POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES
Pol. J. Food Nutr. Sci. 2005, Vol. 14/55, SI 1, pp. 69-73

COMPARATIVE ANALYSIS OF FATTY ACID PROFILE AND CHOLESTEROL CONTENT OF EGG YOLKS OF DIFFERENT BIRD SPECIES

Małgorzata Kaźmierska[1], Bogdan Jarosz[2], Małgorzata Korzeniowska[1], Tadeusz Trziszka[1], Zbigniew Dobrzański[3]*

[1]Department of Animal Product Technology,
[2]Department of Chemistry, Faculty of Food Science;
[3]Department of Animal Hygiene and Environment, Faculty of Biology and Animal Science;
Agricultural University of Wrocław, Wrocław

Key words: fatty acids, cholesterol, eggs, hen, quail, ostrich, pheasant, duck

The aim of the study was to analyse the content of fatty acids, cholesterol and products of cholesterol oxygenation in eggs collected from different bird species, *i.e.* hens, quails, pheasants, ostriches and ducks. Lipids were extracted from homogeneous yolk samples with a mixture of methylene chloride: :methanol and processed to esters by the methylation reaction. The analysis of fatty acid profile was performed using gas chromatography GC/MS (Agilent Technologies). Separation of fatty acids was carried out in a column DB-225 MS. Cholesterol content was analysed using standard method with a gas chromatograph, 5890 Series II Hewlett Packard. Moreover, analyses of the cholesterol oxidation products were carried out using HPLC (Agilent Technologies). The results of the study showed that the average content of saturated fatty acids in all analysed eggs was at a level of 28%. Monounsaturated fatty acids content of egg yolks ranged between 46% and 47%. Eggs collected from hens and quails were characterised by significantly lower content of monounsaturated fatty acids. A higher concentration of polyunsaturated fatty acids from group n-6 was observed in hen and pheasant eggs. Quail egg yolks had the highest level of n-3 polyunsaturated fatty acids. The best ratio of n-6/n-3 fatty acids was counted for quail eggs (5.6). In all other analysed bird eggs, the ratio was significantly higher. The highest cholesterol content was observed in ostrich and duck eggs, whereas the lowest in quail eggs. The oxidation products of cholesterol were not detected in all analysed eggs, which indicates perfect protective functions of the egg components.

INTRODUCTION

Nature does not know any food material more perfect than an egg. When a fertilized egg is provided with warmth, it transforms into a living organism. This fact indicates that all substances necessary for its creation are contained in the egg and proves its perfect biological composition [Trziszka, 2000]. Hen eggs, most frequently used for human consumption, have already been thoroughly studied and there is still not enough information on the profile of fatty acids in eggs from other domestic birds. Triacylglycerols (63.1%) and phospholipids (26.9%) are the dominant lipids of hen eggs. The content of fatty acids is *ca*. 26.6 g/ 100 g yolk. Monoenic (MUFA) acids – 46.9% and polyenic (PUFA) acids – 22.4% are the dominant ones, whereas saturated acids (SFA) constitute the remaining 30.7% [Dobrzański, 2000; Pisulewski, 2000]. Fatty acids are elements of cell membranes and cell organellas where they influence the permeability of nutrients to the cells of human organism [Ziemlański, 1997]. More and more attention has recently been paid to the role of polyenic fatty acids (PUFA), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in human and animal nutrition [Bartnikowska & Kulasek, 1994]. They regulate the biochemical and physiological processes in organisms. The role of PUFAs becomes more important as they are not synthesized in human organism and have to be delivered with food [Bartnikowska & Kulasek, 1994; Ziemlański & Budzyńska-Topolowska, 1991; Pisulewski, 2000]. From the nutritional point of view, the ratio of n-6 to n-3 acids, which should range from 1:1 to 4:1, is especially important [Yannakopoulos & Tserveni-Gousi, 1986].

The cholesterol contained in yolk is controversial as it is related to the risk of cardiovascular diseases. However, the results of numerous studies clearly indicate that cholesterol does not cause arteriosclerosis [Trziszka, 2000]. It is not cholesterol itself, but its oxidated forms which cause a number of diseases.

A specific cytochrome P450 participates in the oxidation of cholesterol and brings about the creation of products referred to as oxysterols [Erickson *et al*., 1977; Tai *et al*., 1999]. From dozens of oxidation products, 7-oxy- and 25--hydroxycholesterol and the products of epoxidation of C=C binding, are the most important [Lyons & Brown, 1999]. It has been known for over 100 years that in the presence of light and air spontaneous, non-enzymatic oxidation takes place, which is relevant for the quality of food rich in cholesterol [Lyons & Brown, 1999]. It should also be stressed that cholesterol is the most important steroidic component of

*Author's address for correspondence: Małgorzata Kaźmierska, Department of Animal Products Technology, Faculty of Food Science, Agricultural University of Wrocław, Norwida 25, 50-375 Wrocław, Poland, tel.: (48 71) 320 54 33; email: malkaz@op.pl

TABLE 1. Content of fatty acids in fresh egg yolk.

Fatty acids	Ostrich	Quail	Pheasant	Duck	Hen
Hexadecanoic (16:0)	23.89^{c}	23.16^{bc}	21.74^{a}	23.63^{c}	22.97^{ab}
Palmitoleic (16:1)	9.61^{d}	5.19^{c}	4.39^{b}	2.37^{a}	2.59^{a}
Stearic (18:0)	4.17^{a}	6.86^{b}	4.68^{a}	6.24^{b}	8.54^{c}
Oleic (18:1)	36.6^{a}	34.19^{a}	43.13^{c}	43.46^{c}	41.36^{b}
Linoleic (18:2) n-6	7.24^{b}	7.82^{b}	9.84^{c}	5.17^{a}	10.72^{c}
α-linoleic (18:3) n-3	0.90^{c}	0.34^{a}	0.69^{b}	0.25^{a}	0.30^{a}
Arachidonic (20:4) n-6	1.90^{d}	1.15^{b}	0.78^{a}	2.54^{e}	1.54^{c}
Docosahexaenoic (22:6) n-3	0.39^{a}	1.27^{c}	0.40^{a}	0.35^{a}	0.56^{b}

animal cell membranes, necessary for proper functioning of cells, tissues and all organism [Accad & Farese, 1998].

The analysis of cholesterol and the products of its oxidation poses some problems. Although most researchers agree that the use of a mixture of chloroform and methanol at a ratio 2:1 gives the best results in most cases, there is no agreed methodology to be used at further stages. It results from a big variety of lipid compounds with a full spectrum of polarity present in lipid fractions making it necessary to use chromatography techniques. Chromatographic purification of extracts and such tests as Liebermann-Burchard tests, which are frequently used, are complicated and not suitable for serial analyses. Moreover, as cholesterol and the products of its oxidation, both in their free and bound forms, do not constitute a homogenous chromatographic fraction, an introductory hydrolysis seems necessary to improve their analysis.

The objective of the study was to evaluate the quality and quantity of fatty acids and cholesterol as well as cholesterol oxidation products in egg yolks collected from different free ranged birds species *i.e.* hen, quail, pheasant, ostrich, duck.

MATERIALS AND METHODS

Materials used in the study were eggs collected from free ranged hens, quails, pheasants, ostriches and ducks eggs. Eggs used in the experiment were fresh, *i.e.* they were not stored under chill condition and they were not older than 21 days. After being delivered to the laboratory, the eggs were manually broken and separated into egg white and yolk. Lipids were then extracted from egg yolks with standard procedure [Folch *et al.,* 1956] using methylene chloride and methanol (2:1). After methylation (14% BF_3 in ethanol), the analysis of fatty acid profile was performed in a gas chromatograph with a spectroscopy mass detector GC/MS (Agillent Technologies). The separation of fatty acids was carried out in a column DB-225 MS (60; 0.25; 0.25). During analysis, special attention was paid to contents of saturated, monounsaturated and polyunsaturated fatty acids and to the ratio of n-6 and n-3 fatty acids in eggs collected from different bird species. Cholesterol content was analysed using standard chromatographic method with a gas chromatograph, 5890 Series II Hewlett Packard. Moreover, analyses of the cholesterol oxidation products were carried out using 7-ketocholesterol, β-epoxycholesterol, 3,5,6-triol, 25-hydroxycholesterol and α-epoxycholesterol standards on a high performance liquid chromatograph (HPLC) in Vaters 2690 Separations Module with detector Vaters 996 Photodiode Array.

RESULTS AND DISCUSSION

An important nutrient of egg yolk are fatty acids occurring in it mostly in the form of glycerides and phospholipids and less frequently in free form, lipoproteides and chemically bound in molecules of other compounds. In the yolk of eggs mono- and polyunsaturated fatty acids, very desired physiologically, are particularly abundant. Palmitic and stearic saturated acids were observed in yolks from all bird species examined in the study (Table 1). The content of palmitic acid did not differ within the experimental groups and was *ca*. 23%. The eggs from hens were characterised by a significantly higher content of stearic acid (8.54%) than the eggs from other species. The total content of monoenic acids was from 40% to 47%. The yolks from ostrich eggs had the highest content of palmitoleinic acid (9.61%), whereas in the yolks from pheasants and ducks oleinic acid was found to predominate (43%) (Table 1). The highest content of polyenic n-6 fatty acids (linolic and arachidonic) was observed in the eggs from hens and pheasants. The yolks from quail eggs were characterised by the highest content of n-3 acids (1.61%).

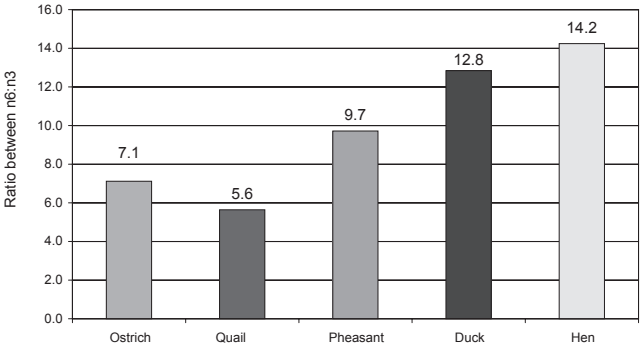

FIGURE 1. Ratio between n6:n3 polyunsaturated fatty acids in egg yolk depending to birds species.

TABLE 2. Cholesterol content in egg yolk from different bird species.

Bird species	Cholesterol content (mg/g yolk)
Hen	13.91[d]
Quail	7.78[b]
Ostrich	16.29[e]
Duck	10.81[c]
Pheasant	6.82[a]

From the nutritional point of view, it is important to determine the n-6/n-3 ratio, which should account for 4-6:1. In our study, the ratio of polyunsaturated fatty acids, PUFA n-6/ /n-3, was most favourable in the quail eggs (5.6:1) (Figure 1). In the eggs from other bird species, the ratio was significantly higher. Koreleski *et al.* [1998] proved the possibility of modifying the ratio n-6/n-3 fatty acids families. The best n-6/n-3 ratio was observed in quail eggs, which indicates their value for human consumption. Favourable, high content of DHA (1.27%) is one of the factors attracting the attention of specialists in human nutrition. It is worth noting that the fat extruded from quail eggs contains the lowest amount of oleinic acid of all the analysed species (34.19%). Panda and Singh [1990] observed a similar level of oleinic acid in eggs from different species of quails.

It s supposed that n-3 and n-6 polyenic fatty acids are metabolized by identical enzymatic systems. On the other hand, none of the representatives of these two families may be transformed into an acid from another family. At high concentrations of linoleic acid, conversion of α-linolenic acid to eicosapentaenoic and docosahexaenoic acids is inhibited. Most probably, the high proportion of linolic acid to linolenic acid in organism, negatively influences the deficiency of long-chain fatty acids [Bartnikowska & Obiedzinski, 1997].

The fat from ostrich eggs was characterised by the highest content of α-linolenic and a low level of linolic acid. The level of palmitoleic acid was also high.

Duck eggs were characterised by the highest fat content. The analysis of the content of acids indicates the highest concentration of arachidonic and oleinic acids and low levels of linolic acid.

Cholesterol content was analysed in our study using a standard GC/MS method. The highest amount of cholesterol was observed in ostrich egg yolk (16.29 mg/g yolk), whilst the lowest in pheasant's eggs. Moreover, relatively small amount of cholesterol was measured in quail and pheasant egg yolks (Table 2). All the results obtained for cholesterol content in bird egg's yolks were lower than those found in literature. Hence, it can be concluded that the methods used in the experiments are not good enough for comparing cholesterol content in egg yolks and these methods should be still modified in order to obtain more comparable results.

Quail eggs are considered to a have lower cholesterol content compared to hen eggs. Stepinska *et al.* [1993] showed a lower cholesterol content in the eggs of green-legged hens when compared to those of Rhode Island Red and Leghorn hens. Niemiec and Świerczewska [1995] and Melluzi *et al.* [1993] reported on the effect of hen genotype on the content of yolk lipid compounds. In the yolk of eggs from quails of different origin, both total cholesterol and free cholesterol as well as cholesterol esters were at a similar level, ranging from 16.25 to 16.70 mg/g yolk. Baumgartner and Simeonova [1992] found a higher cholesterol content in eggs originated from quails of heavier lines (20.46 mg/g yolk), compared to that of light birds, *i.e.* from 16.20 to 18.31 mg/g yolk. The cited data are far higher compared to the results of own observations, both in respect of heavier quails (Pharaoh and White variety) and the lighter ones (Golden variety). Witkowski *et al.* [1987] demonstrated clear differences in cholesterol content in 1 g yolk between quail eggs from long and short laying cycles. These authors suggested also differences in cholesterol concentration in quail eggs depending on the size and colour of shell spots. The effect of quail origin on the content of triglycerides in 1 g yolk was found in the reported study. Significantly more triglycerides were found in eggs of Golden variety quails.

The mean cholesterol content of yolk reached 16.41 mg/g appearing similar to the value found in guinea fowl (16 mg/g) and intermediate between those reported for chicken (15-19 mg/g) and ostrich (13 mg/g) [Reiner *et al*., 1995; Horbańczuk *et al*., 1999].

In addition, using HPLC for quantification, Jiang *et al.* [1991] obtained 11.7 mg cholesterol/g yolk of hen's egg, whereas Beyer and Jensen [1989a] 11.0 mg/g yolk and 11.7 mg/g yolk, the average weight of an egg was about 60 g in all cases. Bragagnolo and Rodriguez-Amaya [2003] reported that cholesterol content of hens egg and quail egg was alike and reached 12 mg/g yolk. The results obtained in our study showed that quail eggs contain considerably less cholesterol than eggs of other bird species.

REFERENCES

1. Accad M., Farese Jr. R.V., Cholesterol homeostasis: A role for oxysterols. Current Biol., 1998, 8, 601-604.
2. Bartnikowska E., Kulasek G., The importance of unsaturated fatty acids in human and animals feeding. Mag. Wet., 1994, 13, 34-38 (in Polish).
3. Bartnikowska E., Obiedzinski M., Unsaturated fatty acids omega-3. Structure, sources, determination, metabolism. Roczn. PZH, 1997, 48, 381-397 (in Polish).
4. Baumgartner J., Simeonova J., Breed or line differences of cholesterol content in quail eggs. 1992, *in*: Proceed. 19th World Poultry Congress. 20-24 September 1992, Amsterdam, The Netherlands, pp. 65-267.
5. Beyer R.S., Jensen L.S., Overestimation of the cholesterol content of eggs. J. Agric. Food Chem., 1989a, 37, 917-920.
6. Beyer R.S., Jensen L.S. Cholesterol content of commercially produced eggs in Georgia. Poultry Sci., 1989b, 68, 1703-1706.
7. Bragagnolo N., Rodriguez-Amaya D.B., Comparison of the cholesterol content of Brazilian chicken and quail eggs. J. Food Comp. Anal., 2003, 16, 147-153.
8. Dobrzański Z., Podstawowe zasady produkcji jaj. 2000, Jajczarstwo, nauka, technologia, praktyka. Praca

zbiorowa. WAR Wrocław, str. 65-133 (in Polish).
9. Erickson S.K., Cooper A.D., Matsui S.M., Gould G.R., 7-ketocholesterol. Its effect on hepatic cholesterogenesis and its hepatic metabolism *in vivo* and *in vitro*. J. Biol. Chem., 1977, 252, 5186-5193.
10. Folch J., Lees, M.Stanley, S., A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 1957, 226, 497-509.
11. Horbanczuk J.O., Sales J., Zieba G., Reklewski T., Celeda T., Kozaczynski K., Lipid cholesterol content and fatty acid composition of ostrich eggs as influenced by subspecies. Archiv für Geflügelkunde, 1999, 63, 234-236.
12. Jiang Z., Fenton M., Sim J.S., Comparison of four different methods for egg cholesterol determination. Poultry Sci., 1991, 70, 1015-1019.
13. Koreleski J., Kuchta M., Ryś R., Sieradzka A., Comparison of the influence of rapeseed oil and fish fat in laying hen nutrition on the level of polyunsaturated fatty acids in egg yolk. Rocz. Nauk. Zootech. 1998, 25, 91-102 (in Polish).
14. Lyons M.A., Brown A.J., 7-ketocholesterol. Int. J. Biochem. Cell Biol., 1999, 31, 369-375.
15. Meluzzi A., Giordani G., Urrai G. F., Sirri F., Cristofori C., Cholesterol level and fatty acid composition of commercial eggs produced in Italy. 1993, *in*: Proceed. 5th Europ. Symp. Quality of Eggs and Egg Products, 4-8 October 1993, Tours, France, pp. 427-432.
16. Niemiec J., Świerczewska E., The effects of the genotype x feed interaction on the quality of eggs. 1995 *in*: Proceed. 6th Europ. Symp. Qual. Eggs and Egg Products, 25-29 September 1995, Zarogoza, Spain, pp. 221-226.
17. Panda B., Singh R.P., Developments in processing quail meat and eggs. World's Poult. Sci. J., 1990, 46, 219-234.
18. Pisulewski P., Wartość odżywcza jaj kurzych oraz współczesne metody jej kształtowania. 2000, *in*: Jajczarstwo, nauka, technologia, praktyka. Wydawnictwo AR we Wrocławiu, str. 189-217 (in Polish).
19. Reiner G., Dorau H.P., Dzapo V., Cholesterol content, nutrients and fatty acid profiles of ostrich (*Struthio camelus*) eggs. Archiv für Geflügelkunde, 1995, 59, 65-68.
20. Stepinska M., Niemiec J., Cywa-Benko K., Egg yolk cholesterol concentration in different hen strains. 1993, *in*: Proceed. 5th Europ. Symp. Qual. Eggs and Egg Products, 4-8 October 1993, Tours, France, pp. 459-464.
21. Tai C.Y., Chen Y.C., Chen B.H., Analysis, formation and inhibition of cholesterol oxidation products in foods: an overview. J. Food Drug Anal., 1999, 7, 243-257.
22. Trziszka T., Przetwórstwo jaj, 2000, *in*: Jajczarstwo, nauka, technologia, praktyka. Wydawnictwo AR we Wrocławiu, str. 291-401 (in Polish).
23. Witkowski A., Zięba J., Niesłony M., Mielanczuk M., Egg yolk cholesterol concentration in quails laying long and short egg clutches. 1987, *in*: Proceed. Inter. Symp. Actual problems of avian genetics. 13-16 April 1987, Smolenice, Slovakia, pp. 212-221.
24. Yannakopoulos A.L., Tserveni-Gousi A.S., Quality characteristics of quail eggs. Br. Poult. Sci., 1986, 27, 171--176.
25. Ziemlański S., Fats in human nutrition. Żyw. Człow. Met. 1997, 24, 2, 35-47 (in Polish).
26. Ziemlański S., Budzyńska-Topolowska J., Food lipids and structural fats. 1991, Wyd. Nauk. PWN, Warszawa (in Polish).

ANALIZA PORÓWNAWCZA ZAWARTOŚCI KWASÓW TŁUSZCZOWYCH ORAZ CHOLESTEROLU W ŻÓŁTKU JAJ RÓŻNYCH GATUNKÓW PTAKÓW

Małgorzata Kaźmierska[1], Bogdan Jarosz[2], Małgorzata Korzeniowska[1], Tadeusz Trziszka[1], Zbigniew Dobrzański[3]

[1]Katedra Technologii Surowców Zwierzęcych,
[2]Katedra Chemii,
[3]Instytut Hodowli Zwierząt; Akademia Rolnicza we Wrocławiu, Wrocław

W pracy podjęto badania nad identyfikacją jakościową i ilościową kwasów tłuszczowych, cholesterolu oraz produktów jego utleniania obecnych w żółtku jaj następujących gatunków ptaków: kura, przepiórka, bażant, struś, kaczka. Do badań użyto jaj świeżych, tj. jaj których okres od momentu zniesienia nie przekraczał 21 dni, bez stosowania procesów chłodniczych. Po dostarczeniu do laboratorium jaja wybijano ręcznie, oddzielano część białkową od żółtka, z którego ekstrahowano tłuszcz metodą standardową z użyciem chlorku metylenu i metanolu. Po przeprowadzeniu reakcji metylacji analizę profilu kwasów tłuszczowych wykonywano przy użyciu chromatografu gazowego wyposażonego w moduł spektroskopu masy GC/MS (firmy Agillent). Do rozdziału frakcji kwasów tłuszczowych stosowano kolumnę DB-225 MS (60;0.25;0.25). W ocenie uwzględniono zawartość nasyconych kwasów tłuszczowych, monoenowych, polienowych oraz stosunek kwasów nienasyconych n-6 do n-3. Zawartość cholesterolu analizowano metodą standardową chromatografii gazowej w urządzeniu 5890 Series II Hewlett Packard. Ponadto, wykonano serie analiz związków powstających w wyniku utleniania cholesterolu metodą wysokosprawnej chromatografii cieczowej (HPLC). W rezultacie przeprowadzonych badań w żółtkach jaj wszystkich analizowanych gatunków ptaków stwierdzono obecność kwasów nasyconych palmitynowego i stearynowego (średnio 28%). Zawartość kwasów monoenowych (palmitooleinowego i oleinowego) kształtowała się na poziomie od 40% do 47%, przy czym w żółtkach jaj kurzych oraz przepiórczych była najniższa i wynosiła średnio 40%. Jak wynika z przeprowadzonych badań najwyższy udział kwasów tłuszczowych polienowych z grupy n-6 stwierdzono w jajach kur i bażantów (13,12% i 11,71%), natomiast żółtka jaj przepiórczych charakteryzowały się najwyższą, ze wszystkich analizowanych jaj, zawartością kwasów z rodziny n-3 (1,61%). Ze względów żywieniowych istotne jest określenie stosunku kwasów tłuszczowych n-6/n-3, który powinien przyjmować wartości 4-6:1. W badaniach własnych najkorzystniejszy stosunek kwasów n-6/n-3 obliczono dla jaj przepiórczych (5,6). Jaja pozostałych gatunków ptaków wykazywały znacząco wyższe wartości analizowanego wyróżnika. Uzyskany w eksperymencie relatywnie wysoki udział nienasyconych kwasów tłuszczowych i korzystny stosunek n-6/n-3 wskazuje na wyższą wartość odżywczą jaj przepiórczych. W wyniku przeprowadzonych badań stwierdzono również, że najwyższą zawartością cholesterolu charakteryzowały się jaja strusie oraz kacze (odpowiednio 16,29 i 10,81 mg cholesterolu w 1 g żółtka), podczas gdy jaja bażancie zawierały 6,82 mg cholesterolu w 1 g żółtka. W jajach wszystkich analizowanych gatunków ptaków nie stwierdzono obecności produktów utleniania cholesterolu, co może świadczyć o doskonałych ochronnych funkcjach składników jaja.