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SELENIUM DEPOSITION IN TISSUES AND EGGS OF LAYING HENS GIVEN SURPLUS OF SELENIUM AS SELENOMETHIONINE

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MOKSNES, KNUT: *Selenium deposition in tissues and eggs of laying hens given surplus of selenium as selenomethionine.* Acta vet. scand. 1983, *24*, 34—44. — Forty-eight Norwegian bred White Leghorn chickens were divided into 6 groups and fed a basal diet containing 0.30 mg Se/kg supplemented with 0, 0.1, 0.5, 1.0, 3.0 or 6.0 mg Se/kg in the form of selenomethionine for 18 weeks. A supplement of only 0.1 mg Se/kg induced significantly higher selenium concentrations in breast muscle and eggs, particularly in the egg white. The increase of selenium in the tissue and egg was proportional to the amounts of selenomethionine added to the feed. In the group given 6.0 mg Se/kg, the selenium concentrations in all tissues and eggs analysed ranged from 4.8 to 7.3 μg Se/g. No signs of toxic effects were observed even at the highest intake of selenium. Excess supply of selenium as selenomethionine to chickens was shown to be more potent than sodium selenite in raising the selenium concentration in tissues and eggs. A supplementation up to 10 times the requirement did not increase the levels of selenium in poultry products to such a degree that they could be considered as a potential risk for human consumption.

dietary selenium; laying hens; selenium concentrations; tissues; eggs.

The chemical composition of the selenium content of the feed has a significant effect on the biological availability of selenium and its deposition in animals' tissues. The main sources of selenium in both animal and human nutrition are organic selenium compounds of plant or animal origin. Selenomethionine constitutes a major part of the selenium content of "non-selenium-accumulator" plants (*Olson et al.* 1970, *Gissel Nielsen* 1980). Inorganic selenium, most commonly sodium selenite, is used as a feed additive in animal nutrition to prevent selenium deficiency diseases. Sodium selenite has been added to poultry feed in Norway since 1980 (*Landbruksdepartementet* 1979).

The effect of graded levels, up to 6 mg Se/kg feed, of sodium selenite has previously been reported (*Moksnes & Norheim* 1982). The scope of the present investigation was to study the effect of graded, high levels of selenomethionine on tissue and egg selenium levels, body weight gain and egg production in laying chickens. A further intention was to evaluate the risk for humans consuming meat and eggs from animals receiving such high levels of dietary selenium in the form of selenomethionine.

MATERIAL AND METHODS

Forty-eight Norwegian bred White Leghorn chickens were divided into 6 groups, each consisting of 8 birds. The birds were 20 weeks old at the start of the experiment and had been fed an ordinary feed including a supplement of 0.1 mg Se/kg as sodium selenite. The birds were individually caged and fed a basal feed of all mash cage laying feed containing 0.30 mg Se/kg (Table 1). The selenium content of the basal feed was established by analysing 3 randomly selected samples, using the same method as for the tissue samples. The basal feed was supplemented with 0, 0.1, 0.5, 1.0, 3.0 or 6.0 mg Se/kg in the form of seleno-DL- methionine. Five birds were slaughtered before the dietary study began in order to establish basal tissue levels.

At the end of the experiment, after 18 weeks (age: 38 weeks), all of the animals were slaughtered and examined for gross- and histopathological lesions. No pathological lesions were found. Tissue samples were taken from all of the birds for selenium analysis. Fifteen eggs from each group were randomly selected and weighed on week 4, 8, 12, 16 and 18 of the feeding period. The total contents of 5 eggs were blended. The whites and yolks of 5 other eggs were separated before pooling and blending. Records were maintained to calculate egg production. The body weight of all birds used in this experiment was monitored. Three chickens died during the experiment: One in the control group due to salpingitis and peritonitis; one in group 0.1 due to granulosa cell tumours and one in group 3.0 due to lymphoid leukosis.

Analytical methods

Samples of breast- and cardiac muscle, liver and kidney were collected from each bird and frozen at —20°C immediately after collection. The egg samples were also stored at —20°C. Material

Table 1. Composition of the diet.

Diet		All-mash cage laying feed 15 %*
Herring meal, extra quality	%	5.5
Meat and bone meal	„	3.5
Soyabean meal, extracted	„	2.4
Ground barley	„	5.0
Ground maize	„	20.0
Maize grits	„	7.8
Ground sorghum	„	14.2
Ground oats	„	17.0
Wheat brand	„	9.6
Grass meal	„	2.5
Fat	„	0.9
Molasses	„	2.0
Mineral mixture	„	9.1
Vitamins	„	0.5
Ground wheat	„	—
Limestone meal	„	—
Guaranteed content:		
Crude protein	%	14—16
Digestible crude protein	„	11—13
Fat	„	—
Metabolisable energy	kcal/kg	2,600—2,720
Calculated content:		
Crude fibre	%	4.4
Fat	„	3.9
Lysine	„	0.74
Methionine + Cysteine	„	0.57
Linoleic acid	„	1.20
Calcium (Ca) per kg	„	3.5
Phosphorus (P) „	„	0.7
Salt (NaCl) „	„	0.5
Selenium (analysed)	mg/kg	0.30
Added per kg:		
Vitamin A	I.U.	12,000
Vitamin D_3	„	2,000
Vitamin E	mg	20
Vitamin K_3	„	0.5
Riboflavin (B_2)	„	5
Folic acid	„	0.5
Pantothenic acid	„	5
DL-Methionine	„	270
Niacin	„	—
Biotin	„	—
Pyridoxine (B_6)	„	—
Choline chloride	„	—
Manganese oxide	„	—

* Commercial concentrate from Arex-Møllesentralen, Oslo.

for analysis was taken in a semifrozen state to prevent loss of fluid from the samples. The tissue and egg samples were analysed according to a modification of a fluorimetric method (*Ihnat* 1974, *Norheim & Nymoen* 1981). Results are expressed as μg Se/g on a wet weight basis. The statistical analyses were calculated on a Compucorp 344 Statistician.

RESULTS

The concentrations of selenium in the tissues analysed increased in all groups of chickens given supplementary selenomethionine (Table 2). When 0.1 mg Se/kg was added to the feed the average concentration in breast muscle increased from 0.20 to 0.29 μg Se/g wet weight and in cardiac muscle from 0.36 to 0.43 μg/g. There were only minor increases in the levels in liver and kidney at this level. The increase in the tissue levels of selenium was directly proportional to the amount of selenomethionine added to the feed (Table 4). In the group given 6.0 mg Se/kg the selenium concentrations in all the tissues analysed ranged from 4.8 to 7.3 μg Se/g.

The concentration of selenium in eggs was also increased by the dietary supplementation of selenomethionine (Table 3). The increases were observed as early as 4 weeks after the start of the experiment. At this time the results showed a broad range. During the rest of the experimental period there were no further increases. A supplement of only 0.1 mg Se/kg induced significantly higher selenium concentrations in the eggs, especially in the egg white. The ratio between the selenium levels in egg yolk and egg white decreased with increasing supplementation. At the end of

Table 2. Tissue selenium content on day of slaughter in laying chickens kept on different dietary levels of selenomethionine (mean±s/g wet weight).

Tissue	Basal diet	Added selenomethionine (mg Se/kg)				
		0.1	0.5	1.0	3.0	6.0
	n=7	n=7	n=8	n=8	n=7	n=8
Breast muscle	0.20±0.01	0.29±0.01	0.65±0.09	1.3±0.06	3.0±0.13	5.4±0.45
Cardiac muscle	0.36±0.02	0.43±0.02	0.78±0.04	1.2±0.04	2.9±0.26	5.4±0.44
Liver	0.58±0.03	0.60±0.03	0.98±0.09	1.6±0.15	3.1±0.71	6.6±0.99
Kidney*	0.63	0.77	1.1	1.5	2.4	5.2

* Each value represents 1 pooled sample of all of the birds in each group.
n = number af analyses.

Table 3. Selenium concentrations (μg Se/g wet weight) in homogenized eggs, egg yolk and egg white as affected by duration of selenium feeding and levels of dietary selenium as selenomethionine. Each value represents 1 pooled sample of 5 eggs.

Added Se (mg/kg)	Sample	Duration of selenium feeding (weeks)				
		4	8	12	16	18
0	Homogenized eggs	0.27	0.32	0.38	0.27	0.24
	Egg yolk	0.66	0.64	0.53	0.54	0.41
	Egg white	0.12	0.12	0.14	0.12	0.13
0.1	Homogenized eggs	0.33	0.40	0.34	0.37	0.36
	Egg yolk	1.1	0.77	0.63	0.66	0.66
	Egg white	0.59	0.27	0.24	0.20	0.22
0.5	Homogenized eggs	0.57	0.82	0.64	0.69	0.69
	Egg yolk	1.3	1.0	0.92	0.90	0.90
	Egg white	0.81	0.75	0.58	0.56	0.58
1.0	Homogenized eggs	1.1	1.2	1.2	1.1	1.1
	Egg yolk	1.3	1.0	1.5	1.4	1.4
	Egg white	0.76	1.0	0.98	1.0	1.1
3.0	Homogenized eggs	2.6	2.6	2.7	2.6	2.3
	Egg yolk	2.8	2.6	2.9	2.9	3.0
	Egg white	2.3	2.9	3.0	2.5	2.2
6.0	Homogenized eggs	4.9	6.2	5.3	4.4	5.0
	Egg yolk	4.8	6.9	5.2	4.6	5.1
	Egg white	4.3	5.9	3.8	4.7	4.9

Table 4. The selenium concentrations (μg Se/g wet weight) in liver (y_1), breast muscle (y_2), cardiac muscle (y_3), kidney (y_4), homogenized egg (y_5), egg yolk (y_6) and egg white (y_7) from laying chickens as a function of the selenium concentrations (mg Se/kg dry weight) added to the ration (x) in the form of selenomethionine.

Regression function	n	r	P
$y_1 = 0.99\ x + 0.50$	45	0.97	< 0.001
$y_2 = 0.87\ x + 0.28$	44	0.99	< 0.001
$y_3 = 0.83\ x + 0.36$	45	0.99	< 0.001
$y_4 = 0.73\ x + 0.66$	6	0.99	< 0.001
$y_5 = 0.81\ x + 0.27$	30	0.99	< 0.001
$y_6 = 0.79\ x + 0.56$	30	0.99	< 0.001
$y_7 = 0.77\ x + 0.20$	30	0.99	< 0.001

n = number of samples analysed, r = correlation coefficient and P = probability of r = 0 (t-test).

the experiment the ratio was 3.2 in the control group and 1.0 in the group given 6.0 μg Se/kg. There were marked positive correlations between the amount of selenium added to the feed and the subsequent levels of selenium found in the eggs (Table 4). In the group given 6.0 mg Se/kg the selenium levels in homogenized eggs were between 4.4 and 6.2 μg/g wet weight.

The supplementation with selenomethionine up to 6.0 mg Se/kg feed had no significant effect on the growth of the chickens. Their mean final body weight ranged from 1614 to 1731 g (Table 5). The egg production was slightly higher in the experimental groups compared to the control group, and the egg weights were higher in the highest supplementary group (Table 5).

Table 5. Effects of different dietary levels of selenomethionine on egg weight, egg production and body weight. The egg production was recorded daily from week 5 to 18 of the experimental period. Differences between the groups were calculated using t-test for dependent data.

Added Se (mg/kg)	Egg weight (g)	No. of eggs per 100 hens per day	Body weight (g)
	n=75		
0	55.5±5.7	68.6±19.5[b]	1614±110
0.1	54.4±5.0	76.5±22.8	1707±164
0.5	56.3±5.1	79.3±23.8	1731±128
1.0	54.3±4.8	79.3±20.3	1633±180
3.0	55.4±3.1	82.0±19.6	1678±103
6.0	57.9±3.7[a]	82.0±19.9	1671±153

[a] Significantly higher than the other groups ($P < 0.01$)
[b] Significantly lower than group 0.1 ($P < 0.01$)

DISCUSSION

The close relationship between the amount of selenomethionine added to the feed and the subsequent levels of selenium found in tissues and eggs of laying chickens in the present investigation is generally in agreement with the experience in practical nutrition. Selenomethionine is the main source of selenium in natural feed ingredients, and there is a strong, positive correlation between the contents of selenium in the feed and the levels found in animals' tissues (*Ku et al.* 1972, *Scott & Thompson* 1971, *Latshaw* 1975). However, quite different results have been reported in a similar experiment in chickens when using sodium

selenite as the selenium source (*Moksnes & Norheim* 1982). The basal levels of selenium in the feed were not equal in these 2 experiments, but taking this into account, it is obvious that selenomethionine at all levels in the feed induce higher tissue levels than sodium selenite, especially in the muscle. The average levels of selenium in muscle increased from 0.15 to 0.29 µg Se/g only when 6.0 mg Se/kg as sodium selenite was added to a basal feed containing 0.17 mg Se/kg for 18 weeks (*Moksnes & Norheim* 1982). The same amount as selenomethionine raised the average muscle levels from 0.20 to 5.4 µg Se/g in the present investigation where the basal feed contained 0.30 mg Se/kg. The differences between selenomethionine and sodium selenite were less pronounced as far as their ability to raise the selenium levels in liver. At the highest supplementary level the concentrations in liver were about 3 times higher following selenomethionine compared to sodium selenite.

The greater ability of high levels of selenomethionine to raise the tissue levels of selenium in laying chicks does not necessarily mean that selenomethionine is more toxic, since selenomethionine may be incorporated into general body proteins in place of methionine (*Mc Connell & Hoffman* 1972).

Osman & Latshaw (1976) found lower selenium levels in liver, kidney and cardiac muscle of chicks when feeding selenomethionine compared to sodium selenite. The selenium contents of breast muscle and pancreas, however, were higher when selenomethionine was fed. Also *Cantor et al.* (1975), *Osman & Latshaw* (1976) and *Gabrielsen* (1978) who studied the availability of natural selenium in feedstuffs and of different selenium compounds, found sodium selenite more efficient than selenomethionine in preventing exudative diathesis in chickens. These findings are not in agreement with the present results and those of *Moksnes & Norheim* (1982). The discrepancy may be explained by the great differences in the selenium levels in the basal feed and the amount of selenium added, which were up to 100 times higher in the present experiments compared to those of the others mentioned.

One possible explanation for the higher availability to low levels of sodium selenite in preventing exudative diathesis has been discussed by *Sunde* (1980) who outlined a scheme of selenium metabolism. He suggested that selenite is generally better than selenomethionine and selenocysteine for the prevention of

selenium deficiency diseases because it is metabolically closer to the form of selenium incorporated into glutathione peroxidase. However, when selenium is given in excess selenomethionine is more potent than sodium selenite in raising the selenium concentration in tissues and eggs because of a possible incorporation of selenomethionine into general body proteins in place of methionine (*Mc Connell & Hoffman* 1972). It seems, therefore, that sodium selenite at low level supplementation is the best choice for preventing deficiency diseases. Selenomethionine, on the other hand, is more potent in building up depots of selenium in the animal body.

If one compares the present results of selenium levels in eggs to similar experiments with sodium selenite (*Moksnes & Norheim* 1982), it is also seen that the levels were highest when selenomethionine was added. *Olson et al.* (1970) found that the selenium content of dried egg white was greater than that of dried egg yolk when selenomethionine was fed as a source of selenium. *Latshaw & Osman* (1975) also found higher selenium concentrations in the egg white when feeding 0.20—0.30 mg Se/kg as naturally occurring selenium to hens. *Moksnes & Norheim* (1982) found a ratio between selenium in egg yolk and egg white of approximately 3.6 when adding sodium selenite to the feed. The ratio did not vary with varying dietary levels of selenium. This is in contrast with the present findings where the ratios decreased with increasing levels of selenomethionine in the feed. This difference in selenium distribution following sodium selenite and selenomethionine supplementation may be due to the fact that egg white proteins are synthesized in the oviduct and yolk proteins are synthesized in the liver (*Latshaw & Osman* 1975).

No signs of toxic effects were observed in the present study even at the highest intake of selenium. This is in agreement with previous experiments using sodium selenite as a source of selenium (*Moksnes & Norheim* 1982). *Ort & Latshaw* (1978) found the most sensitive criterion for toxic effects of sodium selenite to be the hatchability of fertile egg which was decreased by levels higher than 5 mg/kg in the feed. At this level the decrease was about 12 %.

The only differences observed between the different groups besides the selenium levels in organs and eggs, were a slightly lower egg production in the control group compared with the groups supplemented with selenium and a significantly higher

egg weight in the group supplemented with 6.0 mg/kg compared to the other groups. It is difficult to draw any firm conclusions from these results, but it is clear that the addition of selenomethionine to the diet of laying chickens did not have any negative effects on egg production and egg weights.

The present findings in chickens and those of *Moksnes & Norheim* (1982) are at variance with results found in lambs given supplementation of sodium selenite and selenomethionine (*Moksnes & Norheim* 1983). In lambs the 2 compounds were about equal in their ability to raise the selenium concentration in the tissues at levels up to 0.5 mg added selenium per kg, but if the selenium supplement was increased to 1.0 mg/kg, selenomethionine was more potent than sodium selenite. This indicates that there are species differences in the way of accumulating different selenium compounds.

In conclusion, excess supply of selenium as selenomethionine is more potent than sodium selenite in raising the selenium concentration in tissues and eggs of laying chickens, especially in muscle and egg white which are the main products used for human consumption. If one intends to increase the selenium content of these products in order to enhance the human intake of selenium, selenomethionine would be much more effective than sodium selenite. In low selenium areas with suboptimal human intake of selenium, increased selenium concentration in the animals products will give a positive contribution to the human intake of selenium. On the other hand, in areas with sufficient selenium intake, a supplementation up to 10 times the minimum requirement in chicks does not, however, increase the levels of selenium in poultry products to such a degree that these could be considered as a potential risk for human consumption.

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SAMMENDRAG

Selenavleiringen i organer og egg hos verpehøns som har fått overskudd av selen i form av selenomethionin.

Førtiåtte verpehøns ble delt i 6 grupper og gitt et grunnfôr som inneholdt 0.30 mg Se/kg (tørrvekt). Dette fôret ble tilsatt henholdsvis 0, 0,1, 0,5, 1,0, 3,0 og 6,0 mg Se/kg i form av selenomethionin, og fôret i 18 uker. Ved avslutningen av forsøket ble det funnet at et tilskudd på

bare 0,1 mg Se/kg forårsaket signifikant høyere selenkonsentrasjoner i brystmuskel og egg, og da særlig i eggehviten. Signifikante positive sammenhenger ble funnet mellom nivåene av selen i organer og egg og den mengden selen som ble tilsatt fôret. I den gruppen som fikk 6,0 mg Se/kg varierte selenverdiene i alle analyserte organer og egg fra 4,8 til 7,3 μg Se/g. Selv i denne gruppen ble det ikke observert noen toksisk effekt av selentilsetningen. Et overskudd av selenomethionin i fôret til verpehøns fører til høyere konsentrasjoner av selen i organer og egg enn et tilsvarende overskudd av natriumselenitt. Det er også vist at et tilskudd på opp til ti ganger minstebehovet ikke fører til selenkonsentrasjoner i produktene som kan medføre noen risiko for konsumentene.

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