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THE EFFECT OF DIET CONTAINING ARSENIC (III) OXIDE ON THE TRAITS OF EGGS *

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ABSTRACT

The main objective of this study was to determine the effect of different arsenic concentration in feed on the physical traits of eggs. Twenty-four Rhode Island Red laying hens were divided into four equal groups. Three groups received feed mixture with additional arsenic in the form of arsenic (III) oxide in the following concentrations: 7.5 mg kg^{-1} (group 1), 15.0 mg kg^{-1} (group 2) and 30.0 mg kg^{-1} (group 3). The control group was fed on diet without additional arsenic. From the third to the seventh day eggs were collected and measured for physical traits. It has been established that during the trial period arsenic concentration in diet had a highly significant effect ($P = 0.0001$) on shell colour, egg mass, height of dense albumen, as well as on the number of Haugh units. The effect on shell mass and shell thickness was significant ($P \leq 0.01$), while there was no significant influence on the colour of yolk. Changes in physical traits of eggs were not significant during this period. The only exception was the change in yolk colour, yet it was not due to arsenic, but to feeding mixture with added colouring the hens had been fed on prior to trial.

Key words: poultry / hens / animal nutrition / feed / arsenic / eggs / mass / height of dense albumen / Haugh units / eggshell / yolk / colour

VPLIV ARZENOVEGA (III) OKSIDA V KRMI ZA KOKOŠI NA LASTNOSTI JAJC †

IZVLEČEK

Proučevali smo vpliv koncentracije arzena v krmi na fizikalne lastnosti jajc. V preskus smo vključili 24 kokoši nesnic pasme rodajland, ki smo jih naključno razdelili v štiri enako velike skupine. Trem preskusnim skupinam smo v krmo dodajali naslednje koncentracije arzena v obliki arzenovega (III) oksida: 7,5 mg kg^{-1} (prva skupina), 15,0 mg kg^{-1} (druga skupina) in 30,0 mg kg^{-1} (tretja skupina). Kontrolna skupina je prejemala krmo brez dodanega arzena. Od tretjega do sedmega dneva preskusa smo zbirali jajca za ocenjevanje fizikalnih lastnosti jajc. Ugotovili smo, da je koncentracija arzena v krmi v tem obdobju zelo značilno ($P = 0{,}0001$) vplivala na barvo jajčne lupine, maso jajc, višino gostega beljaka ter število Haughovih enot. Vpliv na maso in debelino lupine je bil statistično značilen ($P \leq 0{,}01$), na barvo rumenjaka pa koncentracija arzena v krmi ni imela statistično značilnega vpliva. Spremembe fizikalnih

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lastnosti s časom v omenjenem obdobju niso bile statistično značilne. Izjema so bile le spremembe v barvi rumenjaka. Te pa so posledica krmljenja kokoši s krmnimi mešanicami z dodanimi barvili v predposkusnem obdobju in brez dodanih barvil v poskusnem obdobju.

Ključne besede: perutnina / kokoši / prehrana živali / krma / arzen / jajca / masa / višina gostega beljaka / Haughove enote / lupina / rumenjak / barva

INTRODUCTION

In normal conditions, the daily quantity of arsenic entering the organism with water and air is negligible as it comes to just a few μg. Feed is the main source of arsenic, but there are quite some differences in the quantity of arsenic intake due to various composition of daily rations. The highest rate is in seafood origin (Environmental …, 1981). Under normal conditions, the arsenic content in eggs is rather low. In our experiment where arsenic was added to diet in the form of arsenic (III) oxide in different concentrations, it has been established that with increasing arsenic concentration in the poultry feedstuff, its concentration in egg yolk and white also increased, but a plateau appears to be reached quite rapidly (Holcman and Stibilj, 1997). According to literature, the trials were so far conducted to determine the influence of arsenic content in hen diet mainly on production and reproduction traits (Andrews *et al.*, 1966; Arnold *et al.*, 1973; Stanley *et al.*, 1994; Vodela *et al.*, 1997), not so much on the physical traits of eggs.

This paper presents the relationship between the arsenic concentration in feedstuff and the following physical egg traits: shell color, egg mass, height of albumen, Haugh units, yolk color, thickness and mass of the shell. Three different arsenic (III) oxide concentrations were added to hen diet and the changes in egg quality were observed during the 5-day trial period.

MATERIAL AND METHODS

Twenty-four Rhode Island Red hens were used in the experiment. They were 49 weeks old at the time of caging. Hens were divided at random into four equal groups of six and they were individually caged. The three experimental groups were fed for 19 days with feed in which arsenic (III) oxide added as follows: 7.5 mg kg^{-1} (group 1), 15.0 mg kg^{-1} (group 2), and 30.0 mg kg^{-1} (group 3), while the control group (group 0) consumed feed without added arsenic. In each component of feed mixture, the content of arsenic was determined (Table 1).

Table 1. The feed mixture composition and arsenic concentration in particular component
Preglednica 1. Sestava krmne mešanice in vsebnost arzena v posameznih sestavinah

Component	Component content in feed mixture, g kg^{-1}	Arsenic concentration, ng g^{-1}
Maize	679.3	13.2
Soybean cake	218.6	22.0
Sodium chloride (NaCl)	0.7	< 10.0
Ca-Na phosphate	15.5	21.5
Premix	5.0	1488.0
Limestone	80.0	42.2
Methionine	0.9	-

The eggs were collected from the 3rd to 7th day (five days) of the experiment to determine the physical traits of eggs, while arsenic concentration in eggs was determined from the 8th to the 19th day (Holcman and Stibilj, 1997). Arsenic concentrations in samples were determined in duplicate by neutron activation analysis (NAA) in its destructive or radiochemical form (RNAA) as described by Byrne and Vakselj (1974) and Byrne (1987). The detection limit for arsenic is less than 1 ng g^{-1}.

Physical egg traits were measured on the equipment set (Technical Services and Supplies Ltd; York). The color of eggshell was determined by reflectometer. Egg mass was measured on the electronic scale to 0.1 g accuracy. The egg was broken into the glass mirror and the height of dense albumen was measured 1 cm from the edge of the yolk by using tripod micrometer. Haugh units were calculated as well. The color of yolk was measured by colormeter, which classifies yolk according to the Roche scale. The shells were rinsed under tap water to eliminate egg white, but not the membranes. Washed shells were dried at room temperature for about 14 days, and after that, they were weighed on electronic scale to 0.1 g accuracy. Using micrometer shell thickness was determined together with both membranes to 0.01 mm accurate. Shell thickness was measured on three points along the equatorial part of the shell. The average value of the three measurements was annotated.

Statistical analysis was conducted on the data using least square method in general linear procedure (GLM) in statistical package SAS/STAT (SAS Institute Inc., 1989). The following model was used in analysis:

$$y_{ijkl} = \mu + S_i + D_j + SD_{ij} + K_{ik} + e_{ijkl}, \qquad [1]$$

where y_{ijkl} is observed physical trait, S_i and D_j represent arsenic concentration in feed (four groups) and successive day of the experiment (five days) as fixed effects with levels. Interaction between S_i and D_j (SD_{ij}) was included, as well. The effect of hen within the group (K_{ik}, 24 hens) was also treated as fixed. Identical, independent and normal distribution was assumed for residuals.

RESULTS

This paper covers analyses of physical traits of eggs between third and seventh day of experiment with arsenic additive in feedstuff, while another paper (Holcman and Stibilj, 1997) represented the arsenic residues in eggs during period from the 8^{th} to 19^{th} day of the same experiment.

The ratio of arsenic concentrations for groups with 7.5, 15.0 and 30.0 mg kg^{-1} feed was 1.0 to 2.0 to 4.0, while the ratio of arsenic in albumen was 1.0 to 2.2 to 5.0, and 1.0 to 2.1 to 5.1 in yolks (Table 2). Concentrations of arsenic in eggs were obviously related to content of arsenic in feed. Arsenic concentrations in fresh sample of egg albumen and yolk were similar within groups. The dry matter of albumen contained more arsenic than dry matter of yolks.

Table 2. The arsenic concentration in fresh and dry matter of albumen and yolk
Preglednica 2. Koncentracija arzena v sveži in suhi snovi beljaka in rumenjaka

Group	Arsenic concentration in fresh sample, ng g^{-1} *		Arsenic concentration in dry matter, ng g^{-1}	
	Albumen	Yolk	Albumen	Yolk
0.0 mg As kg^{-1}	0.4 ± 0.1	0.7 ± 0.3	3 ± 1	2 ± 1
7.5 mg As kg^{-1}	24 ± 4	28 ± 6	196 ± 31	59 ± 16
15.0 mg As kg^{-1}	54 ± 10	67 ± 7	423 ± 68	143 ± 12
30.0 mg As kg^{-1}	135 ± 32	133 ± 23	958 ± 122	303 ± 47

* = total number of samples for each experiment group was nine

The darkest shells were observed in the group with the highest arsenic concentration (37.8 %, group 3); there were slight differences between other groups (Table 3). Standard deviations for shell color were comparable among groups with values between 5.6 % and 7.9 %. Average egg mass was the highest in group 3 (59.6 g), and the lowest in group 2 (55.0 g). Also in regard to the height of dense albumen and Haugh units, group 3 with the highest arsenic concentration deviated upwards. The group averages for the color of yolk on Roche scale ranged between 10.3 and 10.9, and were similar. The lowest mass and eggshell thickness was in group 2, followed by the eggs of group 3; eggs of the control as well as the group 1 had the thickest and heaviest shell (Table 3).

Table 3. Mean values and standard deviations of physical traits of eggs
Preglednica 3. Srednja vrednost in standardni odklon za fizikalne lastnosti jajc po skupinah

Trait	Group, mg As kg^{-1}			
	0.0	7.5	15.0	30.0
No. of observations	23	25	18	26
Shell colour, %	43.0 ± 5.6	44.1 ± 6.6	45.1 ± 7.9	37.8 ± 5.6
Egg mass, g	56.1 ± 2.6	58.1 ± 4.1	55.0 ± 2.2	59.6 ± 2.6
Height of dense albumen, mm	5.6 ± 0.8	5.8 ± 1.0	6.0 ± 0.9	6.9 ± 1.3
Haugh units	74.7 ± 6.0	74.8 ± 8.0	77.6 ± 7.1	82.4 ± 8.2
Yolk colour, Roche	10.3 ± 1.3	10.9 ± 1.1	10.4 ± 1.2	10.5 ± 0.9
Shell mass, g	5.3 ± 0.6	5.4 ± 0.4	5.0 ± 0.5	5.3 ± 0.4
Shell thickness, 10^{-2} mm	36.9 ± 4.1	37.5 ± 2.3	35.4 ± 3.6	36.2 ± 2.6

Arsenic concentration in feed had highly significant influence on the shell colour, on egg mass, height of dense albumen and Haugh units (P = 0.0001, Table 4), and significant effect on the mass (P = 0.023) and thickness of the shell (P = 0.0054). The effect of arsenic on the color of yolk showed only trends (P = 0.0714). Five day trial period had no significant influence on the observed egg traits (Table 4). An exception was the color of yolk as it changed significantly (P = 0.0001) during the five days of experiment. Interaction of arsenic concentration in hen diet and trial period had no significant influence on any of the observed physical egg traits (Table 4).

Table 4. Effects on physical traits of eggs (P-values)[a]
Preglednica 4. Vplivi na fizikalne lastnosti jajc (p-vrednosti)

Trait	Source of variability			
	Arsenic content	Time	Group * time interaction	Hen
	3[b]	4	12	19
Shell color, %	0.0001	0.3935	0.4123	0.0001
Egg mass, g	0.0001	0.6975	0.1479	0.0001
Height of dense albumen, mm	0.0001	0.3586	0.8032	0.0001
Haugh units	0.0001	0.1928	0.7447	0.0001
Yolk color, Roche	0.0714	0.0001	0.9221	0.0001
Shell mass, g	0.0023	0.5135	0.5688	0.0001
Thickness of shell, mm	0.0054	0.1027	0.4265	0.0001

[a] = probability, [b] = degrees of freedom

Hen in the group had highly significant influence on the studied physical egg traits (P = 0.0001). Differences among animals were large. However, the sample was too small to draw conclusions about hen differences. The main reason to include the effect of hen in the model was to reduce residual variance and therefore, to reduce standard error of estimates in other effects.

Table 5. Least square means, estimated differences between groups with standard errors (above diagonal) and statistical significances of differences from multiple comparison after Scheffe (below diagonal) for physical traits of eggs

Preglednica 5. Tehtane srednje vrednosti, ocenjene razlike med skupinami s standardnimi napakami (nad diagonalo) in statistična značilnost razlik testa več sredin po Scheffeju

Trait	Group, mg As kg^{-1}	LSM[a] ± SEM[b]	Group, mg As kg^{-1}			
			0.0	7.5	15.0	30.0
Shell, color, %	0.0	42.53 ± 1.05		–2.15 ± 1.46	–2.10 ± 1.65	4.91 ± 1.45
	7.5	44.69 ± 1.01	0.5391		0.05 ± 1.64	7.07 ± 1.42
	15.0	44.63 ± 1.28	0.6590	1.0000		7.02 ± 1.63
	30.0	37.62 ± 1.00	0.0145	0.0001	0.001	
Egg mass, g	0.0	56.43 ± 0.49		–2.13 ± 0.68	1.43 ± 0.77	–2.99 ± 0.67
	7.5	58.00 ± 0.47	0.0273		3.56 ± 0.76	–0.86 ± 0.66
	15.0	55.00 ± 0.60	0.3377	0.0003		–4.42 ± 0.76
	30.0	59.43 ± 0.47	0.0007	0.6389	<.0001	
Height of dense albumen, mm	0.0	5.64 ± 0.15		–0.08 ± 0.21	–0.27 ± 0.24	–1.44 ± 0.21
	7.5	5.75 ± 0.15	0.9833		–0.18 ± 0.24	–1.35 ± 0.21
	15.0	5.91 ± 0.19	0.7471	0.9002		–1.17 ± 0.24
	30.0	7.08 ± 0.15	<.0001	<.0001	0.0001	
Haugh units	0.0	74.80 ± 1.01		0.51 ± 1.40	–2.37 ± 1.59	–8.59 ± 1.39
	7.5	74.28 ± 0.98	0.9873		–2.88 ± 1.58	–9.10 ± 1.37
	15.0	77.17 ± 1.24	0.5347	0.3506		–6.22 ± 1.57
	30.0	83.39 ± 0.96	<.0001	<.0001	0.0030	
Yolk color, Roche	0.0	10.50 ± 0.13		–0.38 ± 0.18	0.13 ± 0.21	–0.03 ± 0.18
	7.5	10.88 ± 0.13	0.2361		0.51 ± 0.21	0.35 ± 0.18
	15.0	10.37 ± 0.16	0.9439	0.1163		–0.16 ± 0.21
	30.0	10.53 ± 0.13	0.9982	0.2973	0.8881	
Shell mass, g	0.0	5.29 ± 0.07		–0.14 ± 0.10	0.34 ± 0.12	0.05 ± 0.10
	7.5	5.43 ± 0.07	0.6203		0.47 ± 0.12	0.19 ± 0.10
	15.0	4.96 ± 0.09	0.0538	0.0022		–0.29 ± 0.11
	30.0	5.24 ± 0.07	0.9738	0.3435	0.1171	
Thickness of shell, 10^{-2} mm	0.0	37.17 ± 0.46		–0.23 ± 0.64	2.29 ± 0.73	1.01 ± 0.64
	7.5	37.41 ± 0.45	0.9873		2.53 ± 0.72	1.25 ± 0.63
	15.0	34.88 ± 0.57	0.0271	0.0107		–1.28 ± 0.72
	30.0	36.16 ± 0.44	0.4768	0.2779	0.3726	

[a] = least squares mean, [b] = standard error of the mean

The darkest shell was observed from group 3 with the highest arsenic concentration in feed, followed by the control group with no added arsenic (Table 5). Group 1 and 2 had almost the same color of shell. The color of shell in the two experimental groups was highly significantly different in comparison to group 3. The difference in the control and the group 3 was significant (P = 0.0145). Eggs from group 3 were the heaviest, the lightest in group 2; the difference was significant (P < 0.0001). Control group deviated significantly (P = 0.0007) from group 3 and group 1 (P = 0.0272). In regard to the height of dense albumen and Haugh units the group 3 highly significantly differed from the control and the other two experimental groups, while the differences between the control, group 1 and group 2 were unsignificant. Unimportant differences in the color of yolk between the groups were observed. Trend in statistically significance showed only the difference between group 1 and group 2. The highest shell mass was noticed in group 1, the lowest in group 2. The difference 0.47 g was highly significant. Mean shell mass differed close to significance between the control and group 2 (P = 0.0538) as well as between group 2 and 3 (P = 0.1171). The thickest shell had the eggs from group 3, the thinnest shell the eggs in group 1. The group 2 significantly varied from the control (P = 0.0271) and group 1 (P = 0.0107), other differences were not significant (Table 5).

Table 6. Least square means, estimated differences between days with standard errors (above diagonal) and statistical significances of differences from multiple comparison after Scheffe (below diagonal) for yolk color

Preglednica 6. Tehtane srednje vrednosti, ocenjene razlike med dnevi s standardnimi napakami (nad diagonalo) in statistična značilnost razlik testa več sredin po Scheffeju za barvo rumenjaka

Trait	Day	LSM[a] ± SEM[b]	Day				
			1	2	3	4	5
Yolk color, Roche	1	11.87 ± 0.16		0.59 ± 0.22	1.69 ± 0.24	2.02 ± 0.21	2.19 ± 0.24
	2	11.27 ± 0.14	0.1354		1.09 ± 0.22	1.42 ± 0.20	1.60 ± 0.23
	3	10.18 ± 0.16	<.0001	0.0003		0.33 ± 0.21	0.51 ± 0.24
	4	9.85 ± 0.14	<.0001	<.0001	0.6582		0.18 ± 0.22
	5	9.68 ± 0.17	<.0001	<.0001	0.3544	0.9592	

[a] = least squares mean, [b] = standard error of the mean

On the first measuring day the values of yolk color ranged from 11.3 (group 2) and 12.2 (group 1), on the fifth day from 9.0 in group 2 to 10.2 in group 1, but interaction between As concentration and days in experiment were not significant. Changes in yolk color during the start of experiment were expected as the hens had been, prior to the trial, fed on wholesome feed mixture that contained premix with added pigment. Feed mixture used in trial was without the pigment. The effect of time is described in detail in Table 4, where day 1 means the first measurement and day 5 the fifth measurement of physical traits. These were taken out the third and seventh day of experiment, respectively.

Differences in shell color, egg mass, shell mass, height of dense albumen, as well as Haugh units and shell thickness between the separate days were not significant (Table 4). Yolk color was most intense on the day 1, later on it gradually got paler (Table 6). Differences between the days 3 and 4 (P = 0.6582), as well as the days 4 and 5 (P = 0.9592) and between days 3 and 5 (P = 0.3544) were not significant, but some trends in difference were shown between days 1 and 2 (P = 0.1354). Other differences between pairs of days were highly significant (P = 0.0001).

DISCUSSION

Estimated mean of shell color in the control group was 42.53 %, and 44.69 %, 44.63 %, 37.62 % in groups 1, 2 and 3, respectively (Table 5). The darkest shell had eggs of hens from the group 3, the lightest shell was in the group 2. Feed concentration 30 mg As kg^{-1} caused change (4.91 %) in the shell color compared to the control group (P = 0.0145) and the other two experimental groups (P = 0.0001 and P = 0.0011) Differences between group 3 and 2 (7.02 %), as well as between group 3 and group 1 (7.09 %) were larger. From the third to the seventh day of arsenic addition to feed mean egg mass was 56.43 g in the control group, 58.56 g in group 1, 55.0 g in group 2, and 59.43 g in group 3 (Table 5). The addition of 30.0 mg As kg^{-1} feed caused increase in mean egg mass during the trial period (2.99 g, P = 0.0007) and 7.5 mg kg^{-1} addition caused increase, too (4.42 g, P = 0.0273). The difference between the control and group 2 was insignificant.

In eggs of the control group, estimated mean height of dense albumen was 5.64 mm, 5.73 mm in group 1, 5.91 mm in group 2, and 7.08 mm in group 3. Mean values of Haugh units were 74.80 (control group), 74.28 (group 1), 77.17 (group 2), and 83.39 (group 3). The addition of 7.5 or 15.0 mg As kg^{-1} feed did not increased the height of dense albumen and the number of Haugh units in comparison to the control group significantly (Table 5). Additional 30.0 mg As in 1 kg of feed increased the height of dense albumen for 1.44 mm ($P < 0.0001$) and the number of Haugh units for 8.59 ($P < 0.0001$). Group 3 had significantly higher values in both traits comparing to groups 1 and 2, too. Andrews *et al.* (1966) studied the effect of arsenic on the quality of egg albumen (Haugh units). In their trial, laying hens were fed on diet with the additional 2 g kg^{-1} of arsanilic acid. They concluded this addition had no effect on Haugh units.

In control group, the least square mean for yolk color was 10.50, and 10.88, 10.37, 10.53 in the group 1, 2, and 3, respectively. Differences between groups in yolk color were unsignificant, standard errors of estimates were large comparing to them. Only the difference between the first and the second experimental group showed some trends (P = 0.1163).

Shells in the control group weighed 5.29 g in average, their thickness was 0.37 mm. Similar were masses of eggs in groups 1 and 3 (5.43 and 5.24 g), while group 2 had estimate of 4.96 g. Shell mass and shell thickness (0.35 mm) was smaller at 15.0 mg As kg^{-1}, while the other two arsenic concentrations had no effect on the mass and thickness of the shell.

Stanley *et al.* (1994) reported that the addition of 25 or 100 μg As to 1 g of feed for mallards (in the form of sodium arsenate) caused 0.1 or 0.2 mm increase in shell thickness compared to the control group, yet the differences were not significant. The addition of 400 μg As to 1 g of feed caused 0.1 mm decrease in shell thickness, but the difference was again not significant. Andrews *et al.* (1966) found no effect of added 2 g kg^{-1} arsanilic acid on shell thickness.

The results of our experiment show that the addition of 30 mg As (in the form of As_2O_3) to 1 kg of feed for laying hens had favorable effect on physical egg traits: egg shell was darker, egg mass was increased, so was the height of dense albumen, and higher was the value of Haugh units.

POVZETEK

V preskus smo vključili 24 kokoši nesnic pasme rodajland, starih 49 tednov. Vseljene so bile v individualne kletke in razdeljene v štiri enako velike skupine. Trem preskusnim skupinam smo v krmo 19 dni dodajali naslednje koncentracije arzena v obliki arzenovega (III) oksida: 7,5 mg As kg^{-1} (prva skupina), 15,0 mg As kg^{-1} (druga skupina) in 30,0 mg As kg^{-1} (tretja skupina). Kontrolna skupina je prejemala krmo brez dodanega arzena. Od tretjega do sedmega dne preskusa smo merili fizikalne lastnosti jajc na elektronskem kompletu aparatur. Koncentracija arzena v krmi je statistično značilno vplivala na barvo lupine, maso jajc, višino gostega beljaka in Haughove enote ($P < 0,001$), na maso in debelino lupine ($P < 0,01$). Jajca iz skupine, ki je v krmi dobivala najvišjo koncentracijo arzena, so bila najtežja in z najtemnejšo lupino. Po večji višini gostega beljaka in večjem številu Haughovih enot je prav tako ta skupina statistično značilno ($P < 0,001$) odstopala od vseh drugih skupin. Razlike v barvi rumenjaka med skupinami so bile majhne, značilna je bila le razlika med prvo in drugo skupino ($P < 0,05$). Največja razlika v masi lupine je bila med prvo in drugo skupino (P = 0,0002). Najdebelejšo lupino so imela jajca prve skupine najtanjšo pa jajca druge skupine**.** Spremembe fizikalnih lastnosti jajc s časom preskusa, to je od tretjega do sedmega dneva, niso bile statistično značilne, z izjemo barve

jajčnega rumenjaka. Ta je s trajanjem preskusa bledel, ker so bile kokoši pred preskusom krmljene s popolno krmno mešanico z dodanimi barvili, v času preskusa pa brez barvil. Kokoš ima statistično zelo značilen vpliv na vse preučevane fizikalne lastnosti jajc.

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