Bulgarian Journal of Agricultural Science, 20 (No 6) 2014, 1472-1476
Agricultural Academy

EGG YOLK LIPIDS CHANGE IN JAPANESE QUAIL GIVEN *TRIBULUS TERRESTRIS* EXTRACT

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Abstract

GRIGOROVA, S., M. NIKOLOVA, D. PENKOV and V. GERZILOV, 2014. Egg yolk lipids change in Japanese quail given *Tribulus terrestris* extract. *Bulg. J. Agric. Sci.*, 20: 1472-1476

The objective of the current research was to study the change of yolk lipid fractions in the eggs of Japanese quails (*Coturnix coturnix japonica*) given different doses of the Bulgarian product *Vemoherb T* (dry extract from the herb *Tribulus terrestris*). A total of 52 female and 16 male Japanese quails of Pharaon breed, at the age of 44 days were randomly divided into four groups – control and 3 experimental (13 female and 4 male in each). All groups were fed *ad libitum* the same compound feed. *Tribulus terrestris* was added to the quails' drinking water in the following daily doses: 4mg/kg body weight for a period of 10 weeks (I^{st} group); 10mg/kg body weight during the first fife weeks of the experiment (II^{nd} group); 10mg/kg body weight for a period of 10 weeks (III^{nd} group). The following indices of egg yolk were determined at the end of the experiment: total lipids – by Bligh and Dyer (1959); phospholipids – by Bartlett (1975); total cholesterol content – by Shoenheimer-Sperry (1950) and fatty acid composition – using the "Perichromm" gas chromatograph.

The content of total yolk lipids in III^{rd} experimental was significantly higher compared to the other two treated groups ($P < 0.05$). However there were no significant differences concerning this parameter between control and experimental groups ($P > 0.05$). The values of total yolk phospholipids were unaffected by addition of different doses TT extract. There was statistically proven decrease of total cholesterol content in the yolk of all experimental groups relative to control group ($P < 001$ for I^{st} and II^{nd} experimental groups and $P < 0.001$ for III^{rd} experimental group). Third experimental group unlike the other groups had higher concentration of linoleic acid ($P < 0.05$).

Key words: Vemoherb T, Japanese quail, egg yolk lipids, total cholesterol, phospholipids, fatty acid composition

Introduction

Cholesterol and fatty acid concentrations of egg yolk vary depending on ration's composition, genetic factors, age and egg production (Guglu et al., 2008,). Concerning nutrition one of the methods developed to change the lipid profile of eggs has been the use of different plant oil sources (flaxseed oil, oregano oil etc.) or herbal extracts (Profirov and Toncheva, 2005; Kazmierska et al., 2007; Grigorova et al., 2009; Mahajan et al., 2010). The annual herb *Tribulus terrstris* (TT) belongs to this group of products. It contains biologically active substances as saponins, flavonoids, alkaloids, tannins, unsaturated fatty acids etc. (Adaikan et al., 2000). The main active substances are steroidal saponins from furostanol type (Kostova and Dinchev, 2005). Predominant among them is protodioscin (Figure 1).

Tribulus terrestris is commonly used in the folk medicine as aphrodisiac and for treatment of erectile disfunction, diabetes, tumors, cardiovascular and other diseases (Chen et al., 2002; Orhan et al., 2004). It was found that this plant has a sizable antioxidative effect (Asenov et al., 1998) as well as blood cholesterol reductive effect (Grigorova et al., 2008a,b; Grigorova et al., 2009). Grigorova et al. (2008a,b) didn't find statistically proven changes of eggs' lipid fraction in laying hens and broiler parents given TT extract in daily dose 10 mg/kg body weight. However, they observed, that the addition of this extract to the compound feed of Guinea fowls in a dose of 10 mg/kg body weight decreased

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significantly ($P < 0.05$) yolk cholesterol content (Grigorova et al., 2009).

Quails are popular in Japan and China, in North America and in some European countries. The early sexual maturity, high egg production, short interval between the individual generations and low feed consumption make quail production increasingly attractive for our farmers (Bakalivanov et al., 2001). In the available literature we did not find any data concerning the effect of TT extract on egg yolk lipid fraction in Japanese quails.

The objective of this work was to study the change of egg yolk lipids in Japanese quails, given different doses of the Bulgarian product *Vemoherb T* (dry extract of *Tribulus terrestris*).

Materials and Methods

The tested herbal extract, produced by *Vemo 99 Ltd*. Company, Sofia, Bulgaria is standardized. It contains (in percent of dry matter): not less than 60% furostanol saponins defined as protodioscin; not less than 10% flavonoids determined as rutin; not less than 10% tannins. The product *Vemoherb-T* is innocuous for humans and animals. Its heavy metals content is ≤ 0.001%.

The present investigation was conducted in the period October-December (Nikolova and Penkov, 2010). A total of 52 female and 16 male, 44 days old Japanese quails (*Coturnix coturnix japonica*) from the breed Pharaon were randomly divided into four groups – control and 3 experimental, 13 female and 4 male quail each. The birds were housed in stainless steel wire cages in an experimental house of the Agricultural University, Plovdiv, Bulgaria on a 16 h lighting schedule, air temperature of 21-24˚C, and relative humidity 70-85%. Water was supplied via vacuum drinkers. The experiment lasted 10 weeks. All groups were fed *ad libitum* the same compound feed for Japanese quails. The ingredients and chemical composition of the diet are shown in Table 1. The forage nutritive value was determined by the conventional Weende analysis. The metabolizable energy was calculated according to WPSA (1989). Experimental groups received Vemoherb-T with the drink water in the following daily doses: 4 mg/kg body weight for a period of 10 weeks (I^{st} group); mg/kg body weight during the first fife weeks of the trial (II^{nd} group); 10mg/kg body weight for a period of 10 weeks (III^{rd} group).

The content of total lipids, total cholesterol and total phospholipids in the yolk was measured in 6 eggs from control group, 15 eggs from I^{st} end II^{nd} experimental groups and 9 eggs from III^{rd} experimental group, collected within two consecutive days at the end of the trial. At the end of experi-

Table 1
Composition and nutritive value of the compound feed for Japanese quail

Ingredients, $g.kg^{-1}$	
Maize	640.10
Soybean meal (44 CP)	220.00
Sunflower meal (37)	50.00
D-C-P	16.00
Limestone	62.00
Salt	2.90
Vitamin-mineral premix	7.00
DL- methionine	2.00
Nutritive value	
$BOEn-_0/AMEn-_0$, MJ/kg	11.80
CP, g	177.70
Lysine, g	9.00
Methionine+cystine, g	8.20
Ca, g	27.50
P, g	4.30

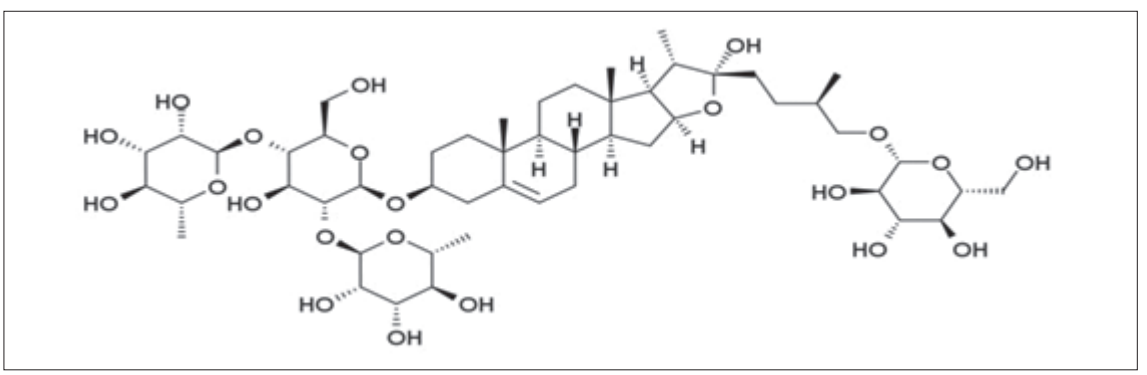

Fig. 1. Formula for protodioscin

ment in 5 eggs from each group was determined the content of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA). The eggs were manually broken and separated into egg white and yolk. Lipids were then extracted from egg yolks using chloroform: methanol mixture in a ratio 2:1 v/v. Total lipids were determined by the method of Bligh and Dyer (1959). The total cholesterol content was determined by the method of Shoencheimer-Sperry, modified by Sperry and Webb (1950). The method of Bartlett (Kates, 1975) was used for yolk phospholipids determination. The fatty acid composition of egg yolk was estimated by Perichrom gas chromatograph with capillary column Supelcowax – 10 (0.32 mm – 30 m) after preliminary esterification.

All data are presented as means with their standard errors. Statistical examination of treatment effects on egg yolk lipids was determined by Excel 2000, single factor, Anova program.

Results and Discussion

The results of egg yolk lipids analysis in control and experimental groups are given in Table 2. The content of total yolk lipids in IIIrd experimental group which was given the tested product in daily dose 10 mg/kg body weight for a period of 10 weeks was significantly higher compared to the other treated groups ($P < 0.05$). However there were no significant differences concerning this parameter between control and experimental groups ($P > 0.05$). The values of total yolk phospholipids were unaffected by addition of different doses TT extract. Grigorova et al. (2008a, 2009) didn't observed statistically proven changes of total yolk lipids and phospholipids in laying hens and guinea fowls given Vemoherb T in daily dose 10 mg/kg body weight. It is visible that total yolk cholesterol level in all experimental groups was significantly lower in comparison with control group ($P < 001$ for Ist and IInd experimental groups and $P < 0.001$ for IIIrd experimental group). Similar reduction of yolk total cholesterol content ($P < 0.01$) was reported by Grigorova et al. in guinea fowls, given 10 mg/kg body weight Vemoherb T for a period of 12 weeks. There is no available literature date concerning the mechanism of TT – induced reduction of yolk cholesterol.

A possible explanation of the observed lower yolk cholesterol level in the experimental quails could be related with their higher laying intensity ($P < 0.05$; $P < 0.001$; $P < 0.001$ for Ist, IInd and IIIrd experimental group respectively) and higher yolk weight, which are the subject of our earlier publication (Nikolova and Penkov, 2010). According Nichols et al. (1963) an inverse relationship exists between yolk size and cholesterol concentration. There is also an inverse relationship between total cholesterol and laying intensity (Bair and Marion, 1978). Yolk cholesterol level analyzed in our experimental material was comparable to that published by Baumgartner and Simeonova, 1992; Bakalivanov et al. (2001); Guglu et al. (2008), which found 19-20 mg cholesterol per g yolk in quails.

Fatty acid profiles of egg yolks from all groups of Japanese quails are shown in Table 3. All experimental groups had a lower content of saturated fatty acids than control group but the differences were not significant ($P > 0.05$). Palmitic acid was the predominant saturated fatty acid (SFA) in all groups (31.21%, 30.33%, 28.20%, 29.06% for control, Ist, IInd and IIIrd experimental groups respectively). The second most dominant SFA was stearic acid (11.20%, 10.43% 9.99%, 8.98% for a control, for Ist, IInd and IIIrd experimental groups respectively). The highest concentration of linoleic acid was established in the egg yolks from IInd exp. group which was given TT in a daily dose of 10 mg/kg body weight for a period of 5 weeks ($P < 0.05$ than control, Ist and IIIrd experimental groups). It should be noted that the essential arachidonic acid is synthesized in a human organism from linoleic acid (Bakalivanov et al., 2001). Similarly highest content of arachidonic acid was found in the IInd experimental group, but this difference was no significant ($P > 0.05$). The other fatty acids in the yolk were unaffected by the treatment of TT extract. Grigo-

Table 2
Lipid fractions of egg yolk in Japanese quails

Parameters	Control group	I exp.group	II exp. group	III exp. group
	n=6	n=15	n=15	n=9
Total lipids, mg/g yolk	34.96 ± 0.55	34.24 ± 0.32 a_1	34.39 ± 0.61 a	36.21 ± 0.46 a, a_1
Total cholesterol, mg/g yolk	22.13 ± 0.54 b, b_1, c	18.90 ± 0.56 b	19.66 ± 0.49 a_2, b_1,	18.72± 0.37 a_2, c
Total phospholipids, mg/g yolk	93.43 ± 2.06	91.54 ± 1.30	91.77± 2.30	95.16 ± 0.99

The value mark of same letters is significant at: a, a_1, a_2 - $P < 0.05$; b, b_1 – $P < 0.01$; c – $P < 0.001$`

Table 3
Total fatty acid composition of egg yolk (n = 5) in Japanese quails (mol %)

Fatty acid		Control group	I exp. group	II exp. group	III exp.group
C14:0	Miristic	0.53±0.06	0.54±0.04	0.51±0.07	0.53±0.05
C16:0	Palmitic	31.21±0.91	30.33±0.72	28.20±0.80	29.06±0.91
C16:1	Palmitoleic	4.27±0.32	5.59±0.81	4.50±0.46	6.47±1.09
C18:0	Stearic	11.20±0.14	10.43±0.71	9.99±0.34	8.98±0.60
C18:1	Oleic	38.59±1.92	39.60±1.11	37.29±0.66	39.42±0.26
C18:2 n-6	Linoleic	11.06±0.89 a	11.08±1.56 a_1	16.14±1.59 a, a_1, a_2	12.57±1.40 a_2
C20:3	Eicosatrienoic	0.74±0.20	0.48±0.06	0.81±0.16	0.46±0.08
C20:4 n-6	Arachidonic	1.79±0.18	1.37±0.26	1.97±0.12	1.84±0.14
C20:5 n-3	Docosapentaenoic	0.31±0.03	0.32±0.09	0.28±0.02	0.39±0.13
C22:6 n-3	Docosahexaenoic	0.30±0.07	0.26±0.04	0.31±0.02	0.28±0.03
Total:		100	100	100	100
SFA		42.94±1.09	41.30±0.80 a_3	38.70±0.80 a_3	38.57±0.88
MUFA		42.86±2.20	45.19±1.88	41.79±1.06 a_4	45.89±1.15 a_4
PUFA		14.20±2.86	13.51±1.65 a_5	19.51±1.63 a_5	15.54±1.44

a, a_1, a_2, a_3, a_4, a_5 _ $P < 0.05$

rova et al. (2008a) reported similar results in eggs of broilers' parents receiving dietary *Vemoherb T* in a daily dose of 10 mg/kg body weight, without any differences in linolenic acid level between the groups. However Grigorova et al. (2009) observed an increase of the content of linolenic acid in the yolk ($P < 0.05$) in experimental guinea fowls, given TT extract in a daily dose of 10 mg/kg body weight as compared to control group.

Conclusions

Based on the present study can be concluded that: The content of total yolk lipids in III[rd] experimental group which was given the tested product in daily dose 10 mg/kg body weight for 10 weeks was significantly higher compared to the other treated groups ($P < 0.05$). However, there were no significant differences concerning this parameter between control and experimental groups ($P > 0.05$). The values of total yolk phospholipids were unaffected by addition of different doses TT extract. There was a statistically proven decrease of total cholesterol content in the yolk of all experimental groups compared with control group ($P < 001$ for I[st] and II[nd] experimental groups and $P < 0.001$ for III[rd] experimental group). Significantly higher value of linoleic acid ($P < 0.05$) was found in the egg yolk of III[rd] experimental group relative to the other groups.

Acknowledgements

The authors are grateful to Mr. Hristo Zlatev (Vemo 99 Ltd, Company, Sofia, Bulgaria) for supplying the product *Vemoherb T* that was used in the present work.

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Received February, 2, 2014; accepted for printing October, 2, 2014.