Effect of a single dose of iodized fatty acid ester Lipiodol® Ultra-Fluid on egg iodine concentrations and egg production

M. Trckova[1], B. Pisarikova[1], P. Suchy[2], I. Herzig[1]

[1]Veterinary Research Institute, Brno, Czech Republic
[2]University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

ABSTRACT: The aim of the study was to test whether a single intramuscular or oral application of iodized fatty acid esters (IFAE), without any iodine supplements in the rations, would increase for a long time iodine concentrations in egg yolk without any drop of egg production and egg quality. The results were compared with those obtained using the conventional iodine source – potassium iodide (KI). After the adaptation period, 10 mg of iodine/kg of live weight were applied to the experimental layers at the age of 21 weeks. The layers were divided into four groups: the group K-KI received iodine orally in the form of potassium iodide; iodine was applied orally in the form of IFAE to the group P-PO; and iodine as IFAE was applied intramuscularly to the group P-IM. The control group (K-0) received no iodine. Increased concentrations of iodine in egg yolk were observed following oral application of KI and IFAE. Maximum concentrations were measured on day 5 following application (12 863 ± 3 269 μg I/kg for K-KI, and 14 037 ± 2 506 μg I/kg for P-PO). Quite different course of changes was recorded following intramuscular application of iodine (group P-IM). Both, the increase and drop of iodine concentrations were slow, maximum values were measured from day 11 till day 35 of the experiment (from 769 ± 426 to 1 163 ± 757 μg of I/kg yolk). Intramuscular application of IFAE resulted in significantly higher levels ($P < 0.05$, $P < 0.01$) of iodine in egg yolk from day 11 till the end of the experiment (on day 154) compared with the group K-0, and from day 14 compared with the group K-KI. At the same time, more fluent course of egg production with maximum at the age of 34 weeks was observed in laying hens. No effect of iodine application on egg production and egg mass was found. Significantly higher weights of eggs ($P < 0.05$, $P < 0.01$) and egg white ($P < 0.05$) were recorded following oral and intramuscular application of IFAE compared with the control group K-0.

Keywords: iodized oil; potassium iodide; egg yolk iodine concentration; egg weight; yolk weight; egg white weight; egg shell weight

Increased supply of iodine in animals diets results in significant increase of this element in foodstuffs of animal origin, which can contribute to the prevention of iodine deficiency in human population. The application of iodised oil in pigs (Herzig *et al*., 2001a) and the effect of humine compounds on iodine utilisation (Herzig *et al*., 2001b) were described in our papers published recently. The substitution of iodine has to be carefully controlled because iodine toxicity can appear as reviewed by Paulikova *et al*. (2002).

In European countries predominantly inorganically bound iodine is used in feed supplements unlike the USA where organic form of ethylenediamine dihydroiodine (EDDI) is used. Iodine bound in oil base in the form of iodized fatty acid esters (IFAE) has been used in the past decade for the prevention of iodine deficiency. The advantage of IFAE is their long-term effect following a single application. This is due to the small and gradual iodine release from lipidic bonds especially, but not exclusively, from fatty tissues during a long time (Heidemann *et al*., 1982). The mechanism by which IFAE release their iodine is not known. It is also uncertain where IFAE deiodinations occure.

Oral application of IFAE is preferred to intramuscular application as less expensive and more easy to

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apply (Phillips and Osmond, 1989). Intramuscular application has a longer iodine release from IFAE than oral application in some (Furnee *et al*., 1997; Wolff, 2001), but not all, studies reported. The rapid appearance of iodine after oral application might be partly due to the presence of bacterial enzymes in the intestine. Other factors that have effect on the efficiency of orally applied IFAE include the type of iodized oil used, the incidence of goiter and intestinal parasitic infections, sex, thickness of fatty tissue, the presence of goitrogens in food and climatic conditions (Furnee, 1997; Furnee *et al*., 1997).

A single oral or intramuscular supplementation with IFAE is used predominantly in humans living in endemic goitrogenic regions where it provides a long-term protection against diseases caused by iodine deficiency (Aquaron *et al*., 1990; Contempre *et al*., 1996; Bellis *et al*., 1996; Delange, 1996; Furnee, 1997). Iodine deficiency can be thus regulated for 3 to 5 years (Hetzel, 1983).

Iodine supplementation of rations for farm animals ensures natural increase of iodine levels in foodstuffs of animal origin. The tolerance of laying hens to accept many-fold higher doses of iodine without apparent disorders of health and egg production allows the production of "iodine supplemented eggs" and easy transfer of iodine to oocytes (Pena *et al*., 1967; Schjeide and Prahlad, 1977). Iodine administered to layers cumulates in both, egg yolk and white but its cumulating in egg yolk is much higher (Nakajima *et al*., 1980). Broader use of eggs as an iodine source is limited by cholesterol contents in egg yolk.

Long-term effect of a single application of IFAE at iodine deficiency was also tested on pigs (Chambon and Chastin, 1993; Herzig *et al*., 2000), sheep (Azuolas and Caple, 1984) and pregnant ewes (Potter *et al*., 1984).

The objective of the study was to test whether a single oral or intramuscular application of iodized fatty acid esters, without iodine supplementation of rations, can increase for a long term iodine concentrations in yolk without any decrease of egg production and quality. We intended to compare the obtained results with those obtained with conventional iodine source potassium iodide (KI).

MATERIAL AND METHODS

Experimental animals and housing

Sixty ISSA Brown pullets aged 16 weeks were used in the experiment. The pullets were housed individually in cages assembled to three-storey batteries of the size 45 × 35 × 47.5 cm which were equipped with feeders, dripping drinkers and through for collecting eggs. A twelve-hours light regime was adopted during the whole experiment and climatic conditions (temperature, moisture) were monitored and controlled. The experiment was carried out in experimental hall of the University of Veterinary

Table 1. Composition of the feed mixture N_1 without iodine supplementation

Component	Content (%)	Component	Content (%)
Corn	35	Monocalcium phosphate	1.2
Wheat	30.7	Premix TKP NP-M var. B[1]	0.5
Extracted soybean meal	15	Feeding salt	0.38
Ground limestone	7.84	D,L-methionine	0.04
Rape meal type 00	5	Lysine-HCl	0.02
Yeast Vitex Q	2.5	L-threonine	0.02
Soybean oil	1.8		

[1]per 1 kg of premix TKP NP-M var. B:
vitamin A 3 000 000 m.j., vitamin D3 600 000 m.j., vitamin E (Alfatokoferol) 14 560 mg, vitamin B1 600 mg, vitamin B2 3 000 mg, vitamin B6 1 000 mg, vitamin B12 6 mg, vitamin K3 900 mg, biotin 40 mg, folic acid 500 mg, niacinamid 10 000 mg, calcium panthotenate 3 000 mg, cholin-chloride 80 000 mg, D,L-methionine 140 g, antioxydant (buthylhydroxyanisol, ethoxyquin) 25 000 mg, Cu ($CuSO_4.5H_2O$) 2 000 mg, Zn ($FeCO_3$) 20 000 mg, Mn (MnO) 20 000 mg, Fe ($FeCO_3$) 10 000 mg, Se (Na_2SeO_3) 60 mg, carrier – wheat flour, calcium carbonate ad 1 kg

and Pharmaceutical Sciences, Brno, Department of Nutrition, Dietetics and Zoohygiene.

Laying hens of all groups were fed throughout the experiment the same complete feed mixture N_1 without iodine supplementation (Table 1) which was produced in two batches (N_1 batch a, N_1 batch b). Feed and drinking water were provided *ad libitum*.

Experimental design

In the adaptation period from week 17 to week 19 of age the development of egg production and number of laid eggs were monitored in individual animals. Before the onset of experimental period, four layers were culled due to an inadequate clutch. Prior to the application of iodized preparations the eggs were taken to determine the initial iodine concentrations in yolk, weights of whole eggs and their parts. The experiment began at the age of 20 weeks, when the layers were divided into 4 groups of 14 animals each. A single iodine supplementation was administered at the age of 21 weeks according to the following pattern:

- group K-0 = without iodine supplementation
- group K-KI = a single oral application of potassium iodide at the dose of 10 mg I/kg of live weight in capsules administered to the tongue root, which corresponds to 13.08 mg KI/kg of live weight
- group P-PO = a single oral application of IFAE at the dose of 10 mg I/kg of live weight in capsules containing Lipiodol® Ultra Fluid (Oleum papaveris) on a carrier – a semi-coarse flour, which corresponds to 0.02 ml Lipiodol/kg of live weight
- group P-IM = a single intramuscular application of IFAE (Lipiodol® Ultra-Fluid) into the left breast muscle at the dose of 10 mg I/kg of live weight, which corresponds to 0.02 ml Lipiodol/kg of live weight

The number of produces eggs was recorded daily in the course of the experiment in all groups of layers; the clutch in percentage and production of egg mass were evaluated in a 14-day periods, and the total production of egg mass during the laying period was evaluated:

$$\text{Clutch in percentage (\%)} = \frac{\text{number of laid eggs} \times 100}{\text{number of days in the monitored period}}$$

$$\text{Production of egg mass (g)} = \text{number of laid eggs} \times \text{mean weight of eggs (g)}$$

To determine iodine concentrations in yolk, egg samples were collected on days 3, 4, 5, 6, 7, 11, 14 and 17 following iodine supplementation, and further from week 4 to 22, egg samples were collected once a fortnight. Ten eggs were sampled from each group. Five pooled samples (one pooled samples was made of two yolks) from each group were used for the analysis. The experiment lasted for 154 days and was terminated at 43 weeks of age of the laying hens; after the experiment the hens were sacrificed.

The contents of dry matter, N-substances, fat, and fibre and ash in feed mixture were determined according to the Regulation No. 222/1996, and the contents of metabolizable energy was calculated before the experiment and in the course of the experiment (Zelenka *et al.*, 1993). Yolk iodine was determined using the alkaline combustion method spectrophotometrically by Sandell-Kolthoff (Bednar *et al.*, 1964). The method is based on Ce^{4+} reduction to Ce^{3+} in the presence of As^{3+} and under catalytic effect of iodine. Mineralization is performed by dry ashing in alkaline environment at 600°C. Thus the total inorganic iodine as well as iodine bound on proteins can be determined. The contents of nutrients, metabolizable energy and iodine in feed mixture N_1 is shown in Table 2.

In egg samples the whole egg weight, weight of separated yolk and albumen, egg shell weight including shell membranes were determined.

Statistical evaluation of the results

The obtained results were analyzed using the statistical and graphical system STAT Plus (Matouskova *et al.*, 1992). Basic statistical characteristics of the files (arithmetic mean, standard deviation) were determined and analyses of variance of simple classification (comparison made by Scheffe test and by Tukey test) were performed as well as regression analyses.

RESULTS AND DISCUSSION

The analyses of feed mixtures confirmed that the contents of basic nutrients and metabolizable energy in the used batches corresponded to the

Table 2. Contens of nutrients, metabolisable energy and iodine per 1 kg of feed mixture N_1 without iodine supplementation

	N_1 batch a		N_1 banch b	
	feed – dry matter		feed – dry matter	
	original	absolute	original	absolute
Dry matter (g)	890.2	1 000	890.6	1 000
Crude protein (g)	163.1	182.9	166.5	185.8
Fat (g)	50.3	56.4	40.4	45.1
Fibre (g)	20.8	23.3	21.8	24.3
Ash (g)	110.0	123.3	109.4	122.1
NFE (g)	547.6	614.1	558.0	622.7
Organic matter (g)	781.8	876.7	786.7	877.9
MEp (MJ)	11.1	12.4	10.9	12.2
Iodine (μg)	132.0	148.0	241.8	269.8

nutrient requirements for laying hens (Zelenka *et al*., 1993).

Iodine concentrations in egg yolk

The dynamics of average iodine concentrations changes in egg yolks are shown in Figure 1. Iodine concentrations in egg yolks of the control group (K-0) without iodine fortification ranged between 24 ± 23 and 179 ± 49 μg/kg.

A marked increase of iodine levels was observed after a single oral application of KI at the dose of 10 mg I/kg of live weight (group K-KI). The maximal mean value 12 863 ± 3 269 μg I/kg of yolk was found on day 5 following iodine application, which represents a 104-fold increase compared to yolk iodine level before KI application (124 ± 68 μg I/kg) (Table 3). A moderate decrease of the values on day 6 and 7 and a considerable decrease on day 11 to only a three-fold value of the initial one were observed. Since day 21 following application, iodine concentrations dropped to the original values. Iodine concentrations were significantly higher compared to the group K-0 on day 5 ($P < 0.01$), day 6 ($P < 0.05$) and 7 ($P < 0.01$).

Similar changes in iodine yolk concentrations were observed after oral application of IFAE (group P-PO). Maximal concentration in the group P-PO 14 037 ± 2 506 μg I/kg of yolk was measured on day

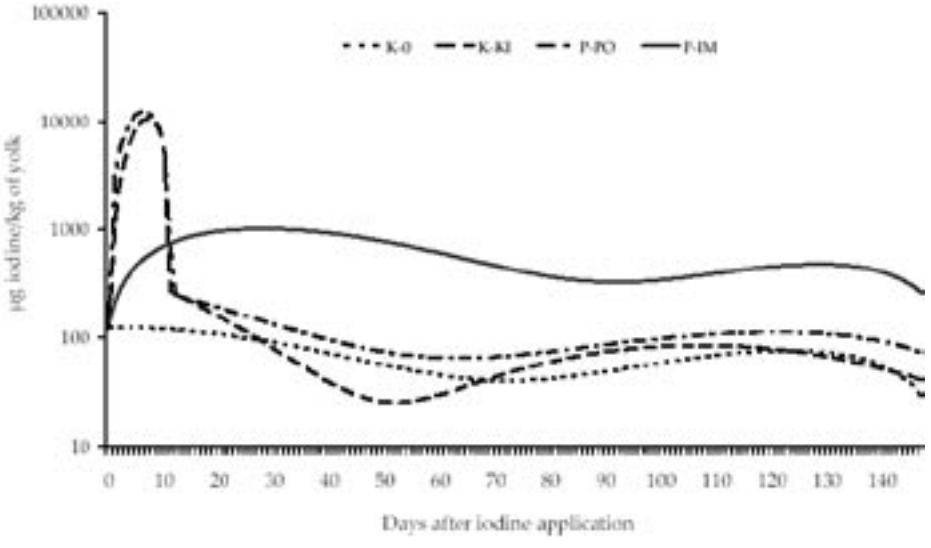

Figure 1. Iodine concentrations in egg yolk (μg/kg)

K-0 = without iodine supplementation
K-KI = a single oral application of KI
P-PO = a single oral application of IFAE
P-IM = a single intramuscular application of IFAE

Table 3. Iodine concentrations in egg yolk (n = 5) after a single application of idized preparations (μg/kg)

Day	K-0	K-KI	P-PO	P-IM
0	97 ± 73	124 ± 68	151 ± 90	81 ± 47
3	146 ± 82	2 721 ± 1 477	4 507 ± 1 953*	99 ± 56
4	160 ± 35	5 660 ± 1 376	11 447 ± 2 033**	207 ± 185
5	131 ± 67	12 863 ± 3 269**	14 037 ± 2 506**	438 ± 125
6	179 ± 49	10 405 ± 3 547*	13 506 ± 1 567**	609 ± 340
7	101 ± 59	8 041 ± 2 790**	8 894 ± 2 461**	847 ± 459
11	137 ± 40	337 ± 145	786 ± 220*	1 163 ± 757**
14	124 ± 34	181 ± 76	337 ± 154	958 ± 534**
17	116 ± 13	198 ± 54	178 ± 119	1 053 ± 250**
21	52 ± 35	88 ± 69	95 ± 22	959 ± 374**
35	54 ± 20	54 ± 23	58 ± 6	1 012 ± 323*
49	71 ± 15	71 ± 25	141 ± 33	702 ± 83**
64	72 ± 23	53 ± 11	92 ± 19	608 ± 146
77	42 ± 30	46 ± 33	75 ± 33	498 ± 122**
91	28 ± 25	40 ± 9	53 ± 22	407 ± 140**
105	64 ± 18	81 ± 21	97 ± 22	407 ± 86**
119	71 ± 54	95 ± 26	111 ± 27	329 ± 31**
133	80 ± 22	93 ± 13	87 ± 23	324 ± 28*
147	24 ± 23	35 ± 9	69 ± 21	277 ± 28**

*significant difference to K-0 ($P < 0.05$)
**significant difference to K-0 ($P < 0.01$)

5 after the application, which represents a 93-fold increase of iodine concentration compared to the level before application (151 ± 90 μg I/kg) (Table 3). Significant changes compared to K-0 were detected on day 3 ($P < 0.05$), 4 ($P < 0.01$), 5 ($P < 0.01$), 6 ($P < 0.01$), 7 ($P < 0.01$) and 11 ($P < 0.05$) of the experiment, suggesting that the effect of a single IFAE application is slightly longer compared to KI.

A different dynamics of changes was observed after intramuscular application of IFAE (group P-IM). Both the increase and decrease of yolk iodine concentrations were slow, the maximal mean values (from 769 ± 426 to 1 163 ± 757 μg I/kg of yolk) were found from day 11 to day 35 of the experiment and they represent 10-fold to 14-fold increase compared to the original concentration (Table 3). Even the last day of the experiment iodine concentration did not decrease to the value found prior IFAE application but was 3 times higher. Significant differences were found between the groups P-IM and K-0 on day 11, 14, 17, 21 ($P < 0.01$), 35 ($P < 0.05$), 49, 77, 91, 105, 119 ($P < 0.01$), 133 ($P < 0.05$) and 147 ($P < 0.01$); between the groups P-IM and K-KI on day 14, 35 ($P < 0.05$), 49, 64 ($P < 0.01$), 77, 91, 105 and 147 ($P < 0.05$), and between the groups P-IM and P-PO on day 3, 4 ($P < 0.01$), 17 and 133 ($P < 0.05$).

These significant changes suggest different action of IFAE in the organism compared with the conventional iodine source – potassium iodide, which consists most probably in formation of a "iodine deposit".

Significant increase of iodine concentrations in yolk is in accordance with the data of other authors (Nakajima *et al*., 1980; Kaufmann *et al*., 1998; Kroupova *et al*., 1999). The effect of a single application of iodine in the form of KI was unstable (Phillips *et al*., 1988). Better results were obtained at repeated or a long-term KI supplementation into the feed mixture. Nakajima *et al*. (1980) did not found any differences in iodine accumula-

tion in eggs during 23 days following a repeated iodine supplementation in feeds (iodized oil from safflower seeds, sea algae and KI). They recorded a sharp increase of yolk iodine concentrations on day 4 following supplementation of KI to the layers. The maximum values, which were found on day 9 to 11, were 60–70 times higher than iodine yolk concentrations in the control group. After termination of iodine supplementation, a sharp drop was recorded on day 5, and on day 11 iodine yolk concentration was the same as in the control. Kroupova *et al*. (1999) mentioned that 8-week application of 3.5 mg I/kg of feed mixture resulted in increase of yolk iodine concentrations to 18 597 ± 1 655 μg I/kg of yolk. Different response to higher iodine doses is influenced by different usability of iodine source (Herzig and Suchy, 1996; Bobek, 1998) but also by the strain (Rys *et al*., 1997) and by the presence of antinutritious substance which have an impact on iodine resorption (Garwin *et al*., 1992; Garber *et al*., 1993).

The results obtained in the experimental groups P-PO and P-IM confirm that intramuscularly applied IFAE have a longer iodine release from lipidic bonds compared with oral application (Wolff, 2001) and therefore the effect of oral IFAE is shorter (Furnee *et al*., 1997). On the other hand, Phillips *et al*. (1988) stated that 9 months after oral application of iodized oil at the dose 2 ml, the distribution of T_4 concentrations was similar to those following intramuscular application. Rapid action of iodine after oral application of IFAE can be partly caused by the presence of bacterial enzymes in the intestine. Further investigation is needed to confirm whether gastrointestinal tissue takes part in rapid deiodization by its own enzymes.

Egg production

Egg production in individual groups of layers is shown in Figure 2, the number of laid eggs and production of egg mass in Table 4. Although iodine supplementation had not significant effect on production of eggs and egg mass, which is in accordance with the findings of Nakajima *et al*. (1980), intramuscular application of IFAE in the group P-IM resulted in more consistent course of egg production with a maximum at 34 weeks of age. A sharp increase of egg production was observed

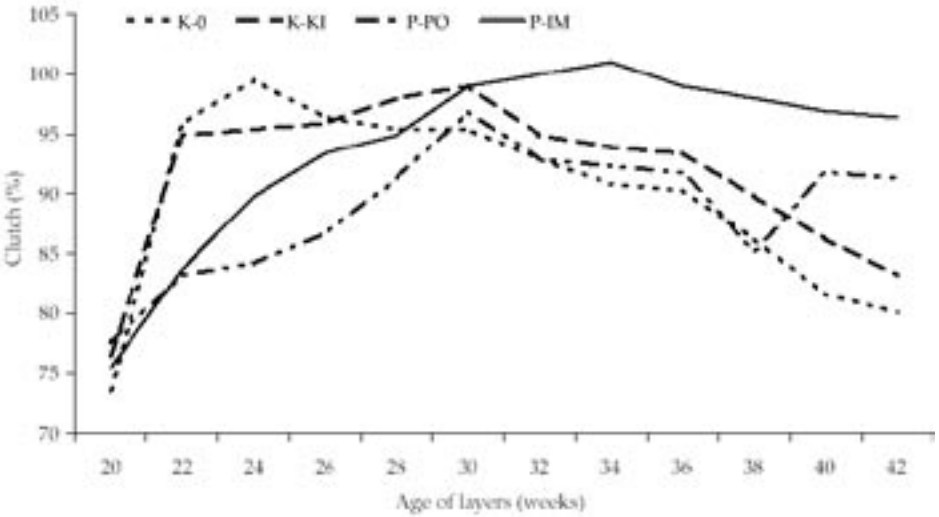

Figure 2. Clutch in percentage

K-0 = without iodine supplementation
K-KI = a single oral application of KI
P-PO = a single oral application of IFAE
P-IM = a single intramuscular application of IFAE

Table 4. Production of egg mass

Group	Number of laid eggs	Production of egg mass (kg)	Production of egg mass (%)
K-0	2 040	114.9	100.0
K-KI	2 083	120.0	104.4
P-PO	2 012	118.5	103.1
P-IM	2 136	126.5	110.1

Table 5. Egg weights (g), (n = 10)

Group	Mean weight of eggs (g) day 64	Mean weight of eggs (g) day 91	Mean weight of eggs (g) day 147
K-0	53.9 ± 5.15	57.5 ± 3.53	61.4 ± 2.51
K-KI	58.3 ± 3.86	60.7 ± 3.21	65.7 ± 5.32
P-PO	59.1 ± 3.34*	62.7 ± 3.01*	64.7 ± 3.48
P-IM	59.9 ± 2.92**	63.5 ± 4.19**	66.4 ± 2.22*

*significant difference to K-0 ($P < 0.05$)
**significant difference to K-0 ($P < 0.01$)

in layers of the group K-0 at the age of 22 weeks with a maximum at the age of 24 weeks and a subsequent decrease since week 26 of age. The layers of the groups K-KI and P-PO reached maximum at 30 weeks of age.

The weights of eggs, yolk, albumen and egg shell

Mean weights of eggs in all groups were increasing during the experiment. A single iodine application in the form of KI had no effect on egg weight and other egg quality characteristics. Single oral and intramuscular applications of IFAE resulted in significant differences in the weights of eggs and albumen compared to the control. The mean weights of eggs in the group P-PO were significantly higher on day 64 and 91 ($P < 0.05$) of the experiment compared to K-0. Intramuscular application of IFAE had a positive effect on egg weight on day 64, 91 ($P < 0.01$) and 147 ($P < 0.05$) (Table 5). Significant differences in albumen weights were only in the group P-PO on day 64 and 91 ($P < 0.05$) compared to the control. Similar changes as in egg weight were recorded in yolk weights, however, no significant differences were found between the groups.

A single intramuscular and to a certain level also oral application of IFAE can ensure long-term increase of iodine concentrations in eggs and stimulate thus the performance under the conditions of iodine deficiency.

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Corresponding Author

Ing. Martina Trckova, Veterinary Research Institute, Hudcova 70, 621 32 Brno, Czech Republic
Tel. +420 533 331 611, fax +420 541 211 229, e-mail: trckova@vri.cz