.50	2.83	1.33
1 mg of I as KIO_3	18.91	68.33	23.50	5.67	2.00	0.50
3 mg of I as KIO_3	20.10	64.17	25.50	5.17	3.17	2.00
5 mg of I as KIO_3	16.38	66.17	25.33	4.83	2.33	1.33
CSRM						
1 mg of I as KI	20.66	69.00	25.83	2.17	1.17	1.83
3 mg of I as KI	18.67	71.17	21.17	5.17	1.33	1.17
5 mg of I as KI	14.00	72.17	21.67	3.33	2.67	0.17
1 mg of I as KIO_3	21.43	67.50	26.00	3.50	1.67	1.33
3 mg of I as KIO_3	21.20	66.67	26.33	5.33	0.67	1.00
5 mg of I as KIO_3	16.00	71.50	21.33	3.83	3.00	0.33
SEM	0.869	1.611	1.279	0.440	0.216	0.162
P-value	0.643	0.958	0.974	0.229	0.423	0.324
Kind of diet						
CSM	17.06	65.69	24.56	6.06[a]	2.47	1.22
CSRM	18.66	69.67	23.72	3.83[b]	1.75	0.97
I source						
KI	16.69	67.97	23.61	5.17	2.08	1.11
KIO_3	18.94	67.39	24.67	4.72	2.14	1.08
Level of I supplement, mg						
1	18.78	66.04	26.33	4.63	1.79	1.13
3	18.43	66.83	23.88	6.08	1.83	1.38
5	16.27	70.17	22.21	4.13	2.71	0.79
Source of variation, *P*-value						
Kind of diet	0.371	0.211	0.608	0.014	0.139	0.582
I source	0.203	0.985	0.780	0.422	0.970	0.934
I level	0.407	0.622	0.460	0.286	0.126	0.243
Kind of diet × I source	0.772	0.779	0.913	0.275	0.872	0.677
Kind of diet × I level	0.277	0.955	0.995	0.404	0.164	0.022
I source × I level	0.626	0.727	0.646	0.551	0.933	0.468
Kind of diet × I source × I level	0.612	0.696	0.683	0.668	0.456	0.808

[a,b]Means within a column marked with different superscripts differ significantly at $P < 0.05$.

[1]WBC = white blood cells; CSM = corn-soybean mixture; CSRM = corn-soybean-rapeseed meal mixture.

Table 7. Hepatic enzyme activity (U/L)[1]

Item	AST	ALT	ALP
Treatment			
CSM			
1 mg of I as KI	262.3	3.95	349.23^{A}
3 mg of I as KI	215.4	2.75	535.42
5 mg of I as KI	234.4	4.68	259.72^{A,a}
1 mg of I as KIO_3	222.4	4.15	380.72^{A}
3 mg of I as KIO_3	199.0	5.42	368.02^{A}
5 mg of I as KIO_3	205.2	3.98	416.20^{a}
CSRM			
1 mg of I as KI	204.9	3.72	385.60^{A}
3 mg of I as KI	204.4	4.07	522.93^{bc}
5 mg of I as KI	259.7	3.47	449.12^{a}
1 mg of I as KIO_3	218.8	3.43	598.07^{bc}
3 mg of I as KIO_3	315.3	5.93	396.67^{a}
5 mg of I as KIO_3	259.8	4.87	735.05^{B,b}
SEM	9.44	0.243	25.90
P-value	0.340	0.339	0.010
Kind of diet			
CSM	223.1	4.16	384.88^{A}
CSRM	243.9	4.25	514.57^{B}
I source			
KI	230.2	3.77	417.00
KIO_3	236.8	4.63	482.45
Level of I supplement			
1 mg	227.15	3.81	428.40
3 mg	233.54	4.54	455.76
5 mg	239.83	4.25	465.02
Source of variation, *P*-value			
Kind of diet	0.271	0.849	0.007
I source	0.726	0.079	0.165
I level	0.858	0.463	0.801
Kind of diet × I source	0.065	0.778	0.213
Kind of diet × I level	0.155	0.466	0.106
I source × I level	0.312	0.119	0.006
Kind of diet × I source × I level	0.540	0.406	0.825

$^{a–c}$Means within a column marked with different superscripts differ significantly at $P < 0.05$.

^{A,B}Means within a column marked with different superscripts differ significantly at $P < 0.01$.

[1]CSM = corn-soybean mixture; CSRM = corn-soybean-rapeseed meal mixture; AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase.

mental factors have not significantly modified these indices. The ALT activity was in compliance with physiological standards (Krasnodębska-Depta, 2005) and was not modified by experimental factors. The activity of alkaline phosphatase showed great diversification from about 260 to 735 U/L and exceeded the recommended range of 184 to 458 U/L. The I supply has not clearly influenced the liver function expressed in terms of the activity of selected enzymes. A similar lack of effect on AST, ALT, and ALP activity of I application in pigs was presented by He et al. (2000, 2002).

Lipid Indices in Blood Serum

Lipid metabolites that were determined in the blood serum of young hens were corrected for healthy hens. The triacylglyceride concentration varied from 8.2 to 10.5 mmol/L (Table 8) and the cholesterol level was within the range 2.8 to 4.8 mmol/L, high-density lipoprotein and low-density lipoprotein fractions were approximately 1.5 to 2.3 and 0.9 to 1.7 mmol/L, respectively. Along with the kind of diet, I supplement and I level did not affect the lipid indices in blood serum. All differences among means for the treatments and experimental factors were nonsignificant. The assayed indices are accurate for laying hens (Krasnodębska-Depta, 2005).

These results are in agreement with the findings of He et al. (2002) that the supply of I, even at higher levels (5–8 mg/kg feed), did not affect the triacylglyceride and cholesterol concentrations in blood serum. Similar to the obtained data, no significant differences among treatments in blood serum cholesterol were obtained by the use of 12 or 24 mg of I supply in the Yalçin et al. (2004) experiment. However, as a result of the application of very high I supplements (up to 500–3,000 mg/kg of feed), dynamically enhanced plasma cholesterol (to about 12 mmol/L) was obtained in the studies of Perry et al. (1989, 1990). Increasing cholesterol levels in serum as a result of high I content in hen diets were also highlighted by Krupová et al. (1998).

Iodine Accumulation in Wet Egg Content

Iodine concentration in wet egg content was determined twice, after 3 and 5 mo of the experiment. Iodine accumulation in egg content was negatively affected by rapeseed meal addition into feed mixture in both sea-

Table 8. Lipid metabolites in laying hens blood serum (mmol/L)[1,2]

Item	TG	CHOL	HDL	LDL
Treatment				
CSM				
1 mg of I as KI	9.85	4.35	1.68	1.26
3 mg of I as KI	8.26	2.90	1.48	0.88
5 mg of I as KI	9.59	4.02	1.92	1.38
1 mg of I as KIO_3	9.91	3.75	1.87	1.29
3 mg of I as KIO_3	10.56	4.85	2.30	1.73
5 mg of I as KIO_3	9.30	3.37	1.60	1.20
CSRM				
1 mg of I as KI	10.39	4.02	1.85	1.38
3 mg of I as KI	10.07	3.40	1.72	1.12
5 mg of I as KI	10.10	3.47	1.83	1.11
1 mg of I as KIO_3	9.50	3.44	1.55	1.16
3 mg of I as KIO_3	9.65	3.41	1.66	1.17
5 mg of I as KIO_3	9.16	2.84	1.55	0.91
SEM	0.209	0.166	0.060	0.063
P-value	0.746	0.444	0.332	0.409
Kind of diet				
CSM	9.58	3.87	1.81	1.29
CSRM	9.81	3.43	1.69	1.14
I source				
KI	9.71	3.69	1.75	1.19
KIO_3	9.68	3.61	1.75	1.24
Level of I supplement, mg				
1	9.91	3.89	1.74	1.27
3	9.64	3.64	1.79	1.22
5	9.54	3.42	1.72	1.15
Source of variation, *P*-value				
Kind of diet	0.587	0.185	0.349	0.244
I source	0.946	0.807	0.957	0.669
I level	0.760	0.525	0.898	0.736
Kind of diet × I source	0.099	0.344	0.070	0.167
Kind of diet × I level	0.932	0.961	0.867	0.670
I source × I level	0.279	0.086	0.071	0.089
Kind of diet × I source × I level	0.569	0.388	0.300	0.440

[1]All differences among means were nonsignificant.

[2]CSM = corn-soybean mixture; CSRM = corn-soybean-rapeseed meal mixture; TG = triglycerides; CHOL = cholesterol; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

sons of egg sampling, but statistically it was confirmed only after 3 mo of the experiment (Table 9). Iodine concentration in eggs of hens receiving rapeseed meal in the ration was 522 and 673 μg/kg, whereas in eggs of hens that did not receive rapeseed meal it was 671 and 841 μg/kg at the first and second time of sampling, respectively. The KI was more effective source of I than KIO_3 in accumulation of this element in wet egg content, especially after 5 mo of treatment (816 vs. 698 μg/kg). Increasing I concentration in the diet of hens led to an increase in I concentrations in egg content and this was confirmed statistically ($P < 0.01$). Data obtained in mo 3 of the experiment are difficult to interpret due to significant interactions between some experimental factors, but after 5 mo of the experiment, all interactions were nonsignificant. Iodine content in animal origin products, including eggs, depends on many factors [e.g., the kind and level of I supplements (Marcilese et al., 1968; Perry et al., 1989; Yue and Kang, 1995; Lewis, 2004; Röttger et al., 2012; Saki et al., 2012), the kind of diet (Wemheuer and Paufler, 1993; Tripathi et al., 2001a; Lichovnikova and Zeman, 2004), the period of additive application (Nair et al., 1998), genetic characteristics of animals (Ryś et al., 1997), animal age, hen laying rates, as well as on the chemical methods used for the determination of I content in biological materials (Knapp et al., 1998; Gąsior and Szczypuła, 2010)]. These dependences have been confirmed by numerous authors, but there have been a range of often differing results concerning rising I concentrations in whole egg, yolk, or albumen after enrichment of diets with I (Ryś et al.,1997; Kaufmann et al., 1998; Krupová et al., 1998; Dobrzański et al., 2001; Opaliński et al., 2012). Similar to our data, Yalçin et al. (2004) reported that increasing I levels in hen diets from 3 to 24 mg/kg caused a clear increase in I content both in egg albumen and egg yolk. Röttger et al. (2012) also assayed progressive increases in I accumulation in yolk (425–3,373 μg/kg) and in albumen (14–124 μg/kg), as well as in whole egg (144–1,304 μg/kg) as a response of laying hens to increasing I supplementation of diets.

In conclusion, the introduction of an I supplement from 1 to 5 mg/kg into the diet of hens enables the significant enrichment of the egg content without breaching European Union regulations. At a mean wet egg content of about 50 g and a supply of 5 mg of I/kg of feed, a consumer may consume in one egg about 51 to 63 μg of I compared with the average recommended daily I consumption per adult person of about 160 μg (WHO, 2001). Physiological indices determined after

Table 9. Iodine accumulation (μg/kg) in wet egg content after 3 and 5 mo of the experiment[1]

Item	I concentration	
	After 3 mo	After 5 mo
Treatment		
CSM		
1 mg of I as KI	327^{ABC}	466^{AB}
3 mg of I as KI	490^{AB}	995^{CD}
5 mg of I as KI	1,168^{D}	1,325^{CE}
1 mg of I as KIO_3	408^{ABC}	271^{AB}
3 mg of I as KIO_3	630^{AE}	618^{A}
5 mg of I as KIO_3	1,006^{D}	1,373^{E}
CSRM		
1 mg of I as KI	113^{C}	269^{AB}
3 mg of I as KI	634^{AE}	644^{AD}
5 mg of I as KI	999^{D}	1,198^{CE}
1 mg of I as KIO_3	192^{BC}	234^{B}
3 mg of I as KIO_3	322^{ABC}	565^{AB}
5 mg of I as KIO_3	871DE	1,129^{CE}
SEM	50.97	61.01
P-value	0.000	0.000
Kind of diet		
CSM	671^{A}	814^{A}
CSRM	522^{B}	673^{B}
I source		
KI	622	816^{A}
KIO_3	571	698^{B}
Level of I supplement, mg		
1	260^{A}	310^{A}
3	519^{B}	705^{B}
5	1,011^{C}	1,256^{C}
Source of variation, *P*-value		
Kind of diet	0.000	0.000
I source	0.167	0.003
I level	0.000	0.000
Kind of diet × I source	0.057	0.136
Kind of diet × I level	0.322	0.618
I source × I level	0.040	0.072
Kind of diet × I source × I level	0.014	0.083

$^{A–E}$Means within a column marked with different superscripts differ significantly at $P < 0.01$.

[1]CSM = corn-soybean mixture; CSRM = corn-soybean-rapeseed meal mixture.

long-term I fortification in blood serum show the correct health status of birds, which may be considered model animals for humans.

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