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DHA Analysis in Different Types of Egg Yolks: Its Possibility of Being a DHA Source for Boar Semen Cryopreservation

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Short Communication

DHA Analysis in Different Types of Egg Yolks: Its Possibility of Being a DHA Source for Boar Semen Cryopreservation

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Abstract

The aim of this study was to investigate the concentrations of Docosahexaenoic acid (DHA) in DHA enriched hen egg yolk (H-DHA) in comparison with egg yolk from chicken, duck, quail, ostrich and crocodile in order to find a source of DHA for boar semen cryopreservation. A pool (10 eggs in each) of DHA enriched hen egg yolk and egg yolks from chicken, duck, quail, ostrich and crocodile were analyzed for fatty acids profile at the Nutrition Laboratory, Mahidol University. Comparing all egg yolks, the highest DHA concentration in egg yolk was found in H-DHA egg yolk (3.7% of total fatty acid) and the lowest was found in ostrich egg yolk (0.4% of total fatty acid). Comparing DHA concentration in all egg yolks (not including H-DHA egg yolk), the highest DHA concentrations were found in duck egg yolk (1.8% of total fatty acid) and quail egg yolk (1.5% of total fatty acid), respectively. In conclusion, H-DHA, duck and quail egg yolks can be an abundant source of DHA for boar semen cryopreservation.

Keywords: boar semen, cryopreservation, docosahexaenoic acid (DHA), egg yolk, fatty acids

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บทคัดย่อ

การวิเคราะห์กรดไขมันชนิด DHA ในไข่แดงชนิดต่างๆ: ความเป็นไปได้ในการเป็นแหล่งของ DHA สำหรับการแช่แข็งน้ำเชื้อสุกร

กัมพล แก้วเกษ * *พนิดา ชนาภิวัตน์*

การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อตรวจประเมินความเข้มข้นของกรดไขมันไม่อิ่มตัวชนิดโดโคซาเฮกซาอีโนอิค (Docosahexaenoic acid, DHA) ในไข่แดงที่มาจากไข่ไก่ที่อุดมด้วยกรดไขมันชนิดโดโคซาเฮกซาอีโนอิกปริมาณสูง (H-DHA) เปรียบเทียบกับไข่แดงที่มาจากไข่ไก่ ไข่เป็ด ไข่นกกระทา ไข่นกกระจอกเทศ และไข่จระเข้ เพื่อหาแหล่งของกรดไขมันชนิดโดโคซาเฮกซาอีโนอิคที่เหมาะสมสำหรับกระบวนการแช่แข็งน้ำเชื้อพ่อสุกร โดยนำไข่ทั้งหมดจำนวน 10 ฟองจากสัตว์แต่ละชนิด แยกไข่แดงออกจากไข่ขาวเพื่อนำไปวิเคราะห์ผลของกรดไขมันชนิดต่างๆ ที่สถาบันวิจัยโภชนาการ มหาวิทยาลัยมหิดล ผลการทดลอง เมื่อเปรียบเทียบความเข้มข้นของกรดไขมันโดโคซาเฮกซาอีโนอิคในไข่แดงทั้งหมดนั้นพบว่า ความเข้มข้นของกรดไขมันโดโคซาเฮกซาอีโนอิคสูงที่สุดในไข่แดงจาก H-DHA (3.7% ของกรดไขมันทั้งหมด) ในขณะที่ไข่นกกระจอกเทศมีระดับความเข้มข้นของกรดไขมันโดโคซาเฮกซาอีโนอิคต่ำที่สุด (0.4% ของกรดไขมันทั้งหมด) และเมื่อเปรียบเทียบความเข้มข้นของกรดไขมันโดโคซาเฮกซาอีโนอิคในไข่แดงทั้งหมด (ไม่รวมไข่ไก่จาก H-DHA) พบว่าไข่แดงจากไข่เป็ดมีระดับความเข้มข้นของกรดไขมันโดโคซาเฮกซาอีโนอิคสูงที่สุด (1.8% ของกรดไขมันทั้งหมด) รองลงมาคือไข่แดงจากนกกระทา (1.5% ของกรดไขมันทั้งหมด) จากผลการทดลองครั้งนี้สรุปได้ว่า ไข่แดงจาก H-DHA ไข่เป็ด และไข่นกกระทาสามารถนำไปใช้เป็นแหล่งของกรดไขมันโดโคซาเฮกซาอีโนอิคสำหรับกระบวนการแช่แข็งน้ำเชื้อพ่อสุกรได้

คำสำคัญ: น้ำเชื้อพ่อสุกร การแช่แข็ง กรดไขมันชนิดดีเอชเอ ไข่แดง กรดไขมัน

ห้องปฏิบัติการน้ำเชื้อ ภาควิชาเวชศาสตร์คลินิกและการสาธารณสุข คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล ถ.พุทธมณฑลสาย 4 ต.ศาลายา อ.พุทธมณฑล จ.นครปฐม 73170
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Introduction

In pig, it has been shown that freezing extender itself plays an important role in the quality of post-thawed semen (Chanapiwat et al., 2009; Kaeoket et al., 2010[a,b]). The primary composition of freezing extender is an egg yolk which has a dominant effect on the protection of sperm from cold shock and maintains viability during freezing (Vishwanath and Shanon, 2000; Muiña et al., 2007). It is well documented that egg yolk consists of cholesterol, in which low-density-lipoprotein (LDL) is a main active ingredient for the protection of sperm from cold shock (Watson, 1981). In boar semen, White (1993) reported that phospholipids in egg yolk protected sperm to some extent from cold shock and also prevented calcium flux into the sperm. Waterhouse et al. (2006) reported that plasma membrane of boar semen is consisted of lipid bilayers which contained both saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA); consequently boar spermatozoa are obviously susceptible to lipid peroxidation during cryopreservation. Recently, Kaeoket et al. (2010[a]) demonstrated that adding Docosahexaenoic acid (DHA) from fish oil, a PUFA, directly into freezing extender improved frozen-thawed boar semen qualities in a concentration dependent manner. However, mixing of fish oil and egg yolk by vortex mixer is a time-consuming. Earlier studies have been reported that feeding laying hen with tuna-fish oil could produce DHA enriched egg yolk (Maldjian 2005) and feeding pig with this fish oil could also improve the quality of fresh boar semen (Paulenz 1999; Rooke et al., 2001). In many countries including Thailand, DHA enriched hen egg is commercially available. However, DHA concentration in this particular laying hen egg yolk has never been investigated. Besides chicken egg, eggs from other species such as duck, quail, ostrich, and crocodile are also available in the market. Therefore, the aim of the present study was to investigate the concentration of DHA in DHA enriched hen egg yolk in comparison with egg yolks from other sources which were chicken, duck, quail, ostrich and crocodile in order to find a source of DHA for boar semen cryopreservation.

Materials and Methods

The research proposal of this project was approved by the Faculty of Veterinary Science-Animal Care and Use Committee (FVS-ACUC)-Mahidol University, No. MUVS-2007-03.

Fatty acid analysis in egg yolk: Ten egg yolks of each species (i.e. hen supplemented with fish oil in feed (H-DHA), duck, quail, chicken, ostrich and crocodile) were separated from the albumin. Filter paper (Whatman No. 1) was used to remove all traces of albumin from the yolks and the yolks were broken and combined. The fatty acid profiles were determined as described by Bathgate et al. (2006) and also followed AOAC 2005. The percent content of the total yolk fat for each fatty acid was determined by calculating the area under the peak and presented as each fatty acid per 100 gram total yolk fat as well as percent (%) of total fatty acid.

Results and Discussion

The percent content of the total yolk fat for each fatty acid are presented as each fatty acid per 100 gram total yolk fat (Table 1), as percent (%) of total fatty acid (Table 2) and as percent of saturated fatty acid (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) (Table 3). The most abundant fatty acid in all egg yolks was oleic acid ranging from 34.9-49.8% of total fatty acids). Comparing the percentage of SFA among avian species, in agreement with Bathgate et al. (2006), we found that quail egg yolk had the highest percentage of SFA but the lowest percentage of UFA. For the percentage of MUFA and PUFA, the highest percentage was found in duck egg yolk and crocodile egg yolk, respectively. Bathgate et al. (2006) suggested that higher percentage of MUFA (C18:1; oleic acid) with its length of fatty acid chain, found in chicken egg yolk might have a specific effect on boar sperm. However, in the present result, the highest percentage of MUFA was found in duck egg yolk (duck versus chicken; 49.8: 45.2). The highest ratio of SFA: UFA and PUFA: SFA were found in crocodile egg yolk (0.65 and 0.58, respectively) (Table 3). Comparing all egg yolks, the crocodile egg yolk had the highest percentage of SFA (39.5% of total fatty acids) and PUFA (23.1% of total fatty acids). As expected, the highest DHA concentration in egg yolk was found in H-DHA egg yolk (3.7% of total fatty acids) and the lowest was found in ostrich egg yolk (0.4% of total fatty acids). This may be due to the fact that the diet of H-DHA chicken contains fish oil, a rich source of DHA. Comparing DHA concentrations in all egg yolks, not including H-DHA egg yolk, the highest DHA concentration was found in duck egg yolk. For the other egg yolks, the DHA concentration in quail egg yolk was higher than chicken, crocodile and ostrich egg yolks. It was shown in freezing stallion semen that duck yolk improved progressive motility and longevity of frozen-thawed semen (Clulow et al., 2004). These indicate that duck and quail egg yolks with its high concentration of DHA can be used in the freezing medium for boar semen cryopreservation instead of chicken egg yolk. This valuable information on the great potential natural source of DHA provided us an encouragement for the utilization of these egg yolks (i.e. H-DHA, duck, quail) in the development of boar semen freezing extender, and egg yolks of ostrich and crocodile were not recommended due to their comparatively low concentration of DHA. It has recently been demonstrated that adding commercial fish oil, a DHA source, directly into freezing extender II yielded a superior quality of frozen-thawed boar semen

Table 1 Composition of fatty acids in different egg yolks presented as gram per 100 grams of egg yolk.

Fatty acids	Carbon atom	Different types of egg yolks					
		H-DHA	Duck	Quail	Chicken	Croc.*	Ostrich
Caproic acid	C6:0 (S)	0	0	0	0	0	0
Caprylic acid	C8:0 (S)	0	0	0	0	0	0
Capric acid	C10:0 (S)	0	0	0	0	0	0
Lauric acid	C12:0 (S)	0.17	0	0	0	0	0.14
Myristic acid	C14:0 (S)	0.21	0.23	0.19	0.22	0.15	0.24
Myristoleic acid	C14:1 (M)	0.14	0	0.11	0.17	0	0
Palmitic acid	C16:0 (S)	4.61	6.25	5.29	5.27	3.77	5.65
Palmitoleic acid	C16:1 (M)	0.55	0.61	0.79	0.63	0.40	0.89
Stearic acid	C18:0 (S)	1.66	1.48	2.21	1.83	1.23	1.41
Oleic acid	C18:1 (M)	7.38	11.0	8.56	9.23	4.40	7.69
Linoleic acid	C18:2, n-6 (P)	2.32	1.77	2.60	3.19	2.33	3.22
Gamma linolenic acid	C18:3, n-6 (P)	0	0	0.07	0	0.20	0
Linolenic acid	C18:3, n-3 (P)	0.19	0.09	0.16	0.21	0.15	0.38
Arachidic acid	C20:0 (S)	0	0	0	0	0	0
Eicosenoic acid	C20:1 (M)	0	0	0	0.09	0	0
Eicosadienoic acid	C20:2 (P)	0	0	0	0	0	0
Eicosatrienoic acid	C20:3, n-6 (P)	0.28	0.91	0.47	0.53	0.5	0.57
Eicosatrienoic acid	C20:3, n-3 (P)	0	0	0	0	0	0
Arachidonic acid	C20:4, n-6 (P)	0	0	0	0	0	0
Eicosapentaenoic acid	C20:5, n-3 (P)	0	0	0	0	0	0
Behenic acid	C22:0 (S)	0	0	0	0	0	0
Erucic acid	C22:1 (M)	0	0	0	0	0	0
Docosahexaenoic acid (DHA)	**C22:6, n-3**(P)	**0.76**	**0.52**	**0.39**	**0.28**	**0.18**	**0.19**
Lognoceric acid	C24:0 (S)	0	0	0	0	0	0
Nervonic acid	C24:1 (M)	0	0	0	0	0	0

H-DHA: DHA enriched egg yolk, *Croc: Crocodile egg yolk, (S): Saturated fatty acid, SFA, (M): Monounsaturated fatty acid, MUFA, (P): Polyunsaturated fatty acid, PUFA

Table 2 Composition of fatty acids in different egg yolks presented as percent (%) of total fatty acid.

Fatty acids	Carbon atom	Different types of egg yolks					
		H-DHA	Duck	Quail	Chicken	Croc.*	Ostrich
Caproic acid	C6:0 (S)	0	0	0	0	0	0
Caprylic acid	C8:0 (S)	0	0	0	0	0	0
Capric acid	C10:0 (S)	0	0	0	0	0	0
Lauric acid	C12:0 (S)	0.2	0	0	0	0	0.1
Myristic acid	C14:0 (S)	0.5	0.5	0.4	0.3	0.4	0.6
Myristoleic acid	C14:1 (M)	0.1	0	0.1	0.1	0	0
Palmitic acid	C16:0 (S)	26.6	28.0	26.2	25.5	29.8	28.8
Palmitoleic acid	C16:1 (M)	2.5	2.3	3.5	2.4	2.4	4.0
Stearic acid	C18:0 (S)	9.1	6.3	10.5	8.4	9.2	6.7
Oleic acid	C18:1 (M)	43.1	49.8	42.8	45.2	34.9	39.4
Linoleic acid	C18:2, n-6 (P)	13.0	7.6	12.6	15.2	18.1	16.2
Gamma linolenic acid	C18:3, n-6 (P)	0	0	0.1	0	0.8	0
Linolenic acid	C18:3, n-3 (P)	0.4	0.1	0.3	0.3	0.4	1.4
Arachidic acid	C20:0 (S)	0	0	0	0	0	0
Eicosenoic acid	C20:1 (M)	0	0	0	0.2	0	0
Eicosadienoic acid	C20:2 (P)	0	0	0	0	0	0
Eicosatrienoic acid	C20:3, n-6 (P)	0.8	3.6	1.8	1.9	3.2	2.4
Eicosatrienoic acid	C20:3, n-3 (P)	0	0	0	0	0	0
Arachidonic acid	C20:4, n-6 (P)	0	0	0	0	0	0
Eicosapentaenoic acid	C20:5, n-3 (P)	0	0	0	0	0	0
Behenic acid	C22:0 (S)	0	0	0	0	0	0
Erucic acid	C22:1 (M)	0	0	0	0	0	0
Docosahexaenoic acid (DHA)	**C22:6, n-3**(P)	**3.7**	**1.8**	**1.5**	**0.6**	**0.6**	**0.4**
Lognoceric acid	C24:0 (S)	0	0	0	0	0.1	0
Nervonic acid	C24:1 (M)	0	0	0	0	0	0

H-DHA: DHA enriched egg yolk, *Croc: Crocodile egg yolk, (S): Saturated fatty acid, SFA, (M): Monounsaturated fatty acid, MUFA, (P): Polyunsaturated fatty acid, PUFA

Table 3 Composition of SFA, MUFA and PUFA from different egg yolks.

Fatty acids	% Total yolk fatty acid					
	H-DHA	Duck	Quail	Chicken	Croc.*	Ostrich
SFA						
C12:0	0.2	0	0	0	0	0.1
C14:0	0.5	0.5	0.4	0.3	0.4	0.6
C16:0	26.6	28.0	26.2	25.5	29.8	28.8
C18:0	9.1	6.3	10.5	8.4	9.2	6.7
C24:0	0	0	0	0	0.1	0
Total	**36.4**	**34.8**	**37.1**	**34.2**	**39.5**	**36.2**
MUFA						
C14:1	0.1	0	0.1	0.1	0	0
C16:1	2.5	2.3	3.5	2.4	2.4	4.0
C18:1	43.1	49.8	42.8	45.2	34.9	39.4
C20:1	0	0	0	0.2	0	0
Total	**45.7**	**52.1**	**46.4**	**47.9**	**37.3**	**43.4**
PUFA						
C18:2, n-6	13.0	7.6	12.6	15.2	18.1	16.2
C18:3, n-6	0	0	0.1	0	0.8	0
C18:3, n-3	0.4	0.1	0.3	0.3	0.4	1.4
C20:3, n-6	0.8	3.6	1.8	1.9	3.2	2.4
C22:6, n-3*	3.7	1.8	1.5	0.6	0.6	0.4
Total	**17.9**	**13.1**	**16.3**	**18.0**	**23.1**	**20.4**
Ratio						
SFA:UFA	**0.57**	**0.53**	**0.59**	**0.52**	**0.65**	**0.57**
PUFA:SFA	**0.49**	**0.38**	**0.44**	**0.53**	**0.58**	**0.56**

H-DHA: DHA enriched egg yolk, *Croc: Crocodile egg yolk, (S): Saturated fatty acid, SFA, (M): Monounsaturated fatty acid, MUFA, (P): Polyunsaturated fatty acid, PUFA

(Kaeoket et al., 2010[a,b]; Chanapiwat et al., 2009, 2012). In addition, this improvement has also been reported for turkey semen (Blesbois et al., 2004) and bull semen (Nasiri et al., 2012; Towhidi and Parks, 2012). It is accepted that DHA has its critical role in maintaining fluidity of sperm plasma membrane during cryopreservation which in turn prevents lipid peroxidation that causes sperm plasma membrane

damage as shown in many studies (Maldjian et al., 2005; Chanapiwat et al., 2009, 2012; Kaeoket et al., 2010[a,b]; Nasiri et al., 2012). However, further investigation on the feasibility of H-DHA egg yolk, duck egg yolk and quail egg yolk as a Lactose-Egg-Yolk (LEY) based freezing extender to improve the quality of frozen boar semen is needed. In addition, in practice, we recommend that the analysis of DHA profile in each batch of egg yolk has to be performed prior to the formulation of the freezing medium since the concentration of DHA in egg yolk may vary from batch to batch. However, it is worth noting that other components such as proteins, phospholipid and cholesterol are also present in egg yolks. Therefore, we cannot exclude the feasibility that they may influence the quality of frozen-thawed boar semen during the freezing process (Watson, 1981; Bathgate et al., 2006; Chanapiwat et al., 2012). With this regard, the analysis of these components should be done at the same time of fatty acid profile analysis.

In conclusion, based on the DHA concentration in egg yolk, H-DHA, duck and quail egg yolks can be a rich source of DHA as Lactose-Egg-Yolk (LEY) based freezing extender for boar semen cryopreservation.

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