The effect of digitonin on egg quality, cholesterol content in eggs, biochemical and haematological parameters in laying hens

E. Tůmová, H. Härtlová, Z. Ledvinka, A. Fučíková

Czech University of Agriculture, Prague, Czech Republic

ABSTRACT: The aim of the present study was to describe the effect of digitonin on egg quality, cholesterol content in eggs, some parameters of serum lipid metabolism and blood picture of laying hens. The experiment was split into 3 groups (8 laying hens per group); group 1 was fed control feed mixture with 16.54% crude protein and 11.61 MJ ME, group 2 received control feed mixture with 0.01% digitonin and supplement of digitonin in group 3 was 0.025%. Digitonin in the amount 0.025% significantly ($P \leq 0.05$) reduced egg weight from 65.07 g in control group to 61.44 g in group 3, white weight (41.21 g vs. 37.96 g), and egg cholesterol content (13.64 mg/g vs. 12.95 mg/g of yolk). Digitonin significantly ($P \leq 0.05$) increased HDL cholesterol (0.43 mmol/l in control group to 0.59 mmol/l in group 3) and triglycerides from 13.47 mmol/l (control) to 16.90 mmol/l (group 3) in blood serum. Significantly ($P \leq 0.05$) lower number of erythrocytes and MCV value in group 3 were observed.

Keywords: digitonin; egg weight; lipid metabolism**;** egg cholesterol; blood picture

Digitonin is a plant steroid that belongs to steroids sapogenin, C_{27}-steroid. Digitonin contains 1 mol of digitogenin, 2 mols of glucose and 1 mol of xylose. Saponins in complex with cholesterol and other 3 β-hydroxysterols form molecular compounds such as cholesterol digitonide. Digitonin and its analogues damage liposomal elements of plasma membranes and cause haemolysis of red cells. These compounds activate granulocytes as well (Nishikawa *et al*., 1984; Sancho *et al*., 1993). Clarke and Hillard (1981) were interested in the effect of digitonin on hepatic cells in broiler chickens. The authors found out that plasma cholesterol disappeared from plasma membranes and it caused perforation of plasma membranes as a result of elimination of plasma cholesterol. Digitonin inactivates acetyl CoA carboxylase by means of its transformation to inactivated form. This process reduces fatty acid synthesis. Haeffaner and Wittmann (1999) observed the effect of cholesterol – digitonide on the growth of ascites tumour cells. According to their description cholesterol – digitonide influenced phospholipase C activity. Phospholipase C activity could be increased synergistically in cholesterol – digitonide treated cells.

Digitonin is known as a substance that influences mainly cholesterol metabolism. The use of this compound results in a lower plasma cholesterol concentration, the modulation of the regulatory mechanism of hepatic metabolism (Ulloa and Nervi, 1985). These authors reported that digitonin decreased liver weight, bile flow and biliary cholesterol secretion in rats. Eastwood and Hamilton (1968) suggested that sterols inhibited bile acid reabsorption, thereby decreasing cholesterol body stores. According to Clarenburg *et al*. (1971) egg cholesterol levels in hens were lowered by 35% when 4% sitosterol was added to a standard diet. However, Bartov *et al*. (1971) found no effect of soybean sterols on egg yolk cholesterol levels. Fisher and Griminger (1967) found slightly higher plasma cholesterol-lowering effects from soybean sterols.

Supported by the Ministry of Education, Youth and Sports of the Czech Republic (Project No. 412100003).

A ignificant inverse relationship was found between serum cholesterol and the cholesterol present in the yolk (Sim and Bragg, 1978). The effect of digitonin on egg quality and lipid metabolism has not been described in available literature until now.

The aim of the present study was to evaluate the effect of digitonin on egg quality, cholesterol content in eggs, selected parameters of lipid metabolism and blood picture of laying hens.

MATERIAL AND METHODS

Twenty-four laying hens Hisex brown were used in this experiment. The experiment was split into 3 groups (8 laying hens per group); group 1 was fed control feed mixture, group 2 received control feed mixture with 0.01% digitonin and feed mixture for group 3 was supplemented with 0.025% digitonin. The composition of feed mixture is given in Table 1. The experiment lasted 16 weeks, from the 28th week of age to the 44th week of age. The laying hens were kept individually in 3-floor cages Big Dutchman Eurovent. There was a floor space of 1 000 cm^2 per hen, 10 cm feeding space and 2 available drinkers per hen. The temperature 20°C and relative humidity 65–70% were maintained for the whole experiment. A 16-hour photoperiod was used. The laying hens had ad libitum access to feed and water.

Some biochemical parameters were measured in blood serum, cholesterol, HDL cholesterol, and triglycerides. Laying hens were bled from the wing vein in 14-day intervals (64 samples per group). Biochemical characteristics were determined by photometrical methods on Hitachi 911 analyser (Hitachi Chemical Diagnostic, Inc.). Cholesterol content was analysed enzymatically by CHOD-POD method. In this method cholesterol esters are hydrolysed by cholesterol esterase and cholesterol oxidase and in the third reaction H_2O_2 is produced determined by Trinder reaction. HDL cholesterol was detected by a direct method using the enzymatic set (Lachema, Brno, Czech Republic) for total cholesterol determination. The method for triglyceride analysis is based on the fact that triglycerides are split by lipoprotein lipase to glycerol and fatty acids. In the analyses glycerol is phosphorylated and oxidised. Triglycerides are determined by GPO-PAP method (sets of Lachema, Brno, Czech Republic).

Haematological parameters, namely erythrocyte number (Er), leukocyte number (Le), mean cell volume (MCV), concentration of haemoglobin (Hb) and haematocrit value (PCV), were determined. All haematological characteristics were detected in blood stabilized with K_2EDTA. The analyses were done using a Coulter Model ZF (Coulter Electronics Ltd, England).

Eggs for measuring the egg quality were sampled every 14 days, 2 days in each collection period

Table 1. Composition of feed mixture

Ingredients	%
Wheat	49.87
Maize	17.00
Fish meal	1.5
Rapeseed meal	4.00
Lucerne meal	2.00
Soybean meal	14,00
Rapeseed oil	1.5
Limestone	5.50
Salt	0.25
Eggshells	3.0
Bolifor MCP-F	0.83
DL-methionine	0.05
Aminovitan SK-C1	0.50
Content of nutrients	
Dry matter	87.36
Crude protein	16.54
Metabolisable energy (MJ)	11.16
Fat	4.09
Fibre	3.48
Ca	3.56
P	0.57

Composition of Aminovitan SK per 1 kg of premix: vitamin A 1 600 000 i.u., vitamin D_3 450 000 i.u., α-tocopherol 3 000 mg, vitamin K_3 300 mg, vitamin B_1 300 mg, vitamin B_2 800 mg, vitamin B_6 400 mg, vitamin B_{12} 2 mg, niacin 4 000 mg, Ca pantothenate 1 200 mg, choline 50 000 mg, biotin 12 mg, folic acid 80 mg, betaine 10 000 mg, DL-methionine 60 g, Co 60 mg, Cu 1 200 mg, Fe 6 000 mg, I 140 mg, Mn 12 000 mg, Zn 10 000 mg, Se 40 mg, butylhydroxytoluene 3 000 mg, ethoxyquin 2 000 mg, butylhydroxyanisole 400 mg, wheat meal ad 1 kg

(12 eggs for each collection period in the group, total 96 eggs per group). Egg weight, yolk weight, white weight, shell weight and shell deformation were determined. Eggshell deformation was measured by means of nondestructive deformation with Columbus M instrument (Simeonovová *et al*., 1992). Egg cholesterol content was determined in yolk by the method of Ingr and Simeonovová (1983).

Data were analysed by one-way analysis of variance ANOVA. Means were compared by Duncan's test. Differences were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

The results of egg quality characteristics are presented in Table 2. Digitonin at the level of 0.025% significantly decreased egg weight ($P \leq 0.05$). Differences between control group and group 3 were more than 3.5 g. On the other hand, egg yolk weight was not affected by digitonin but white weight was significantly reduced ($P \leq 0.05$) in group 3 with higher digitonin content. Digitonin in the amount of 0.025% significantly decreased eggshell weight ($P \leq 0.05$) but did not influence eggshell deformation. No literary sources for comparison of egg quality and digitonin content are available. Egg cholesterol content was diminished by digitonin in group 3 significantly ($P \leq 0.05$). Similar effects of sterols were described by Singh *et al*. (1972), who found that desmosterol accumulated in the blood replaced cholesterol in the egg yolk. Weiss *et al*. (1967) suggested that the egg could represent an excretory mechanism for an excess of cholesterol in the blood.

The performance of biochemical characteristics is presented in Table 3. Serum triglycerides and HDL cholesterol level were significantly ($P \leq 0.05$) higher in group 3 (0.025% digitonin). A higher sup-

Table 2. Selected egg quality characteristics

Measurement	Group		
	1 – control $n = 96$	2 – 0.01% digitonin $n = 96$	3 – 0.025% digitonin $n = 96$
Egg weight (g)	65.07[a]	65.94[a]	61.44[b]
Yolk weight (g)	16.64	16.77	16.37
White weight (g)	41.21[a]	41.49[a]	37.96[b]
Shell weight (g)	7.22[b]	7.69[a]	7.14[b]
Shell deformation (μm)	31.72	34.67	32.30
Cholesterol content (mg/g)	13.64[a]	13.14[ab]	12.95[b]

[a,b]statistically significant differences ($P < 0.05$) in the same row are designated by different superscripts

Table 3. Average values of some characteristics of lipid metabolism

Measurement	Group		
	1 – control $n = 64$	2 – 0.01% digitonin $n = 64$	3 – 0.025% digitonin $n = 64$
Cholesterol (mmol/l)	4.59	3.4	7.53
HDL cholesterol (mmol/l)	0.43[b]	0.42[b]	0.59[a]
Triglycerides (mmol/l)	13.47[b]	12.94[b]	16.90[a]

[a,b]statistically significant differences ($P < 0.05$) in the same row are designated by different superscripts

Table 4. Average values of haematological characteristics

Measurement	Group		
	1 – control $n = 64$	2 – 0.01% digitonin $n = 64$	3 – 0.025% digitonin $n = 64$
Er (T/l)	3.15[a]	2.88[ab]	2.83[b]
Le (G/l)	17.65	18.41	16.59
PCV (%)	33.57	30.73	28.71
MCV (f/l)	123.71[a]	122.03[a]	118.00[b]
Hb (g/100 ml)	17.17	16.64	16.19

[a,b]statistically significant differences ($P < 0.05$) in the same row are designated by different superscripts

plement of digitonin increased the content of serum cholesterol but the difference between the control group and group 3 was insignificant. The higher level of serum triglycerides shows that digitonin in the content of 0.025% influences fatty metabolism, namely lipolysis. The place where fatty acids are synthetised is the liver in birds (Leveile *et al*., 1975). HDL elements are acceptors of free cholesterol from tissues. The higher level of HDL cholesterol could be caused by increased cholesterol transport from yolk because we recorded a significantly lower cholesterol content in groups with digitonin.

A significantly ($P \leq 0.05$) lower number of erythrocytes and MCV value (Table 4) were observed in group 3 (0.025% digitonin). At the same time we recorded insignificantly lower concentrations of haemoglobin and haematocrit in this group. According to literature (Sancho *et al*., 1993; Mensies *et al*., 1999) it is possible that digitonin damages erythrocytes.

Our results confirmed that digitonin influenced several parts of fatty metabolism. The supplement of 0.025% of digitonin increased serum HDL cholesterol and triglycerides, decreased egg cholesterol content. An inverse relationship between serum cholesterol and cholesterol present in the yolk corresponded with findings of Sim and Bragg (1978). In the blood digitonin significantly reduced the number of erythrocytes and MCV value. Digitonin affected egg weight, white weight and egg cholesterol content.

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Received: 03–06–12
Accepted after corrections: 03–12–02

ABSTRAKT

Vliv digitoninu na kvalitu vajec, obsah cholesterolu ve vejcích, biochemické a hematologické ukazatele u slepic nosného typu

Cílem pokusu bylo posouzení vlivu digitoninu na kvalitu vajec, obsah cholesterolu ve vejcích, vybrané parametry tukového metabolismu a krevní obraz u slepic nosného typu. Pokus byl rozdělen do tří skupin (osm slepic ve skupině), skupina 1 byla krmena kontrolní krmnou směsí s 16,54 % N-látek a 11,61 MJ metabolizovatelné energie, skupina 2 dostávala kontrolní směs s doplňkem 0,01 % digitoninu a skupina 3 s doplňkem 0,025 % digitoninu. Digitonin v množství 0,025 % signifikantně ($P \leq 0,05$) snížil hmotnost vajec z 65,07 g v kontrolní skupině na 61,44 g ve skupině 3, hmotnost bílku (41,21 g vs. 37,96 g) a obsah cholesterolu ve vejcích (13,64 mg/g vs. 12,95 mg/g žloutku). V krevním séru digitonin významně ($P \leq 0,05$) zvýšil HDL cholesterol (z 0,43 mmol/l v kontrolní skupině na 0,59 mmol/l ve skupině 3) a obsah triglyceridů z 13,47 mmol/l (kontrola) na 16,90 mmol/l (skupina 3). Byl zjištěn průkazně ($P \leq 0,05$) nižší počet erytrocytů a hodnota MCV ve skupině 3.

Klíčová slova: digitonin; hmotnost vajec; metabolismus lipidů; cholesterol ve vejcích; krevní obraz

Corresponding Author

Prof. Ing. Eva Tůmová, CSc., Katedra chovu prasat a drůbeže, Česká zemědělská univerzita v Praze,
165 21 Praha 6-Suchdol, Česká republika
Tel. + 420 224 383 048, e-mail: tumova@af.czu.cz