National Library of Canada
Acquisitions and Bibliographic Services Branch
395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale du Canada
Direction des acquisitions et des services bibliographiques
395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Canada

MATERNAL DIETARY FATTY ACIDS: EFFECTS ON REPRODUCTION AND EMBRYO LIPID METABOLISM IN JAPANESE QUAIL

(Coturnix coturnix japonica)

by

Niceas Carlos Vilchez

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILLMENT OF

THE REQUIREMENT FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

Department of Animal Science (Nutrition)

McGill University, Montreal, Quebec

ISBN 0-315-80449-1

Canada

Suggested short title:

DIETARY FATTY ACIDS AND QUAIL REPRODUCTION

VILCHEZ

Suggested short title:

DIETARY FATTY ACIDS AND QUAIL REPRODUCTION

VILCHEZ

Dedicated to:

My mother Virgilia, my brothers Obcis and Melitón, my sister Viima, my wife Gladys and my son Paul David.

The people of my village Huáchac (Huancayo-Perú), which is located in the heart of the "Los Andes" Mountain (3,212 m.a.s.l.), with whom I shared my childhood and adolescence, and from whom I learned to understand and overcome some of the hardships of life.

Dedicado a:

Mi madre Virgilia, mis hermanos Obcís y Melitón, mi hermana Vilma, mi esposa Gladys y mi hijo Paul David.

La gente de mi pueblo Huáchac (Huancayo-Perú), situado en el corazón de la cordillera de "Los Andes" (3,212 m.s.n.m), con quienes compartí mi infancia y adolecencia, y de quienes aprendí a comprender y sobreponerse a algunas de las dificultades de la vida.

ABSTRACT

Doctor of Philosophy Animal Science (Nutrition)

Niceas Carlos Vilchez

MATERNAL DIETARY FATTY ACIDS: EFFECTS ON REPRODUCTION AND EMBRYO LIPID METABOLISM IN JAPANESE QUAIL (Coturnix coturnix japonica)

Japanese quail hens were used to study the effect of feeding palmitic, oleic or linoleic acids on the reproductive performance, tissue fatty acid composition and embryo lipid metabolism. Quail fed palmitic acid consumed more feed than those fed either oleic or linoleic acids. The highest level of reproductive performance was observed in quail fed palmitic acid followed by those fed oleic and linoleic acids. The highest level of embryo survival, observed in the palmitic acid fed group, was associated with more rapid mobilization and assimilation of yolk material by the embryo during incubation and it was not related to changes in eggshell quality. High levels of oleic and linoleic acids were found in egg yolk, plasma and liver lipids from quail fed oleic and linoleic acids, respectively. However, feeding palmitic acid resulted in elevated levels of palmitoleic acids in all three tissues. The fatty acid profiles of phospholipid, triglyceride and cholesterol esters of embryonic tissues were consistently influenced by the fatty acid composition of the yolk lipids and the stage of development. Feeding palmitic acid promoted more retention of labeled fatty acids in embryo lipids. Labeled oleic acid was preferentially esterified in the cholesterol ester fraction of yolk sac membrane lipids, and it appears that this fatty acid is utilized to a great extent by the quail embryo during its development.

Résumé

Docteur en Philosophie — Zootechnie (Nutrition)

Niceas Carlos Vilchez

LES ACIDES GRAS DANS L'ALIMENTATION DE LA MERE: EFFETS SUR LA REPRODUCTION ET LE METABOLISM DES LIPIDES DE L'EMBRYON CHEZ LA CAILLE JAPONAISE (Coturnix coturnix japonica)

L'ajout alimentaire d'acide palmitique, oléique ou linoléique a été expérimenté chez des cailles japonaises en ponte afin d'en déterminer les performances reproductives et d'examiner la composition tissulaire d'acides gras et le métabolisme lipidique de l'embryon. Les cailles nourries avec l'acide palmitique ont consommé plus d'aliment que celles ayant reçu de l'acide oléique ou linoléique. La meilleure performance reproductive a été obtenue chez les cailles nourries avec l'acide palmitique, suivi de celles avec l'acide oléique et enfin, celles avec l'acide linoléique. Le meilleur taux de survie des embryons, issus de mères nourries avec l'acide palmitique, était associé avec une mobilization et une assimilation plus rapide du jaune de l'oeuf par l'embryon durant l'incubation; cette survie n'était pas function de modifications de la qualité de la coquille. Des concentrations élevées d'acide oléique et linoléique ont été déposées dans le jaune de l'oeuf, le plasma et les lipides hépatiques chez les cailles supplémentées avec l'acide oléique ou linoléique, respectivement. Cependant, l'ajout d'acide palmitique alimentaire se réfléta par des concentrations élevées d'acide palmitoléique dans ces trois tissus. Les profils d'acides gras des phospholipides, des triglycérides et des esters de cholésterol des tissus embryonnaires furent régulièrement influencés par la nature des acides gras des lipides du jaune de l'oeuf et par le stade de développement. L'ajout d'acide palmitique favorisa une rétention accrue des acides gras radioactifs dans les lipides de l'embryon. L'acide oléique radioactif se fit incorporer particulièrement dans la fraction du cholésterol estérifié des lipides membranaires du sac vitellin; il semblerait que cet acide gras soit abondamment utilisé par l'embryon de la caille durant son développement.

RESUMEN

Doctor en Philosofía Ciencia Animal (Nutrición)

Niceas Carlos Vilchez

ACIDOS GRASOS EN LA DIETA MATERNA: EFECTOS EN REPRODUCCION Y METABOLISMO DE LIPIDOS EN EMBRIONES DE CODORNICES (Coturnix coturnix japonica)

Effectos de la inclusión de ácido palmítico, oleico o linoleico en la dieta materna sobre la perfomance reproductiva, la composición de ácidos grasos de los tejidos y el metabolismo de lípidos en los embriones fueron determinados en codornices en reproductión. Codornices alimentadas con ácido palmítico consumieron más que aquellas que recibieron ácido oleico o linoleico. Mejor perfomance reproductiva se observó en codornices alimentadas con ácido palmítico seguidos por el ácido oleico y linoleico. La mejor tasa de sobrevivencia embrionaria obtenido con el ácido palmítico estuvo asociado con una mayor movilización y asimilación de la yema por el embrión durante la incubación y no fué influenciado por la diferencia en la calidad de la cáscara del huevo. Altos niveles de ácido oleico y linoleico se observaron en los lípidos de la yema, del plasma y del hígado de codornices alimentadas con ácido oleico y linoleico, respectivamente. La inclusión de ácido palmítico en la dieta resultó en altos niveles de ácido palmitoleico en los tres tejidos. La composición de ácidos grasos de los fosfolípidos, triglicéridos y ésteres de colesterol de los tejidos embrionarios fueron influenciados por la composición de los ácidos grasos de la yema y por el estado de desarrollo del embrión. La inclusión de ácido palmítico en la dieta promovió una mayor retención de ácidos grasos radioactivos en los lípidos del embrión. El ácido oleico radioactivo fué esterificado preferentemente con el colesterol y este ácido graso fué utilizado en alto grado por el embrión durante su desarrollo.

This thesis is presented as a series of 5 papers corresponding to Sections III.1 to III.5. McGill University Guidelines Concerning Thesis Preparation state: "While the inclusion of manuscripts co-authored by the candidate and others is not prohibited by McGill, the candidate is warned to make an explicit statement on who contributed to such work and to what extent, and supervisors and others will have to bear witness to the accuracy of such claims before the oral committee. It should be noted that the task of the external examiner is made more difficult in such cases, and it is the candidate's interest to make authorship responsibilities perfectly clear."

Section III.1 and III.2 and Section III.3 were published and Section III.4 was accepted for publication and is in press. Sections III.1, III.2 and III.3 are authored by C. Vilchez, S. P. Touchburn, E. R. Chavez, and C. W. Chan. Section III.4 is authored by C. Vilchez, S. P. Touchburn, E. R. Chavez, and P. C. Lague. Drs. S. P Touchburn and E. R. Chavez were the student's co-supervisors. Dr, P. C. Lague provided valuable advice in measuring eggshell quality. Mr. C. W. Chan, Research Associate, provided valuable guidance in the management of the incubators and in gas-liquid chromatography techniques. Section III.5 will be submitted for publication with the student's supervisors as co-authors.

TABLE OF CONTENTS

	Page
Acknowledgments	i
Claim of originality and contribution to knowledge	ii
List of tables	iv
I. GENERAL INTRODUCTION	1
II. REVIEW OF LITERATURE	4
2.1. Dietary fat effect on egg production and egg weight	4
2.2. Dietary fat effect on fertility and hatchability	8
2.3. Dietary fat and egg yolk lipid composition	12
2.4. Dietary fatty acids and plasma lipoproteins	14
2.5. Lipid metabolism in the avian embryo	17
2.6. Dietary fat and changes in membrane composition and function	19
III. EXPERIMENTAL SECTION	22
1. DIETARY PALMITIC AND LINOLEIC ACIDS AND REPRODUCTION OF JAPANESE QUAIL (Coturnix coturnix japonica)	23
ABSTRACT	
INTRODUCTION	24
MATERIALS AND METHODS	26

RESULTS 29

DISCUSSION 32

ACKNOWLEDGEMENT 37

2. EFFECT OF FEEDING PALMITIC, OLEIC, AND LINOLEIC ACIDS TO JAPANESE QUAIL HENS (Coturnix coturnix japonica). 1. REPRODUCTIVE PERFORMANCE AND TISSUE FATTY ACIDS 46

ABSTRACT 46

INTRODUCTION 47

MATERIALS AND METHODS 49

RESULTS AND DISCUSSION 54

ACKNOWLEDGEMENTS 63

3. EFFECT OF FEEDING PALMITIC, OLEIC, AND LINOLEIC ACIDS TO JAPANESE QUAIL HENS (Coturnix coturnix japonica). 2. MATERNAL DIETS AND STAGE OF INCUBATION ON THE LIPID METABOLISM OF QUAIL EMBRYOS 72

ABSTRACT 72

INTRODUCTION 73

MATERIALS AND METHODS 75

RESULTS AND DISCUSSION 78

ACKNOWLEDGEMENTS 88

4. RESEARCH NOTE:EGGSHELL QUALITY IN JAPANESE QUAIL FED DIFFERENT FATTY ACIDS 96

ABSTRACT 96

INTRODUCTION 97

MATERIALS AND METHODS 98

RESULTS AND DISCUSSION 99

ACKNOWLEDGEMENT 101

5. METABOLISM OF [1-^{14}C]-PALMITATE, -OLEATE, AND -LINOLEATE IN INTACT QUAIL EMBRYOS AS AFFECTED BY MATERNAL DIET 103

ABSTRACT 103

INTRODUCTION 104

MATERIALS AND METHODS 106

RESULTS AND DISCUSSION 108

IV. GENERAL DISCUSSION 117

V. CONCLUSIONS 122

VI. LITERATURE CITED 125

ACKNOWLEDGMENTS

The author gratefully acknowledges the invaluable advice, assistance, patience, friendship and economical support of Drs. S. P. Touchburn and E. R. Chavez throughout the course of this program. The advice and friendship of Dr. S. Kubow of the School of Dietetics and Human Nutrition is also to be noticed.

My sincere appreciation to Mr. C. W. Chan for his encouragement, assistance and friendship.

Thanks to Dr. P. Lague for providing me the opportunity to use the facilities of the Poultry Unit, for the French version of the abstract and for his willingness to help.

Thanks to Dr. V. Gavino of the Université de Montréal and Dr. S. Sparace of the Department of Plant Science (McGill University) for their advice and assistance for the duration of Experiment 5.

Thanks to Carroll Themba Khombe for typing the tables.

Acknowledgment is also given to the staff and fellow graduate students of the Department of Animal Science for their friendship and ongoing encouragement.

Thanks to the Ministère de L'Enseignement supérier et de la Science du Québec, The consulate of Perú in Montreal and the Department of Animal Science for their contribution to grant me the tuition fee waiver

Finally, my deepest gratitude to my wife Gladys and my son Paul for their support, patience and encouragement during the course of my studies;

CLAIM OF ORIGINALITY AND CONTRIBUTION TO KNOWLEDGE

To the author's knowledge, the following aspects of this study constitute original contributions to knowledge:

1. Development of a semipurified diet and management practices which facilitated near-maximal egg production and fertility of Japanese quail.

2. The use of Japanese quail as an animal model for poultry to study the effects of fats or fatty acids on reproduction and embryo lipid metabolism.

3. Development of a method to separate monoglycerides from phospholipids by thin layer chromatography.

As far as the author is aware, the following findings have not been previously reported and expand the knowledge of the nutrition of Japanese quail:

1. Demonstration that reproductive performance was not impaired when diets containing .7 to .8% of linoleic acid were fed.

2. Report showing an exceptionally high rate of egg production and fertility over a long-term experiment.

3. Demonstration that feeding palmitic acid, a saturated, non-essential fatty acid, enhanced the rate of egg production, egg mass output, synthesis of lipoproteins, embryo survival and hatchability of fertile eggs.

4. Demonstration that mobilization of yolk lipids to the embryo during incubation was affected by the type of fatty acid fed to the hen.

5. Report of the effects of dietary fatty acids on fatty acid composition of egg yolk, plasma and liver lipids of the hen.

6. Report of the effects of maternal diet and stage of embryonic development on the fatty acid composition of the lipid classes of yolk sac membrane and extrahepatic embryonic tissues.

7. Report of the effects of the type of fatty acid included in the diet on eggshell quality.

8. Demonstration that there was a preferential mobilization from the yolk to the embryo, oxidation by the embryo and incorporation into lipid classes of embryonic tissues during incubation when labeled fatty acids were injected into the egg yolks.

LIST OF TABLES

Table		Page
1.1	Composition of the low-fat basal mix and analysis of experimental diets	38
1.2	Analyzed fatty acid composition of the experimental diets	39
1.3	Distribution of the treatment groups and number of animals in each reproduction phase	40
1.4	Reproductive performance of quail fed diets containing 3% palmitic (P) or 3% linoleic acid (L) from 7 to 10 wk of age (Phase 1)	14
1.5	Reproductive performance of quail fed diets containing 3% palmitic (P) or 3% linoleic acid (L) from 10 to 13 wk of age (Phase 2) with analysis	42
1.6	Reproductive performance of quail fed diets containing 3% palmitic (P) or 3% linoleic acid (L) from 13 to 16 wk of age (Phase 3) with analysis	43
1.7	Effect of dietary palmitic (P) or linoleic (L) acids on the fatty acid composition of plasma and egg yolk lipids of Japanese quail hens (pooled data of Phases 1, 2, and 3)	44
1.8	Effect of dietary palmitic (P) or linoleic (L) acids on total plasma phosphorus of Japanese quail hens	45
2.1	Composition of the low-fat basal mix and analysis of experimental diets	64
2.2	Analyzed fatty acid composition of the experimental diets	65
2.3	Effect of palmitic, oleic, and linoleic acids on the reproductive performance of Japanese quail hens from 8 to 32 wk of age (24-wk production period)	66
2.4	Effect of palmitic, oleic, and linoleic acids on the fatty acid composition of egg yolk lipids of Japanese quail	67
2.5	Effect of palmitic, oleic, and linoleic acids on the weight, dry matter, total lipid, and cholesterol content of egg yolks of Japanese quail	68

2.6	Effect of palmitic, oleic, and linoleic acids on the final body weight, liver weight and total plasma phosphorus of Japanese quail	69
2.7	Effect of palmitic, oleic, and linoleic acids on the major fatty acids of blood plasma lipids of Japanese quail	70
2.8	Effect of palmitic, oleic, and linoleic acids on the major fatty acids of liver lipids of Japanese quail	71
3.1	Effect of maternal diet (MD) and stage of development (DEV) on the weights of yolk plus yolk sac membrane (TY), yolk sac membrane (YSM), yolk, liver, and extrahepatic tissues (ET) of quail embryos	89
3.2	Major phospholipid fatty acids of yolk sac membrane of quail embryos as affected by maternal diet (MD) and stage of development (DEV)	90
3.3	Major phospholipid fatty acids of extrahepatic tissue of quail embryos as affected by maternal diet (MD) and stage of development (DEV)	91
3.4	Major triglyceride fatty acids of yolk sac membrane of quail embryos as affected by maternal diet (MD) and stage of development (DEV)	92
3.5	Major triglyceride fatty acids of extrahepatic tissue of quail embryos as affected by maternal diet (MD) and stage of development (DEV)	93
3.6	Major cholesterol ester fatty acids of yolk sac membrane of quail embryos as affected by maternal diet (MD) and stage of development (DEV)	94
3.7	Major cholesterol ester fatty acids of extrahepatic tissue of quail embryos as affected by maternal diet (MD) and stage of development (DEV)	95
4.1	Effect of palmitic, oleic, and linoleic acids on feed consumption, and on weight, specific gravity, and thickness of shell plus membrane of eggs of Japanese quail	102

5.1 Oxidation of [1-^{14}C]-palmitate, -oleate, and -linoleate by quail embryos from hens fed diets containing different fatty acids 114

5.2 Distribution of radioactivity from [1-^{14}C]-palmitate, -oleate, and -linoleate in the yolk plus yolk sac lipid classes of quail embryos from hens fed diets containing different fatty acids 115

5.3 Distribution of radiactivity from [1-^{14}C]-palmitate, -oleate, and -linoleate in embryo lipid classes of quail embryos from hens fed diets containing different fatty acids 116

I. GENERAL INTRODUCTION

Dietary fat plays at least two main roles for the living animal: 1) it is a concentrated source of metabolizable energy, and 2) it supplies fatty acids which are essential components of cell membranes as well as essential precursors for the synthesis of eicosanoids.

Many investigations in poultry have shown improvements in rate of growth, rate of egg production, efficiency of feed utilization and increased egg weight resulting from the addition of fat to the diets. In recent studies here with turkey hens (Vilchez et al., 1990; De Henau et al. 1985) and broiler breeders (Gilbert et al., 1991) it was observed that isocaloric replacement of a portion of the carbohydrate by fats or oils in the breeder diets reduced embryonic mortality during the latter stage incubation, thus improving hatchability of fertile eggs.

In spite of the amount of research done on the effectiveness of adding fat to the diets of poultry, the role of a particular fatty acid on a given reproductive parameter has not been identified. Among fatty acids, linoleic acid has been the most studied (Balnave, 1971a). In fact, the dietary requirement of chicken, turkey, quail, and other species of poultry for linoleic acid has been established by the National Research Council (NRC, 1984), but there is no information concerning the dietary

requirements of these species for fatty acids other than linoleic acid. Experiments carried out by Whitehead (1981), Scragg et al. (1987) and Vilchez et al. (1990a) indicate the possibility that supplemental dietary fatty acids such as palmitic or oleic acids, may be as good as linoleic acid in maintaining appropriate reproductive performance.

The composition of dietary lipids significantly influences the composition of egg yolk lipids, with the polyunsaturated fatty acids (PUFA) having the most dramatic effects (Noble, 1987a). Futher, in recent years many studies have demonstrated the effects of dietary fat on the composition of membrane phospholipids, which is accompanied by changes in different membrane-bound enzyme activities (Spector and Yorek, 1985). Therefore, as the avian embryo depends entirely on the yolk lipid during its development, it may be assumed that modification of the fatty acid composition of the yolk lipids, particularly of the phospholipids, by dietary means would also alter the activities of different enzymes in embryo tissue affecting its development and livability.

The general objectives of the present research are outlined as follows:

1. To determine the effects of the inclusion of fatty acids differing in the degree of saturation and essentiality on reproduction of Japanese quail hens.

2. To determine the appropiate fatty acid composition of the diets of quail hens which, in turn, will influence the fatty acid composition of the egg yolk lipids in order to maximize the reproductive performance.

3. To examine the effects of the maternal diet on the lipid metabolism of quail embryos during incubation.

II. REVIEW OF LITERATURE

2.1 Dietary fat effect on egg production and egg weight

The addition of fats and oils to the diets of chicken or turkey hens results in variable responses in feed efficiency, egg production and egg weight depending on the source of the supplemental fat or oil. In some cases, the addition of fat improved the energetic efficiency of egg production (Jackson et al., 1969; Rosebrough et al., 1980; Harms and Wilson, 1983). In other cases the energetic efficiency was not significantly changed (Horani and Sell, 1977) or was reduced (Sell et al., 1979). These responses are probably related to the plane of nutrition of the hen in a particular experiment. Thus, when fat is added to correct an energy deficiency, the energy consumed will be used productively, improving the energetic efficiency. Conversely, when the addition of fat increases the caloric content excessively in relation to the nutrient content of the diet, it will result in an over consumption of energy by the hen, resulting in a gradual increase in body fat content and a decline in energetic efficiency.

Numerous studies concerning the effectiveness of corn oil on egg production and egg weight have been conducted since Jensen et al. (1958) reported that both egg production and egg weight could be improved by the addition of corn oil to practical and semi-purified carbohydrate-based diets. Thus, the addition of corn oil to diets containing different sources of carbohydrates significantly increased egg weight

regardless the source of grain. Egg production, however, was not affected by dietary treatment (Shutze et al., 1962). Furthermore, the addition of 5% of either corn, tall or safflower oils to a semipurified low-fat laying hen diet improved egg weight (Shutze and Jensen, 1963).

Feeding laying hens diets containing corn oil (5%) or a combination with menhaden oil (50:50) resulted in an increase in egg weight and egg production rate when compared to diets low in linoleic acid (Marion and Edwards, 1964). In a similar experiment, Bray (1967) demonstrated that the inclusion of 8 parts of corn oil for 20 parts of starch increased egg weight. It was postulated that the increased egg weight may be due to an accelerated lipid deposition in the growing follicle because of the availability of fatty acids absorbed from the gut, and that corn oil had an effect which was not attributable to the difference in dietary energy (Edwards and Morris, 1967). In this regard, laying hens fed a diet supplemented with corn starch produced more eggs than the equivalent (in terms of calories) addition of corn oil; however, egg size was greater with the corn oil supplementation (Balnave, 1971a). Recently, March and Macmillan (1990) hypothesized that linoleic acid, or longer-chain fatty acids of the linoleic-acid family are required for the synthesis of the lipoproteins that can be transported to the ovary for uptake by the developing ova.

Balnave and Weatherup (1974) observed that the addition of corn oil to the diets of laying hens resulted in a small increase in egg weight when birds were fed

conventional rearing diets. Supplements of linoleic acid in excess of the requirement have little effect on egg weight in birds with adequate reserves of linoleic acid (Balnave, 1982). Furthermore, once the requirement for linoleic acid had been met, part of the extra linoleate may be used by the bird as a source of energy, which would explain some results where linoleic acid supplementation had a beneficial effect on both egg production and egg egg weight (Balnave, 1971b).

The linoleic acid in excess of the requirement for normal body functions is not specifically required for maximum egg size (Shannon and Whitehead, 1974), which would suggest that egg size can not be maximized if the amount of linoleic acid available is not adequate to support maximum rate of synthesis of lipoproteins (March and McMillan, 1990). However, unsaturated fats rich in other fatty acids, such as oleic acid, might have a beneficial effect on egg weight or egg production or both. Thus, egg production rate and average egg weight of laying hens fed a diet supplemented with 3% of olive oil were similar to those receiving the same amount of corn oil. Therefore, it appears that the parameter egg weight does respond to the presence of readily absorbable fatty acids such as linoleic and oleic acids (Whitehead, 1981; Scragg et al., 1987).

Egg production rate was not significantly affected when hens were fed isocaloric and isonitrogenous diets containing 0, 3, or 6% animal-vegetable fat blend (A-V fat) (Sell et al., 1987). In the same study, a similar response was obtained when hens were

fed non isocaloric diets containing either 3 or 6% A-V fat. The inclusion of A-V fat or crude soybean oil, at 1 and 8% levels, in the diets of laying hen diets also resulted in no significant difference in egg production, but egg weight tended to be heavier at the higher dietary fat level (Huyghebaert, 1988). Likewise, the inclusion of graded levels of palm oil (0, 3, 6 and 9%) in the laying hen diets did not affect egg production and egg weight. Contrary to these observations, Pourreza et al. (1988) reported that the inclusion of 3% of palm oil and 3.2% of olein in the diets of brown egg layers resulted in an increased egg output and lipid content of the yolk. Fat supplementation (the high fat diet containing 8.7% total fat) prior to the onset of sexual maturity of laying hens (Hoyle and Garlich, 1987) resulted in higher initial egg weights than for birds fed a diet with no added fat (high carbohydrate diet, 0.99% total fat); however, egg production rate was not affected by dietary treatment.

The addition of either A-V fat, corn oil or olive oil to the diets of turkey hens had no significant effect on egg production rate (Grizzle et al., 1982; Whitehead and Herron, 1988; Vilchez et al., 1990) or an improved response (Harms and Wilson, 1983) when compared with their respective control diets without added fat. Average egg weight from turkey hens fed 5% of corn oil was heavier than those fed 5% of olive oil (Vilchez et al., 1990), but Whitehead and Herron (1988) found no difference in egg weight from turkey hens fed diets containing 3% of either corn oil or olive oil added.

Calvert (1969) reported that both egg production and egg weight were significantly reduced by feeding a linoleic acid deficient diet to adult Japanese quail. Likewise, quail fed a semi-purified low-fat basal mix (0.6% of linoleic acid) plus 3% corn starch had significantly lower egg production and egg weights than quail fed the same basal diet but supplemented with 3% of either corn oil, palmitic, oleic or linoleic acids (Vilchez et al., 1990a).

In summary, the addition of corn oil to the laying hen diets, in most cases, resulted in an improvement of egg weight, but not necessarily of egg production. The positive effect of corn oil on egg weight has been attributed to its linoleic acid content. However, some studies indicated that egg weight can also respond to the presence of other fatty acids such as palmitic or oleic acids. This fact opens the possibility to re-evaluate the nutritional properties of the so-called non essential fatty acids on both egg production and egg weight.

2.2. Dietary fat effect on fertility and hatchability

The possibility of the presence of an unidentified factor in corn oil necessary for fertility and hatchability was indicated by Jensen et al. (1958). Subsequent investigations conducted by Marion and Edwards (1962) demonstrated that the addition of corn oil to a low-fat diet significantly increased hatchability of fertile eggs. Jensen and Shutze (1963) and Menge et al. (1965a) reported depressed hatchability

of eggs from hens suffering a esential fatty acid (EFA) deficiency. They concluded that the linoleic acid present in corn oil is the factor necessary for reproduction since fertility and hatchability increased as the linoleic acid increased in the diet of the breeder hens.

Increased levels of linoleic acid supplied by safflower oil had a significant stimulatory effect on the reproduction of the EFA-deficient hens (Menge et al., 1965b). In the same study it was found that the addition of menhaden oil promoted an even greater response than did safflower oil. Since the linoleic acid contents were the same in both dietary treatments, the response was assigned to the polyunsaturated fatty acids (PUFA) present in the menhaden oil. On the other hand, a high incidence of embryonic mortality and nearly zero hatchability was found in eggs from EFA-deficient hens. It was hypothesized that a deficiency of linoleic acid produces a malposition in the embryo and also a lack of strength to completely break out of the shell (Calvert, 1967).

Menge (1968) indicated that approximately 1% of linoleic acid is required for hatchability of fertile eggs. This author reported, however, that only 0.125% of linoleic acid is required for production of fertile eggs. Two different studies (Balnave, 1971a; Whitehead, 1981) reported that the specific requirement of laying hens for linoleic acid is not greater than 0.9% of the diet, while the National Research Council (NRC, 1984) lists the requirement of chicken, turkey and quail hens for linoleic acid

as 1% of the diet. This recommendation also includes the male breeders.

Feeding graded levels of tallow (2.5,5, 7.5,10 and 12.5%) to turkey breeder hens resulted in no significant differences in fertility or hatchability that could be attributed to the dietary fat (Creger et al., 1970) A similar response was obtained by Rosebrough et al. (1980) when they fed turkey hens with diets containing different levels of fat substituted isocalorically for corn meal. The addition of 6% A-V fat (Potter et al., 1978) or 4% A-V fat (Grizzle et al., 1982) to the diets of turkey hens had no significant effect on hatchability of fertile eggs when compared with the control diets containing no added fat. The addition of corn oil to the diet of turkey hens, already containing 1.2% of linoleic acid, did not further improve the rate of egg production or hatchability (Cooper and Barnet, 1968).

The addition of 8% of animal fat to the diets of turkey hens significantly improved percent of fertility when compared to the control diet (without added fat) or the 4% added fat diet (Harms and Wilson, 1983). Hatchability of fertile eggs was not affected by dietary treatment. A further study conducted by Harms et al. (1984) demonstrated that feeding 3% of animal fat to growing turkeys (12 to 32 weeks of age) improved hatchability of fertile eggs during the laying period when compared to those fed a diet without added fat during the same experimental period, but fertility was not affected by dietary treatments during the growing period.

Studies with turkey hens involving different protein levels, calorie:protein ratios and supplemental A-V fat demonstrated that the inclusion of 3.5% A-V fat in the diet significantly improved hatchability of fertile eggs (De Henau et al., 1985). Since feed intake was not affected by dietary treatment, it was suggested that fat per se has some physiological effect other than its caloric contribution to the diet. A subsequent study (Vilchez et al., 1990) showed that the isocaloric inclusion of either A-V fat, corn oil or olive oil to the diets of turkey hens decreased embryonic mortality, particularly during the latter stage of incubation, when compared to the control diet without added fat. Hatchability of fertile eggs was not significantly different among treatments, but it was observed that hatchability in birds fed olive oil tended to be higher than those fed either A-V fat or corn oil. A similar observation was obtained by Whitehead and Herron (1988) when turkey breeder hens were fed diets containing 3% of either corn oil or olive oil.

When Japanese quail were fed a semi-purified low-fat basal mix (0.6% of linoleic acid) plus 3% of corn oil, palmitic acid, oleic acid or linoleic acid the levels of hatchability of fertile eggs were not significantly different among treatments, but they were all significantly higher than that of quail fed the same basal mix plus 3% of corn starch. Percent fertility was not affected by any of the dietary treatments (Vilchez et al., 1990a)

In summary, the parameters fertility and hatchability are severely affected only

when birds are fed a linoleic acid deficient diet for a long period. These parameters are not affected when birds are fed conventional diets supplemented with different sources of fat. However, the addition of fats or oils to the diets of turkey hens result in a reduced embryonic mortality between 8 and 28 days of incubation. Further, adding different commercial fatty acids to a semi-purified low-fat basal mix resulted in a response similar to those obtained when corn oil was added, which would indicate the need for an evaluation of the importance of each fatty acid on parameters such as fertility and hatchability.

2.3. Dietary fat and egg yolk lipid composition

Manipulation of dietary lipid composition can, in most cases, be readily translated into effects on yolk lipid profile. Thus, the proportion of linoleic and linolenic acids in the egg yolk lipids increased with increasing amounts of these acids included in the diets (Marty and Reiser, 1961). Eggs from laying hens fed 15% hydrogenated coconut oil contained considerable quantities of lauric, myristic and myristoleic acids, and significantly less arachidonic acid than eggs from hens fed 15% safflower oil (Christie and Moore, 1972).

Incorporation of a range of vegetable oils rich in oleic acid, linoleic acid or PUFA of the n-3 series into the diets of laying hens (Yu and Sim, 1987; Guenter et al., 1971; Sell et al., 1968; Donaldson, 1967; Thomsen, 1966; Chen, 1964), turkey hens

(Vilchez et al., 1990; Couch et al., 1973), and quail hens (Vilchez et al., 1990a) resulted in a significant increase in their concentrations in the yolk lipid. However, changes in the levels of PUFA are commonly balanced by proportional changes in the level of mono-unsaturated fatty acids, such as palmitoleic and oleic acids. Thus, Sim et al. (1973) reported that palmitoleic and oleic acid available for egg yolk formation are reduced by the presence of a large quantity of linoleic acid, suggesting that a homeostatic mechanism in the liver inhibits the de novo synthesis of these mono-unsaturated fatty acids. Furthermore, it appears to stimulate the synthesis of saturated fatty acids in order to maintain a specific ratio of saturated-to-unsaturated fatty acids during a period of high PUFA ingestion. A similar observation was reported by Vilchez et al. (1990a) in egg yolk lipids of Japanese quail fed linoleic or linolenic acids.

In summary, of the major lipid constituents, fatty acid profile is most readily altered by the diet. In addition, if the diet contains a high concentration of PUFA, the proportions of mono-unsaturated fatty acids are reduced. This indicates that the bird has a mechanism to maintain an appropriate saturated-to-unsaturated fatty acid ratio compatible with the embryonic development. In fact, the impact of the fatty acid composition of egg yolk lipids on the hatchability of fertile eggs has been suggested (Tullett, 1990; Vilchez et al., 1990; Vilchez et al., 1990a; Donaldson and Fites, 1970). The mechanism(s) by which yolk fatty acid composition affects hatchability has not been determined.

2.4. Dietary fatty acids and plasma lipoproteins

There is a considerable body of information on qualitative and quantitative aspects of the serum lipoproteins in avian species, primarily in the chicken, but also in turkeys, geese and, to a limited extent, in Japanese quail (Chapman, 1980). The main source of nutrients for the developing embryo in egg-laying species is the egg yolk. Egg yolk proteins are synthesized under control of estrogen in the liver. Estrogens permit the hepatocyte to transcribe apoprotein II, and all very low density lipoproteins (VLDL) have this added characteristic (Miller and Lane, 1984). Estrogens also augment total hepatic VLDL formation by increasing the de novo fatty acid synthesis (Dashti et al., 1983).

Studies carried out with the fowl indicate that plasma VLDL and vitellogenin are the two main precursors of yolk lipid components (Perry and Gilbert, 1979; Patterson et al., 1962). It has been postulated that VLDL, vitellogenin and the vitamin-binding proteins are deposited into the yolk by a receptor mediated endocytosis (Griffin et al., 1984). Lipoproteins that are synthesized by the liver in response to estrogens are thought to be deposited mainly in the ova because other tissues of the body appear to be limited in their ability to use them (Gormall and Kuksis, 1973; Griffin et al., 1982). In fact, a recent evidence indicates that the presence of apolipoprotein VLDL-II on VLDL inhibits lipolysis of these triglyceride-rich lipoproteins in the laying hen (Schneider et al., 1990).

The effects of dietary fats and oils on the quality and quantitity of plasma lipoproteins and cholesterol have been intensively studied in mammalian species. Recent data indicate that much attention has been put on individual lipoproteins: VLDL, high density lipoproteins (HDL) and low density lipoproteins (LDL). Saturated fatty acids and cholesterol have been demonstrated to raise the LDL levels with palmitic acid being considered the major cholesterol-raising saturated fatty acid in the diet (Grundy and Denke, 1990). However, replacing lauric acid plus myristic acid from coconut oil with palmitic acid as palm oil induced a significant increase in HDL-cholesterol with a trend toward a decrease in LDL, suggesting that palmitic acid may enhance HDL production (Lindsey et al., 1990).

Feeding mono-unsaturated fatty acids, i.e.oleic acid, showed favourable effects when substituted for saturated fat acids in the diet. Mono-unsaturated fatty acids reduce LDL-cholesterol, but do not lower HDL-cholesterol (Grundy, 1989). There is no report indicating that oleic acid decreases the VLDL levels. Dietary linoleic acid has been reported to reduce concentrations of VLDL (Grundy, 1975). Like PUFA from fish oil, linoleic acid also decreases the formation of VLDL but to a lesser extent.

Unlike in mammalian species, limited reports exist concerning the effects of dietary fats and oils on the quality and quantity of plasma lipoproteins in avian species. Thus, a negligible amount of VLDL was found in the plasma of chickens fed

a ration containing 10% corn oil when compared with those fed the same diet but supplemented with 1% of cholesterol. The VLDL in plasma from the cholesterol fed group was the predominant lipoprotein and carried 72% of the total plasma lipids. The VLDL was very low in triglycerides (6%) and high in total cholesterol; the level of LDL was not changed, but the concentration of total lipids and phospholipids in the HDL fraction decreased significantly in the cholesterol-fed group (Kruski and Narayan, 1972).

An experiment conducted with laying hens (Evans et al., 1977) showed that the percentage of plasma VLDL of hens fed 2.5% cottonseed oil was higher (80 vs 22.3%) than that of the control group. The opposite figure was observed in the percentage of plasma LDL (8.5 vs 65.9%). The percentage of HDL was not affected by the diets. The higher percentage of LDL was associated with increased levels of stearic acid in their lipids.

In summary, it has been demonstrated in mammalian species that the plasma VLDL concentration is influenced by the type of fat or fatty acids fed to the experimental animals. Thus, saturated fatty acids are considered to increase lipoprotein synthesis whereas PUFA are considered to cause the opposite effect. Further, it is also known that plasma VLDL is considered to be the yolk precursor in egg-laying species. However, there is limited information concerning the effects of different types of fatty acids on the levels of lipoproteins in poultry breeders and the

relationship of these effects with reproduction.

2.5. Lipid metabolism in the avian embryo

The avian embryo contains only a small amount of carbohydrate; thus, the embryonic diet is a low-carbohydrate, high fat diet (Donaldson et al., 1971). Thus, the avian embryo derives over 90% of its caloric requirement from fatty acid oxidation. It also requires fatty acids for synthesis of phospholipids for membrane formation and synthesis of triglyceride for energy storage. The exact mechanism of lipid absorption from the yolk by the developing embryos still not well defined.

Noble and Moore (1964) reported that the amount of lipid transported in the first 13 days of incubation of chicken eggs is about 350 mg whereas in the last week of incubation, the uptake of lipid accelerates dramatically to reach about 1 g per day. At the 17th. day of incubation, the distribution of membrane triglycerides of the yolk sac membrane is virtually identical with that of yolk triglicerides (Noble and Moore, 1967a). The latter suggests not only the absorption of intact molecules but also that that is the major method of absorption. Furthermore, the uptake of phospholipids seems not to require hydrolysis (Noble and Moore, 1967b). The uptake of intact molecules is enhanced as incubation progresses (Siek and Newburg, 1965).

In accordance with its possible role in lipid uptake by the yolk sac membrane,

Noble et al. (1984) demonstrated the ability of the yolk sac membrane to synthesize cholesterol esters, in particular cholesteryl oleate. Previous work (Noble and Moore, 1967a) has shown that during the period between days 13 and 17 of incubation, the proportion of oleic acid within the cholesterol esters of the membrane dramatically increased, but that there were no further changes in fatty acid composition during the last 4 days of incubation.

The yolk sac membrane exhibits high levels of both stearyl-CoA and linoleyl-CoA desaturases. The high rate of desaturation due to the high levels of these two desaturases in the yolk sac membrane, increases the accumulation of mono-unsaturated fatty acids and PUFA in the liver of the embryos (Noble and Shand, 1985). The activity of both enzymes is reported to decrease with the approach of hatching.

The lipids absorbed by the yolk sac membrane are transported by the blood stream to the embryo where they can be utilized for growth or the production of energy. Moreover, the lipids can also be modified and new classes of lipids formed in order to maintain a given ratio of saturated to unsaturated fatty acids (Donaldson, 1981). The fatty acids of the embryo lipids are more saturated than those of the yolk from which the embryo developed. The latter is thought to be due to the low level or absence of the desaturase activity in the embryonic liver (Donaldson et al., 1971).

Comparative studies of the fatty acid composition of the phospholipid classes within the yolk, yolk sac membrane, liver, and extrahepatic tissues (Abad et al., 1976; Nakagawa, 1982) are consistent with an extensive degradation of yolk phospholipids following assimilation and resynthesis into products which are either utilized for embryonic development or as a source of energy (Budowski et al., 1961; Donaldson, 1981).

In summary, the yolk lipid constitutes a source of both energy and essential tissue components such as phospholipids for the developing embryo. The accelerated transfer of lipids from the yolk to the embryo during the latter stage of incubation is reflected in the lipid composition of embryonic tissues. Limited information exists on lipid metabolism in developing embryos of species other than chickens. The effects of maternal diet and stage of development on the utilization of the yolk material and their effect on lipid classes of embryonic tissues have not been adequately addressed.

2.6. Dietary fat and changes in membrane composition and function

It is well established that the fatty acid composition of the membrane phospholipids can be modified in vivo by both dietary means and by intensive mechanisms such as desaturation and elongation reactions (Spector and Yorek, 1985). Feeding rats diets rich in PUFA was shown to increase the content of these fatty acids in a variety of membranes such as endoplasmic reticulum (Hammer and Wills,

1979) and plasma membrane (Morson and Clandinin, 1986).

The role of dietary fat on membrane structure and cell function has been documented (Berdanier, 1988; Clandinin et al., 1985; Clandinin et al., 1991). The changes in the extent of membrane fatty acid unsaturation result in a decrease in the phase transition temperature of membrane lipids (Innis and Clandinin, 1981). Membrane functions which are influenced by membrane fluidity include the insulin receptor sensitivity (Ginsberg et al., 1982), sodium-potassium ATPase activity (Solomonson et al., 1976), glucagon-stimulated adenylate cyclase activity (G-SACA) (Morson and Clandinin, 1986), calcium-ATPase acitivity (Hidalgo et al., 1986) and transport of hexoses (Legendre et al., 1980; Thomson et al., 1987).

Modifications in the dietary ratio of saturated to PUFA (Thomson et al., 1987) have early (3 days) effects on intestinal transport function in rats. Thus, jejunal uptakes of glucose, lauric, stearic, oleic and linoleic acids are greater in rats fed fed high PUFA than high saturated fatty acids. Thomson et al. (1989) reported that changes in the dietary PUFA to saturated fatty acid ratio influence the activity of intestinal lipid metabolizing enzymes which alter the type of fatty acid incorporated into membrane phospholipids.

The G-SACA was significantly influenced by the type of fatty acid present in the phospholipids (Neelands and Clandinin, 1983). Thus, as the level of oleic acid and

total mono-unsaturated fatty acids in phosphatidylcholine and phosphatidylethanolamine increased, a corresponding increase in G-SACA was observed. On the contrary, total n-6 unsaturated fatty acids in phosphatidylcholine inversely correlated with G-SACA. A decreased G-SACA in plasma membrane was associated with high dietary levels of linoleic and linolenic acids (Clandinin et al., 1985).

In rodents, utilization of dietary fats high in very long chain mono-unsaturated fatty acids has been correlated with changes in energetic efficiency and rate of oxygen consumption (Hornstra, 1972). Changes in efficiency in mitochondrial energetics and whole body conservation of energy in chicks fed diets containing high or low erucic acid rapeseed oils was also demonstrated by Renner et al. (1979). These studies and others (Clandinin, 1978) indicate the existance of a dynamic mechanism which associates changes in dietary fat, membrane structure and metabolic conservation of dietary energy.

In summary, dietary fat significantly influences the fatty acid composition of the membrane lipids. Changes in fatty acid profile of total phophospholipids and their different classes are associated with changes in the membrane-bound enzyme activities. Therefore, it is possible to modulate the activity of a given enzyme by feeding different type of fats and oils according to a particular purpose.

III. EXPERIMENTAL SECTION

1. DIETARY PALMITIC AND LINOLEIC ACIDS AND REPRODUCTION OF JAPANESE QUAIL (Coturnix coturnix japonica)[1]

C. VILCHEZ, S.P. TOUCHBURN, E.R. CHAVEZ, and C.W. CHAN

Department of Animal Science, Macdonald College of McGill University, and McGill Nutrition and Food Science Centre

Ste. Anne de Bellevue, P.Q., Canada, H9X 1C0

ABSTRACT An experiment was conducted to evaluate the effects of two diets containing 3% of either palmitic acid (Diet P) or linoleic acid (Diet L) on the sensitivity of the reproductive performance, fatty acid composition of both plasma and yolk lipids and total plasma phosphorus of Japanese quail. A 9-wk production period was divided into three phases of 3 wk each: 1) 7 to 10 wk, 2) 10 to 13 wk, and 3) 13 to 16 wk of age. Eighty male plus female pairs of quail were used. The response to dietary treatments appeared not to be influenced by diet fed in the preceding phase. When data were pooled by diet fed in each phase, feed consumption in Phases 2 and 3 was higher ($P < .05$) in birds fed Diet P than those fed Diet L. In Phase 2, egg production was higher ($P < .05$) in the groups fed Diet P than those fed Diet L. In Phase 3, birds fed Diet P exhibited lower embryonic mortality and higher hatchability

[1] As published, 1990 Poultry Sci. 69:1922-1930.

($P < .05$) than those fed Diet L. Fatty acid compositions of plasma and egg yolk lipids from birds fed Diet L had higher ($P < .05$) levels of linoleic acid than those fed Diet P. In contrast, those fed Diet P did not show higher levels of palmitic acid in their plasma or yolk lipids. Instead, they exhibited higher ($P < .05$) levels of palmitoleic and oleic acids. These results indicate that Diet P, which had only .8% linoleic acid, appeared to be sufficient for overall reproductive performance, suggesting that this level satisfied the requirement of linoleic acid or that oleic acid apparently derived from palmitic acid is sparing the function of linoleic acid for normal reproduction.
(**Key words**: quail, free fatty acids, plasma, yolk, reproduction)

INTRODUCTION

Considerable information has been published concerning the influence of the addition of a source rich in linoleic acid on the reproductive performance of chickens (Menge et al., 1965; Calvert, 1967; Balnave, 1971) and Japanese quail (Calvert, 1969). In most cases, the responses of hens fed the diets containing linoleic acid were compared with those fed either fat-free diets or fat-containing diets but deficient in this essential fatty acid. Better performances were often obtained with hens fed diets supplemented with linoleic acid.

Likewise, there is evidence that indicates that saturated fatty acids are less

digestible and absorbable than the unsaturated fatty acids (Garrett and Young, 1975), which, in turn, would result in a decreased availability of metabolizable energy to the birds. Howevwer, very few reports have dealt with the use of saturated fatty acids on the performance of laying or breeding hens. Thus, Machlin and Dudley (1962) reported that high levels of myristic acid (15%) in the diet significantly depressed egg production and hatchability of fertile eggs. The depression in egg production, but not hatchability, was reversed by the addition of 1.4% of linoleic acid to the diet.

More recently, Atteh and Leeson (1985) observed no significant differences in egg production and egg weight of laying hens fed diets supplemented with 8% of either palmitic acid, oleic acid or a 50:50 mixture of palmitic and oleic acids. In addition, a previous study in the authors' laboratory (Vilchez et al., 1990) has shown that the addition of 3% palmitic acid to the diet of Japanese quail resulted in a performance similar to those fed diets containing 3% of either corn oil, oleic acid or linoleic acid.

From the results of several studies carried out with rats, it was postulated that polyunsaturated fatty acids (PUFA) promote oxidation, whereas saturated fatty acids, such as palmitate, promote lipid storage and liver lipoprotein synthesis (Beynen and Katan, 1985). Bacon et al. (1982) stated that total plasma phosphorus is a good measure of the plasma very low density lipoprotein fraction, which is a yolk lipid precursor (Bacon, 1981) of quail hens. However, there is no information on the effect of dietary free fatty acids on the yolk lipid precursor of quail hens.

The objectives of this experiment were to determine 1) the response of Japanese quail fed a semipurified low-fat basal diet supplemented with either palmitic or linoleic acids, as free fatty acids, at different phases during the reproductive period, and 2) the changes in fatty acid compositions of both plasma and egg yolk lipids and total plasma phosphorus in relation to these treatments.

MATERIALS AND METHODS

Reproductive Performance

At 4 wk of age, 80 male plus female pairs of Japanese quail, divided into two groups of 40 pairs each, were placed in wire quail cages (one pair per cage). Each groupwas fed a diet containing 97% semipurified low-fat (1.3% fat containing .8% linoleic acid) basal mix plus 3% of either palmitic acid (Diet P) or linoleic acid (Diet L) from 4 to 7 wk of age. The composition of the low-fat basal mix and the analyzed fatty acid composition of the experimental diets are presented in Tables 1.1 and 1.2, respectively.

A 9-wk production period, starting at 7 wk of age, was divided into three phases of 3 wk each: 7 to 10 wk, 10 to 13 wk, 13 to 16 wk of age. In Phase 1, quail continued on the same diets as used from 4 to 7 wk of age. In Phase 2, each group of Phase 1 was divided into two groups. One continued on the same diet; the other

switched to the alternate diet. In Phase 3, each group was further divided into two groups. One continued on the same diet as in Phase 2, the other switched to the alternate diet. The distribution of the treatment groups and the number of animals in each phase are shown in Table 1.3.

Plasma and Yolk Fatty Acids and Total Plasma Phosphorus

In a second experiment, 96 female and 48 male Japanese quail, at 4 wk of age, were placed in a battery brooder.[2] In Phase 1, they were divided into two groups with 48 females and 24 males each; In Phase 2, they were divided into four groups with 24 females and 12 males each; in Phase 3, they were further divided into eight groups of 12 females and 6 males each. These birds received the same dietary treatments as indicated in Table 1.3.

Individual blood samples from 16, 8 and 4 female quail in each treatment group (Table 1.3) were collected at 10, 13 and 16 wk of age, respectively. Approximately 2 mL of blood were taken by heart puncture into heparinized tubes and centrifuged at 5 C at 1500 x g for 15 min to separate the plasma. The plasma lipid was extracted by using the method of Folch et al. (1957) modified by the addition of .01% of butylated hydroxy toluene. Methyl esters of the lipid extract were prepared according to Morrison and Smith (1974). Fatty acid compositions of plasma lipids were

[2]Petersime Incubator Co., Gettysburg, OH 45328.

determined by gas-liquid chromatography (Bitmann et al., 1984). Fatty acids were identified by comparison of retention times with standards and expressed as percentages of fatty acid methyl esters distributions. Total plasma phosphorus was determined following the procedure indicated by Strong et al. (1978).

Twenty, 10 and 5 eggs from birds of each treatment group (Table 1.3) were randomly collected at 10, 13 and 16 wk of age, respectively. Each egg was broken out and the yolk separated. Individual yolk lipid was extracted, methylated and analyzed for fatty acid composition following the same procedures as for the plasma.

General Procedure

The birds were provided 14 h of light daily during the breeding period. Feed and water were available ad libitum. To prevent lipid peroxidation, the experimental diets were mixed every 10 days and stored at 5 C. The hens were mated naturally. Eggs from pair-mated hens were collected daily, identified, weighed and stored for incubation. The sound-shelled eggs were set weekly. Because the quail egg shell is colored, tinted and blotched, accurate assessment of the condition of the embryo by candling is not possible. Thus, instead of candling, unhatched eggs were opened on Day 18 and examined macroscopically to identify the apparent infertility and early (0 to 7 days of incubation) and late (7 to 18 days of incubation) embryonic mortalities.

Individual records were obtained for egg production, fertility, hatchability, and embryonic mortality at 7 and 18 days of incubation. Body weight and feed consumption were recorded for each 3-wk phase. Only the female body weights are presented. The feed consumption was the record of both female and male in the same cage assuming that they consumed the same amount of feed. The dietary effects on males were not considered in this experiment.

Statistical Analysis

Treatment differences in Phase 1 and the pooled data by diet within Phases 2 and 3 were subjected to analysis of variance using the general linear model (GLM) procedure in SAS (1986). All percent data were subjected to arcsin transformation prior to analysis. Linear contrasts were used to test the switch-over effects in Phases 2 and 3 (Steel and Torrie, 1980).

RESULTS

Reproductive Performance

Because the analysis of variance used to determine the significance of the interactions among phases was significant for some parameters (data not included), the results are presented for each phase independently. Data on the reproductive

performance of quail fed diets containing palmitic and linoleic acids from 7 to 10 wk (Phase 1), 10 to 13 wk (Phase 2) and 13 to 16 wk of age (Phase 3) are presented in Tables 1.4, 1.5 and 1.6, respectively.

In Phase 1, feeding either Diet P or Diet L had no significant effect ($P > .05$) on any measured parameter (Table 1.4). In Phase 2 (Table 1.5), birds fed Diet P in Phase 1, then switched to Diet L, exhibited significantly lower ($P < .01$) feed consumption and egg production than those maintained on Diet P. The other reproductive parameters were not affected. In contrast, birds fed Diet L in Phase 1 and then switched to Diet P had significantly higher ($P < .05$) feed consumption than those maintained on Diet L. The other parameters were not affected.

Data pooled by diet fed during Phase 2 (Table 1.5) indicated that birds receiving Diet P had significantly higher ($P < .05$) feed consumption and egg production than those on Diet L. There were no significant differences in any of the other parameters.

In Phase 3 (Table 1.6), quail fed Diet P during Phases 1 and 2 then switched to Diet L showed lower ($P < .01$) feed consumption and egg weight than those maintained on Diet P. No effects were observed in the other parameters. When birds were fed Diet P and Diet L during Phases 1 and 2, respectively, then switched to Diet P, they exhibited significantly higher ($P < .01$) feed consumption, embryo livability

from 0 to 7 days of incubation, and hatchability than those that continued on Diet L. Likewise, quail fed Diet L during Phases 1 and 2 then switched to Diet P showed increased ($P<.01$) feed consumption as compared to those that continued on Diet L, but no other differences were observed. Data pooled by diet fed during Phase 3 show that birds fed Diet P exhibited higher feed consumption, higher embryo livability from 7 to 18 days of incubation and higher hatchability than those fed Diet L ($P<.05$).

Fatty Acid Composition of Plasma and Egg Yolk Lipids

Fatty acid compositions of plasma and yolk lipids among 10 (Phase 1), 13 (Phase 2), and 16 wk of age (Phase 3) were not significantly different among treatment groups receiving the same diet in each phase (See Table 1.3 for experimental design). Therefore, the data were pooled by diet (P or L) across the three phases for plasma and yolk lipids (Table 1.7).

Plasma concentrations of myristic and palmitic acids were not significantly different ($P>.05$) between diets. Birds fed Diet P had significantly higher ($P<.05$) levels of palmitoleic and oleic acids, but lower ($P<.05$) stearic and linoleic acids than those fed Diet L. Higher concentrations of palmitoleic, oleic and linolenic acids ($P<.05$), but lower myristic, palmitic, stearic, and linoleic acids ($P<.05$), were observed in yolks from birds fed Diet P than in yolks from birds fed Diet L. The arachidonic acid concentrations were not influenced by dietary treatment.

Total Plasma Phosphorus

The total plasma phosphorus contents in Phases 2 and 3 were not significantly different among treatment groups receiving the same diet. Therefore, data pooled by diet fed in each phase are presented in Table 1.8.

Dietary treatment had no significant effect ($P > .05$) on the total plasma phosphorus throughout the experiment (Table 1.8). However, plasma from birds fed Diet P had consistently higher total phosphorus than those fed Diet L, and the values decreased as the age of the birds increased.

DISCUSSION

Feed consumption was not affected by dietary treatment during Phase 1 (Table 1.4); however, it was significantly influenced in Phase 2 (Table 1.5) and in Phase 3 (Table 1.6). An increased feed consumption was observed in all but one group of quail fed Diet P as compared with those fed Diet L. The greater feed consumption in birds fed Diet P may be due to an apparent lower energy value because of the well-known low absorbability of palmitic acid (Garrett and Young, 1975). Atteh and Leeson (1985) also found that laying hens fed a diet supplemented with only palmitic acid (8%) consumed more feed than birds on diets supplemented with oleic acid (8%) and a mixture (50:50) of palmitic and oleic acids. They

concluded that laying hens could not utilize palmitic acid efficiently when it is the major source of fat in the diet. They also reported that feeding palmitic acid resulted in a significant loss of body weight of hens over a 7-wk period. In the present study, however, such a loss of body weight was not observed in any of the 3-wk phases as indicated in Tables 1.4, 1.5 and 1.6. It is possible that the level of palmitic acid used in the present experiment (3%) was not high enough to cause any detrimental effect because the energy deficit was compensated by a significant increase in feed consumption.

Egg weight was commonly considered to be sensitive to the level of linoleic acid present in the diets of chickens (Balnave, 1971; Whitehead, 1981; Scragg et al., 1987). In most cases, average egg weight was increased as the level of linoleic acid in the diet increased. The results of the present study do not support those observations since the average egg weight from quail fed Diet P (.8% linoleic acid) was not significantly different from those on Diet L (2.7% linoleic acid). Similar observations were previously obtained in quail (Vilchez et al., 1990a) and in laying hens (Atteh and Leeson, 1985) when diets containing palmitic acid were compared with diets containing linoleic and oleic acids, respectively.

In Phase 2, switching from Diet P to Diet L or from Diet L to Diet P resulted in no significant changes in fertility, embryonic mortality and hatchability as demonstrated by linear contrasts (Table 1.5). Egg production , however, decreased

considerably when birds were switched from Diet P to Diet L. The authors have no explanation for this response considering that egg production of this particular group was high and not statistically different in Phase 3 (Table 1.6) when they were fed either Diet P (Treatment 3, P-L-P) or Diet L (Treatment 4, P-L-L).

In Phase 3, egg production, fertility and late embryonic mortality were not affected by switching from Diet P to Diet L or from Diet L to Diet P (Table 1.6). Embryo mortality increased, however, from 0 to 7 days of incubation and hatchability was reduced in birds on Treatment 4 (P-L-L) as compared with those on Treatment 3 (P-L-P). Although these were the only parameters to reach statistical significance, a close observation of the data indicate that most groups of birds fed Diet P tended to have high egg production, low embryonic mortality, (particularly from 7 to 18 days of incubation), and high hatchability as compared with those receiving Diet L. In fact, data pooled by diet fed in Phase 3 (Table 1.6) clearly show these tendencies. It also shows late embryonic mortality to be significantly lower, resulting in better hatchability, than those birds fed Diet L. The increased livability of embryos from birds fed Diet P may be due to the high levels of oleic acid present in their yolk lipids (Table 1.8). Donaldson and Fites (1970) demonstrated the significance of oleic acid in eggs on embryo survival. Likewise, Noble (1987b) indicated the role of cholesterol esters of oleic acid on lipid transport and assimilation from the yolk during embryo development. Therefore, in this experiment, a high level of oleic acid in the yolk together with the ability of yolk sac membrane to synthesize cholesterol esters (Noble

et al., 1984) may have enhanced the formation of lipoprotein complexes required for the lipid transport from the yolk to the embryo. This enhancement of formation of lipoprotein complexes may have resulted in a better utilization of the yolk material given that it is estimated that more than 90% of the energy requirement for embryo development is derived from oxidation of yolk lipid fatty acid (Freeman and Vince, 1974).

It has long been demonstrated in chickens that the fatty acid compositions of both plasma and egg yolk lipids reflect the fatty acid composition of their diets (Machlin et al., 1962; Thomsen, 1966). The results of the present study with quail agree with those early observations. The levels of linoleic acid in both plasma and egg yolk (Table 1.7) were significantly influenced by the concentration of this fatty acid present in the diet (Table 1.2). Decreased concentrations of palmitoleic and oleic acids as well as increased proportions of myristic, palmitic and stearic acids were also observed in plasma lipids and, more particularly, in yolks from birds fed Diet L. These observations are in accordance with those reported in chickens (Thomsen, 1966; Balnave, 1968; Sim et al., 1973) in which the synthesis of monounsaturated fatty acids is considerably reduced, whereas the synthesis of saturated fatty acids is increased in order to maintain a specific saturated to unsaturated ratio during a period of PUFA ingestion.

Higher concentrations of palmitoleic and oleic acids were observed in both

plasma and egg yolk lipids of birds fed Diet P as compared with those fed Diet L. These higher levels may be due to the availability of their immediate precursor, palmitic acid, which will undergo desaturation to form palmitoleic acid or elongation and desaturation to form oleic acid (Vance and Vance, 1985). As indicated earlier, these differences may also be due to the ingestion of a relatively high level of PUFA (Diet L). From this experiment, it is not possible to determine which of the two alternatives is the predominant one. Linolenic acid in egg yolks from birds fed Diet P was higher than from those fed Diet L throughout the experiment. This difference may be due to the relatively high content of this fatty acid in Diet P (Table 1.2). However, a similar tendency was also observed in eggs from laying hens fed a diet containing hydrogenated coconut oil (with 0% linolenic acid) as compared to those from hens fed safflower oil (Machlin et al., 1962).

In conclusion, the results of this experiment show that feeding quail a semipurified low-fat basal mix supplemented with 3% palmitic acid (Diet P) resulted in increased feed consumption as compared with consumption by quail fed the same basal mix supplemented with 3% linoleic acid (Diet L). In spite of the fact that Diet P had lower linoleic acid (.8%) than recommended for breeder quail (1%; National Research Council, 1984), the performance of quail fed Diet P equaled and even surpassed those fed Diet L. Switching from Diet P to Diet L did not result in any significant change in most parameters, except lowered feed consumption (in each phase) and reduced egg production (Treatment B, Table 1.5). However, switching

from Diet L to Diet P had no significant effect in any parameter except feed consumption, which increased in all cases. Fatty acid compositions of both plasma and egg yolk lipids from birds fed Diet L had high concentrations of linoleic acid. In contrast, birds fed Diet P showed less palmitic acid in their yolk lipids and elevated palmitoleic and oleic acids in both plasma and yolk lipids. The observed elevated egg yolk oleic acid coupled with its importance in synthesis of cholesteryl oleate for transport across the yolk sac membrane (Noble, 1987b) provides a likely explanation of the improved embryonic livability and hatchability on the palmitic acid supplemented diet. Total plasma phosphorus was not affected significantly by dietary treatment. Thus, differences due to diet cannot be attributed to increased yolk precursor output from the liver.

ACKNOWLEDGEMENT

This research was supported by the Ministere de l'Agriculture des Pecheries et de l'Alimentation du Quebec.

TABLE 1.1. Composition of low-fat basal mix and analysis of experimental diets

Ingredient and analysis	(%)	
Ground yellow corn	24.0	
Corn starch	30.6	
Alfalfa meal (15% CP)	10.0	
Isolated soybean protein (67.8% CP)	26.0	
Calcium carbonate (38%)	4.8	
Calcium phophate, mono-basic (21% P)	3.6	
Choline chloride (70%)	.3	
DL-Methionine (98%)	.2	
Vitamin-mineral premix[1]	.3	
Sodium chloride	.2	
	Experimental diets[2]	
Determined analyses	P	L
Crude protein, %	19.3	19.6
Calcium, %	2.5	2.5
Total phosphorus, %	.7	.7
Ether extract, %	4.3	4.4
Palmitic acid, %[3]	2.7	.3
Linoleic acid, %[3]	.8	2.7

[1] Provides the following per kilogram of diet: vitamin A, 8000 IU; vitamin D_3, 1,700 ICU; DL-α-tocophery acetate, 5.6 mg; menadione sodium bisulfite, 1.2 mg; vitamin B_{12}, .005 mg; biotin, .16 mg; folacin, 1.26 mg; thiamine, 2 mg; pyridixine, 4 mg; niacin, 70 mg; riboflavin, 5 mg; calcium pantothenate, 20 mg; iron, 20 mg; manganese, 54 mg; copper, 7 mg; zinc, 68 mg; iodine, 1.035 mg; selenium, .19 mg; butylated hydroxytoluene, 100 mg.

[2] Palmitic acid (Diet P): 97% basal + 3% palmitic acid; linoleic acid (Diet L): 97% basal + 3% linoleic acid.

[3] ICN Biomedicals, Inc., Cleveland, OH 14128. Purity: palmitic acid, 92.2%, linoleic acid, 64.4%.

TABLE 1.2. Analyzed fatty acid composition of the experimental diets[1]

	Diets[2]	
Fatty acid	P	L
Lauric	.52	...
Myristic	3.10	3.34
Palmitic	63.27	6.40
Palmitoleic	...	.29
Stearic	1.86	1.46
Oleic	8.74	22.84
Linoleic	19.44	61.10
Linolenic	2.13	1.51

[1] As percentage of total methyl esters.

[2] P = diet containing 3% palmitic acid; L = diet containing 3% linoleic acid.

TABLE 1.3. Distribution of the treatment groups and number of animals in each reproduction phase.

Phase 1		Phase 2		Phase 3	
7 to 10 wk of age		10 to 13 wk of age		13 to 16 wk of age	
Treatment[1]	n[2]	Treatment	n	Treatment	n
P	40	A (P-P)	20	1 (P-P-P)	10
L	40	B (P-L)	20	2 (P-P-L)	10
		C (L-P)	20	3 (P-L-P)	10
		D (L-L)	20	4 (P-L-L)	10
				5 (L-P-P)	10
				6 (L-P-L)	10
				7 (L-L-P)	10
				8 (L-L-L)	10

[1] P = diet containing 3% palmitic acid; L = diet containing 3% linoleic acid.

[2] n = number of female quail.

TABLE 1.4. Reproductive performance of quail fed diets containing 3% palmitic (P) or 3% linoleic acid (L) from 7 to 10 wk of age (Phase 1)

Diet	FC[1]	BWI	EW	EP	FER	Embryo mortality		Hatch
						7-day	18-day	
	(g/day)	(g per phase)	(g)			%		
P	20.8[2]	13.0	10.6	82.6	92.6	15.4	11.4	74.0
L	20.3	12.9	10.4	84.2	93.8	13.2	15.8	71.0
SEM	.2	3.0	.2	2.6	1.3	1.5	1.9	2.4

[1] FC = feed consumption; BWI = body weight increase; EW = egg weight; EP = egg production; FER = fertility; Hatch = hatchability.
[2] Values are means of 40 observations.

TABLE 1.5. Reproductive performance of quail fed diets containing 3% palmitic (P) or 3% linoleic acid (L) from 10 to 13 wk of age (Phase 2) with analysis.

Treatment	FC[1]	BWI	EW	EP	FER	Embryo mortality 7-day	Embryo mortality 18-day	Hatch
	(g/day)	(g per phase)	(g)			%		
A (P-P)	23.2[2]	3.1	11.6	90.8	93.9	5.0	7.9	87.1
B (P-L)	21.4	5.7	11.5	77.2	95.0	8.3	10.1	81.6
C (L-P)	23.1	6.3	11.4	88.1	94.1	6.8	10.5	82.7
D (L-L)	22.3	4.4	11.6	89.0	94.8	5.6	13.1	81.3
Analysis ofvariance								
Source of variation								
Linear contrast		Probability						
A vs B	**	NS	NS	**	NS	NS	NS	NS
C vs D	*	NS	NS	NS	NS	NS	NS	NS
Data pooled by diet fed during Phase 2[3]:								
P	23.1[a]	4.7	11.5	89.4[a]	94.0	5.6	9.3	84.8
L	21.0[b]	5.1	11.6	83.3[b]	94.9	6.8	11.7	81.4
SEM	.2	2.9	.08	2.5	1.6	1.3	1.5	2.0

[a,b] Means within columns with no common superscripts differ significantly (P<.05).

[1] FC = feed consumption; BWI = body weight increase; EW = egg weight; EP = egg production; FER = fertility; Hatch = hatchability.

[2] Values are means of 20 observations.

[3] Values are means and SEM of 40 observations.

* P<.05.

** P<.01.

TABLE 1.6. Reproductive performance of quail fed diets containing 3% palmitic (P) or 3% linoleic acid (L) from 10 to 13 wk of age (Phase 2) with analysis.

Treatment	FC[1]	BWI	EW	EP	FER	Embryo mortality		Hatch
						7-day	18-day	
	(g/day)	(g per phase)	(g)			%		
1 (P-P-P)	25.6[2]	2.2	12.2	95.8	93.1	9.7	6.6	83.7
2 (P-P-L)	22.2	2.6	11.4	88.6	94.1	12.2	11.3	76.5
3 (P-L-P)	23.9	2.1	12.0	96.4	93.8	4.1	4.5	91.4
4 (P-L-L)	22.6	1.5	12.2	88.9	95.2	12.6	9.6	77.7
5 (L-P-P)	23.9	.7	11.6	83.3	93.9	8.2	8.2	83.6
6 (L-P-L)	23.7	3.8	11.7	90.0	95.0	8.9	14.2	77.0
7 (L-L-P)	24.9	-1.8	11.7	91.9	94.8	9.1	8.8	82.1
8 (L-L-L)	23.4	-3.9	12.1	84.3	94.2	8.2	16.1	75.7
Source of variation	Probability							
Linear contrast								
1 vs 2	**	NS	**	NS	NS	NS	NS	NS
3 vs 4	**	NS	NS	NS	NS	*	NS	*
5 vs 6	NS	NS	NS	NS	NS	NS	NS	NS
7 vs 8	*	NS	NS	NS	NS	NS	NS	NS
Data pooled by diet fed during Phase[3]:								
P	24.4	.8	11.8	91.5	93.9	8.1	7.3	84.6
L	23.2	.3	11.9	87.9	94.6	10.3	12.7	77.0
SEM	.2	.4	.08	2.0	1.2	1.4	1.5	2.0

[a,b] Means within columns with no common superscript differ significantly (P<.05).
[1] FC = feed consumption; BWI = body weight increase; EW = egg weight; EP = egg production; FER = fertility; Hatch = hatchability.
[2] Values are means of 20 observations.
[3] Values are means and SEM of 40 observations.
* P< .05
** P< .01

TABLE 1.7. Effect of dietary palmitic (P) or linoleic (L) acids on the fatty acid composition of plasma and egg yolk lipids of Japanese quail hens (pooled data of Phases 1, 2 and 3)

Sample and	Fatty acid[1]							
diet	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:4}$
Plasma[2]								
P	6.5	25.7	5.0[a]	8.4[b]	41.5[a]	13.0[b]		
L	7.5	26.3	3.6[b]	9.2[a]	30.9[b]	22.7[a]		
SEM	.6	.4	.2	.2	.5	.3		
Yolk[3]								
P	8.0[b]	25.8[b]	7.9[a]	6.9[b]	41.2[a]	6.9[b]	.20[a]	1.5
L	9.0[a]	29.8[a]	6.7[b]	8.4[a]	30.2[b]	13.4[a]	.15[b]	1.5
SEM	.3	.2	.2	.1	.4	.2	.01	.02

[a,b]Means within columns and samples with no common superscripts differ significantly ($P<.05$).

[1]Values are percentage of total methyl esters.

[2]Values are means of 48 observations per diet.

[3]Values are means of 60 observations per diet.

TABLE 1.8. Effect of dietary palmitic (P) or linoleic (L) acids on total plasma phosphorus of Japanese quail hens.

Phase	Diet		Phase mean	SEM
	P	L		
	(μg/ml)			
1	453[1]	411	432	26
2	432	409	420	28
3	363	355	359	15

[1]Values are means of 16 observations.

2. EFFECT OF FEEDING PALMITIC, OLEIC, AND LINOLEIC ACIDS TO JAPANESE QUAIL HENS (Coturnix coturnix japonica). 1. REPRODUCTIVE PERFORMANCE AND TISSUE FATTY ACIDS[1]

C. VILCHEZ, S. P. TOUCHBURN, E. R. CHAVEZ, AND C. W. CHAN

Department of Animal Science, Macdonald College of McGill University, and McGill Nutrition and Food Science Centre, Ste. Anne de Bellevue, Quebec, H9X 1C0, Canada.

ABSTRACT A study was conducted to evaluate the effects of diets containing 5% of either palmitic acid (Diet PA), oleic acid (Diet OA), and linoleic acid (Diet LA) on reproductive performance, fatty acid composition of egg yolk, plasma, and liver, and total plasma phosphorus of Japanese quail. Each diet was fed to 20 individually caged hens from 5 wk of age. A 24-wk production period started at 8 wk of age. Fertile eggs for incubation were obtained by placing at random a male in the cage with the female for 15 to 20 min twice per week. The males were kept in separate individual cages and fed a turkey grower diet throughout. Feed consumption, egg production, egg output, and the number of chicks per hen were higher ($P < .05$) in birds fed Diet PA than in those fed Diet OA or Diet LA. Hatchability was not

[1] As published, 1991 Poultry Science 70:2484-2493.

different ($P > .05$) between Diet PA and Diet OA, but they were higher ($P < .05$) than that of Diet LA. Quail weight at hatch from birds fed Diet LA was heavier ($P < .05$) than those from Diet OA, but not different ($P > .05$) from those fed Diet PA. Total plasma phosphorus concentration was higher ($P < .05$) in birds fed Diet PA than in those fed Diet LA. High levels of oleic and linoleic acids were found in egg yolk, plasma and liver lipids from birds fed Diet OA and Diet LA, respectively. Feeding Diet PA resulted in elevated levels of palmitoleic acid in all three tissues. The highest overall reproductive performance on Diet PA suggests that palmitic acid has some physiological role in reproduction. The sustained near-maximal levels of egg production and fertility achieved in this trial indicate the superiority of the mating procedure, which would also permit testing the response of male and female birds while minimizing injuries incurred by the females.

(**Key words**: quail, reproduction, fatty acids, plasma, liver)

INTRODUCTION

In contrast to reports on the effect of feeding different oils and animal-vegetable fat blend to chickens, few reports have dealt with the use of saturated fatty acids on the performance of laying or breeding hens. Machlin and Dudley (1962) reported that high levels of myristic acid (15%) in the diet significantly depressed egg production and hatchability for fertile eggs. The depression in egg production, but not hatchability, was reversed by the addition of 1.4% of linoleic acid. Atteh and Leeson

(1985) observed no significant differences in egg production and egg weight of laying hens fed diets supplemented with 8% of either palmitic acid, oleic acid or a 50:50 mixture of palmitic and oleic acids. In a low-linoleic acid diet of Japanese quail, the inclusion of 3% palmitic acid resulted in a performance similar to those fed diets containing 3% of either corn oil, oleic acid, or linoleic acid (Vilchez et al., 1990a). In a subsequent switch-over experiment, Vilchez et al. (1990b) compared the effect of the inclusion of 3% of either palmitic acid or linoleic acid in the diets of breeding Japanese quail. Their data showed that feeding 3% palmitic acid resulted in a higher reproductive performance than feeding 3% linoleic acid. The duration of the above mentioned experiments was less than 10 wk. Therefore, it seemed important to measure the effects of feeding fatty acids of different saturation to laying or breeding hens for a longer period of time.

The objective of the present experiment was to investigate further the response of Japanese quail hens to a semipurified low-fat low-linoleic acid basal diet supplemented with either palmitic, oleic, or linoleic acids in terms of 1) reproductive performance; 2) changes in fatty acid compositions of egg yolk, plasma, and liver lipids; and 3) total plasma phosphorus and yolk cholesterol content in relation to these treatments.

MATERIALS AND METHODS

Reproductive Performance

At 5 wk of age, 60 female Japanese quail (mean BW of 126 $\pm$ .38 g) divided into three groups of 20 birds each, were placed one per cage in wire quail laying cages (152 x 229 x 175 mm). Each group was fed a diet containing 97% semipurified, low-fat (1.3% fat containing .7% linoleic acid) basal mix plus 3% of either palmitic acid (Diet PA), oleic acid (Diet OA), or linoleic acid (Diet LA) from 5 to 32 wk of age. The composition of the low-fat basal mix and the analyzed fatty acid composition of the experimental diets are presented in Tables 2.1 and 2.2, respectively.

Twenty male quail of the same age (5 wk) were placed in individual wire quail cages (one per cage) located in the same room as the females. They were fed a commercial turkey grower diet (23% CP) thoroughout the experimental period.

The birds were provided 16 h of light daily during the breeding period. Feed and water were available for ad libitum intake. To prevent lipid peroxidation, the experimental diets were mixed every 10 days and stored in sealed containers at 5 C. Birds were fed fresh feed daily.

Starting at 7 wk of age, birds were mated naturally, but unlike the previous

experiments (Vilchez et at., 1990a,b), this was accomplished by placing at random a male in the cage with the female for 15 to 20 min. Each female was mated every 3rd day. At this rate each male was used once a day, six times per week. Starting at 8 wk of age, eggs were collected daily, identified, weighed, and stored for incubation. The sound-shelled eggs were set weekly. Because the quail eggshell is colored, tinted, and blotched, accurate assessment of the condition of the embryo by candling is impossible. Therefore, unhatched eggs were opened on Day 18 and examined macroscopically to identify the apparent infertility and early (0 to 7 days of incubation) and late (7 to 18 days of incubation) embryonic mortality.

Individual records were obtained for egg production , fertility, hatchability, and embryonic mortality at 7 and 18 days of incubation. Body weight was recorded at the beginning and the end of the experiment. Feed consumption was recorded daily. Body weights at hatch of quail chicks from each group of hens fed the experimental diets were recorded every second hatch until the 20th hatch.

Cholesterol Content and Fatty Acid Composition of the Yolk Lipids

Eggs from 10 individual hens per treatment were collected for 2 consecutive days (20 eggs per treatment) at 18 wk of age. Eggs from the same bird were broken out and the albumen and yolk separated. The last traces of albumen were removed by rolling the yolk on filter paper. Yolk weight was recorded. After the yolk material was thoroughly mixed using a glass rod, 1 g of yolk sample was freeze-dried to

determine its dry matter content. Yolk total lipid content was determined gravimetrically in the freeze-dried yolk sample after extraction using chloroform:methanol (2:1, vol/vol) solution (Folch et al., 1957). The remaining fresh yolk material of each bird was pooled, resulting in 10 yolk samples per treatment that were used to determine both the cholesterol content and the fatty acid composition of the egg yolk lipids.

Lipids of .2 g yolk was extracted by the method of Folch et al. (1957), modified by the addition of .01% of butylated hydroxytoluene to the chloroform:methanol (2:1, vol/vol) solution. Methyl esters of the lipid extract were prepared according to Morrison and Smith (1974). Fatty acid composition of yolk lipids was determined by gas-liquid chromatography (GLC) using a Hewlett-Packard 5711A gas chromatograph (GC), fitted with automatic sampler 7671A, integrator 3380S, and FID detector.[2] Separation of fatty acid methyl esters was achieved using a glass column about 2 m long packed with 10% SP-2340 on 100/120 mesh Chromosorb W-AW.[3] Fatty acid methyl esters were identified by comparison of retention times with standards[4] and expressed as percentages of methyl esters.

For the determination of the egg cholesterol content, .2 g yolk sample was

---

[2]Hewlett Packard Co., Avondale, CA 19311.

[3]Supelco Inc., Bellefonte, PA 16823.

[4]Nu-Chek-Prep, Elysian, MN 56028.

used. The total lipid was extracted (Washburn and Nix, 1974), hydrolyzed (Heuck et al., 1977), trimethylsilylated (Marcel and Vezina, 1973), and analyzed for cholesterol by GLC (Marcel and Vezina, 1973) using B-sitosterol as internal standard. The characteristics of the GC were described in the previous section. The separation of B-sitosterol and cholesterol was achieved using a glass column about 1 m long packed with 3% OV-1 on 80/100 mesh Chromosorb W-AW.[3]

Fatty Acid Compositions of Plasma and Liver Lipids and Total Plasma Phosphorus

At the end of the experiment (32 wk of age), individual quail BW were recorded. Individual blood samples from 16 female quail in each treatment were collected. Approximately 2.5 mL of blood were taken by heart puncture into heparinized tubes and centrifuged at 5 C at 1,500 rpm for 15 min to separate the plasma. After blood sample was collected, the birds were killed by cervical dislocation and the livers removed, blotted with paper towel, and their weights recorded.

Plasma (.1 mL) and liver (.1 g) samples from eight birds of each dietary group were used to determine the fatty acid composition of both plasma and liver lipids. Fatty acid methyl esters were prepared following the method indicated by Lepage and Roy (1986). Fatty acid composition was determined by GLC using the GC described in the preceding section. Fatty acid methyl esters were separated by using a Supelcowax 10 fused silica capillary column (30 m, .53 mm ID, 1.0 μm film).[3] Fatty

acid methyl esters were identified by comparison of retention times with standards[4] and expressed as percentages of fatty acid methyl esters.

Total plasma phosphorus concentration was determined using a modification of the method of Strong et al. (1978). A .1-mL sample of plasma was diluted to .6 mL in saline and 2 aliquots of .2 mL each were transferred to micro-Kjeldahl flasks. The diluted samples were wet ashed until clear and colorless using 2 mL of 70% nitric acid and .5 mL of 70% perchloric acid. Standards containing known amounts of phosphorus were prepared and ashed in the same manner. Four milliliter of vanado-molybdate reagent (Association of Official Agricultural Chemists, 1965) were added to each flask and the absorbance was determined at 400 nm with a Beckman DU-50 spectrophotometer.[5]

Statistical Analyses

The statistical model for the reproductive performance data was a randomized complete block design in a split plot arrangement, where dietary treatment was the main plot unit and age was the sub-plot unit (Steel and Torrie, 1980). For the other parameters evaluated in this experiment, the statistical model was a randomized complete block design. Analysis of variance was carried out on data using the General Linear Models (GLM) procedure (SAS Institute, 1986). Significant

[5]Beckman INstruments, Inc., Fullerton, CA 92634.

differences of means were tested using Duncan's multiple range test (Duncan, 1955). All percentage data were subjected to arc sine transformation prior to analyses.

RESULTS AND DISCUSSION

Data on the reproductive performance of quail fed diets containing palmitic, oleic and linoleic acids, over a 24-wk production period, are presented in Table 2.3. Neither fertility nor late embryonic mortality were significantly affected by dietary treatment ($P > .05$).

In agreement with earlier observations (Vilchez et al., 1990a,b), feeding palmitic acid to quail hens resulted in an increased feed consumption as compared with those fed either oleic or linoleic acids (Table 2.3). However, the average feed consumption in the current experiment is higher than those previously reported. The difference may be due to the fact that data reported in the present study represent feed consumption of single female quail, whereas the values in previous experiments were calculated on a bird-day basis because of the presence of a male bird in each cage (Vilchez et al., 1990a,b). It is recognized that the female would consume more than the male. Thus, an average feed consumption of 15.5 g per bird per day was reported when adult male Japanese quail were fed ad libitum consumption (Shapira et al., 1978). On the contrary, feed consumption of laying Japanese quail was reported to vary from 20 to 26 g per bird per day (Santoma, 1989).

The greater feed consumption in birds fed Diet PA may be related to an apparent lower energy value of the feed because of the low absorbability of palmitic acid (Garrett and Young 1975). In laying hens, Atteh and Leeson (1985) also found that birds fed diets supplemented with palmitic acid consumed more feed than those fed oleic acid or a 50:50 mixture of palmitic and oleic acids. They concluded that laying hens could not utilize palmitic acid efficiently when it is the major component of fat in the diet. In fact, in the present study, an analysis of quail feces for the total fat content as well as its fatty acid composition by the method of Sukhija and Palmquist (1988) showed that feces from quail hens fed Diet PA had sixfold more fat (2.65%) than those fed either Diet OA (.44%) or Diet LA (.41%); moreover, the palmitic acid in the fat of feces of quail fed Diet PA represented around 90% of the total fatty acid methyl esters.

Japanese quail are capable of maintaining a rather high rate of egg production under optimum conditions, but that production is very sensitive to dietary and environmental changes (Wilson et al., 1961). In the present study, a high rate of egg production was observed with the highest corresponding to those fed Diet PA (96.0%), followed by those fed Diet OA (93.2%), and Diet LA (87.5%) (Table 2.3). High rates of egg production are also reported in the literature: 90% over a 45-day production period (Yamane et al., 1980); 94.3% over a 35-day production period (Allen and Young, 1980); 94.3% over a 60-day production period (Sakurai, 1981). Unlike these previous studies, however, the values reported in the present study

represent the average egg production of a 168-day production period. To the authors' knowledge this is the first report showing a high rate of egg production over a long term feeding experiment with Japanese quail breeder hens.

The high average egg weight observed in birds fed Diet LA are consistent with the data reported in chickens (Balnave, 1971; Whitehead, 1981; Scragg et al., 1987). In most cases, average egg weight increased as the level of linoleic acid in the diet increased. However, the results of the present study do not support the data previously reported in quail (Vilchez et al., 1990a,b) as well as in laying hens (Atteh and Leeson, 1985) where diets containing palmitic acid were compared with diets containing oleic and linoleic acids. On the contrary, a comparison of the estimated weight of egg produced per hen on a daily basis (Table 2.3), clearly shows that quail hens fed Diet PA had the highest egg output (10.85 g), followed by those fed Diet OA (10.53 g) and then Diet LA (10.06 g). Similar observations have not been reported in poultry.

In the present study, embryonic mortality from 0 to 7 days of incubation was higher than that occurring from 7 to 18 days of incubation. These figures contrast with previous reports (Vilchez et al., 1990a,b) in which late embryonic mortality was higher than early embryonic mortality. Explanation for this difference is not readily apparent. However, a response similar to our present results has been reported by Narahari et al. (1988).

The high total embryonic mortality and low hatchability of fertile eggs observed in birds fed Diet LA as compared to those fed either Diet PA or Diet OA (Table 2.3) contrast with those reported by Vilchez et al. (1990a) who found no significant differences in these parameters when quail were fed diets containing either palmitic, oleic or linoleic acids. However, the data of the present study support those reported by Vilchez et al. (1990b) in which quail fed a diet supplemented with linoleic acid resulted in lower hatchability when compared with those fed a diet supplemented with palmitic acid. They suggested the increased livability of embryos from birds fed palmitic acid may be due to the high levels of oleic acid present in their egg yolk lipids.

Donaldson and Fites (1970) demonstrated the significance of oleic acid in eggs on quail embryo survival. Tullett (1990) reported that hatchability was adversely affected when stearic acid represented more than 12% and the oleic acid less than 40% of the total fatty acids in the yolk and when the ratio of stearic to oleic acid exceeded .25. In addition, Noble (1987b) indicated the role of cholesterol esters of oleic acid on lipid transport and assimilation from the yolk during embryo development. Therefore, in the present study, the high levels of oleic acid and lower stearic to oleic acid ratios in egg yolks from birds fed Diet PA and Diet OA (Table 2.4), together with the ability of the yolk sac membrane to synthesize cholesterol esters (Noble et al., 1984), may have enhanced the formation of lipoprotein complexes required for the lipid transport from the yolk to the embryo. Freeman and

Vince (1974) indicated the significance of the yolk fatty acids as energy source during embryonic development.

In the current study, the estimated number of quail chicks per hen was significantly different among treatments (Table 2.3). The highest value corresponded to the group fed Diet PA (122 chicks), followed by those fed Diet OA (115 chicks), and Diet LA (100 chicks). The low number of chicks per hen observed in the group fed Diet LA is related to both its low egg production rate and its low hatchability of fertile eggs as compared with the other two treatments. The average body weight of quail chicks at hatch was higher for those from hens fed Diet LA than those from hens fed Diet OA, but it was not different from those fed Diet PA. The heavy body weight of quail chicks from hens fed Diet LA can be attributed to the high average egg weight observed in this group. In chickens, hatching weight is highly correlated with egg size at setting (Shanawany, 1984). In quail, significant correlations among egg, embryo, and hatch weights have been reported (Marks, 1975). However, Yannakopoulos and Tserveni-gousi (1987) indicated that egg weight did not affect the hatching weight if egg weight was above 11 g.

Average yolk weight, its dry matter content and total lipid content were not significantly affected by dietary treatment (Table 2.5). However, the values obtained in this experiment are in agreement with those reported for the Japanese quail by Roca et al. (1984). It has been demonstrated in chickens that the percentage of total

lipid content of egg yolk is not significantly influenced by the type or amount of dietary lipids (Marion and Edwards, 1962; Vogtmann and Clandinin, 1975). Likewise, cholesterol content in egg yolks was not different among treatments (Table 2.5), but the values reported herein are similar to those indicated in previous studies (Bitman and Wood, 1980). It was reported that dietary fat has little effect on cholesterol deposition in the egg yolk. Thus, when laying hens were fed diets containing corn oil, lard, or hydrogenated coconut oil (Chung et al., 1965) there was no increase in egg yolk cholesterol. However, feeding cholesterol to laying hens in most cases results in an increase in egg yolk cholesterol levels. Hebert et al. (1987) demonstrated no difference in egg yolk cholesterol when laying hens were fed diets supplemented with 6% of either oleic or linoleic acids. The type of fatty acid fed did not affect egg yolk weight.

Dietary treatment significantly affected the total plasma phosphorus concentration (Table 2.6). The lipids synthesized in the liver of the laying female are transported by the plasma in the form of lipoprotein complexes to the ovary where they are deposited intact in the follicles (Christie and Moore, 1972; Hillard et al., 1972). Bacon et al. (1982) stated that total plasma phosphorus is a good measure of the plasma very low density lipoprotein fraction, which is a yolk lipid precursor (Bacon, 1981) of quail hens. Beynen and Katan (1985) indicated that polyunsaturated fatty acids promote oxidation, whereas saturated fatty acids, such as palmitate, promote lipid storage and liver lipoprotein synthesis. In fact, Hood (1990)

demonstrated that liver from quail given a diet containing saturated fat (beef fat) synthesized significantly more fatty acids than quail fed a diet containing polyunsaturated fatty acids (tuna oil).

It is well recognized in chickens that the fatty acid composition of plasma, egg yolk, and liver lipids reflect the fatty acid composition of their diets (Machlin et al., 1962; Thomsen, 1966). More recently, Vilchez et al. (1990a,b) demonstrated that dietary fatty acids significantly influence the fatty acid composition of both plasma and egg yolk lipids of Japanese quail. The results of the present study confirm those early observations.

The levels of linoleic and oleic acids in egg yolk (Table 2.4), plasma (Table 2.7) and in the liver lipids (Table 2.8) were significantly influenced by the concentrations of these fatty acids in Diet LA and Diet OA, respectively (Table 2.2). Increased concentrations of palmitic and stearic acids as well as decreased concentrations of palmitoleic and oleic acids were also observed in yolk lipids from birds fed Diet LA. A similar tendency was observed in the fatty acid composition of the plasma and liver lipids. It was previously reported in chickens that feeding diets containing high levels of polyunsaturated fatty acids result in decreased synthesis of monounsaturated fatty acids and increased synthesis of saturated fatty acids, respectively (Balnave, 1968; Sim et al., 1973).

Higher concentrations of oleic acid were observed in yolk, plasma, and liver lipids in hens fed Diet PA as compared with those fed Diet LA, but they were lower than those fed Diet OA (Tables 2.4, 2.7, and 2.8). Feeding Diet PA resulted in high concentrations of palmitoleic acid in yolk and plasma lipids only. The high levels of palmitoleic and oleic acids in tissues from birds fed Diet PA may be due to the presence of their immediate precursor, palmitic acid, which will undergo desaturation to form palmitoleic acid or elongation and desaturation to form oleic acid (Vance and Vance, 1985). The presence of a high level of arachidonic acid in the liver lipids of birds fed Diet PA is surprising since the level of its immediate precursor, linoleic acid, in Diet PA is almost 4 times lower than that in Diet LA (Table 1.1). There are two possible explanations for this response. First, it is probable that under a situation of low linoleic acid , the synthesis of arachidonic acid in the hepatic tissue may be more efficient or maximized; however, there is no evidence to support this hypothesis. Second, feeding diets high in saturated fatty acid, such as Diet PA, may increase the synthesis of polyunsaturated fatty acids in order to maintain an appropriate saturated to unsaturated ratio in the tissue lipids. The latter would be an opposite situation to those reported in cases of feeding diets high in polyunsaturated fatty acids (Balnave, 1968; Sim et al., 1973).

In summary, the inclusion of 3% of either palmitic or oleic acids in the diets of quail breeder hens results in an improved reproductive performance when compared with those fed a diet supplemented with 3% linoleic acid. The high egg production

rate observed in hens fed Diet PA may be due to their high concentrations of lipoproteins as indicated by total plasma phosphorus. Noble (1987b) indicated the importance of the synthesis of cholesterol ester of oleic acid for the lipid transport across the yolk sac membrane. In the present study, the low stearic to oleic acid ratio in the egg yolk lipids was coupled with elevated egg yolk oleic acid. The above provide a likely explanation of the improved embryonic livability and hatchability obtained in hens fed diets supplemented with either palmitic or oleic acids. The fatty acid compositions of egg yolk, plasma, and liver lipids were significantly influenced by the fatty acid composition of the diets. Parameters such as yolk weight, yolk lipid content, yolk cholesterol content, final body weight, liver weight were not significantly affected by dietary treatments.

The results of the present experiment also suggest that maintaining female and male quail in separate individual cages, and mating each female twice a week for only 15 to 20 min, maximize egg production rate and fertility. This improvement may be due to a reduction in the stress caused by the aggressive sexual activity of the male resulting in injury to the hen when housed in pairs (personal observations) and to the agonistic behaviour leading to establishment of peck orders commonly observed in quail maintained in colony cages (Gildersleeve et al., 1987). Moreover, the quail husbandry followed in the present study would allow the isolation and identification of each bird when used in experiments designed to test the response of either the female or the male quail.

ACKNOWLEDGMENTS

This research was supported by the Ministere de l'Agriculture des Pecheries et de l'Alimentation du Quebec. The assistance of Suzanne Drouin is greatly appreciated.

TABLE 2.1. Composition of the low-fat basal mix and analysis of experimental diets.

Ingredients and analyses	Basal diet	Experimental diets[1]		
		PA	OA	LA
	(%)			
Ground yellow corn	24.0			
Corn starch	29.8			
Alfafa meal (15% CP)	10.0			
Isolated soybean protein (67.8% CP)	26.0			
Calcium carbonate (38% Ca)	4.8			
Calcium phosphate, mono-basic (21% P)	3.6			
Vitamin-mineral premix[2]	1.0			
Choline chloride (70%)	.3			
DL-methionine (98%)	.3			
Sodium chloride	.2			
Determined analyses				
Crude protein		19.7	19.6	19.8
Calcium		2.5	2.5	2.5
Total phosphorus		.7	.7	.7
Ether extract		4.4	4.3	4.3
Palmitic acid[3]		3.2	.4	.3
Oleic acid[3]		.3	2.3	1.0
Linoleic acid[3]		.7	1.1	2.6

[1] Diet PA = 97% basal mix plus 3% palmitic acid; Diet OA = 97% basal mix plus 3% oleic acid; Diet LA = 97% basal mix plus 3% linoleic acid.

[2] Provides the following per kilogram of diet: vitamin A, 8,000 IU; cholecalciferol, 1,700 ICU; DL-a-tocopheryl acetate, 5.6 mg; menadione sodium bisulfite, 1.26 mg; vitamin B_{12}, .005 mg; biotin, .16 mg; folacin, 1.26 mg; thiamine, 2 mg; pyridoxine, 4 mg; niacin, 70 mg; riboflavin, 5 mg; calcium pantothenate, 20 mg; iron, 20 mg; manganese, 54 mg; copper, 7 mg; zinc, 68 mg; iodine, 1.035 mg; selenium, .19 mg; butylated hydroxytoluene, 120 mg.

[3] ICN Biomedicals, Inc, Cleveland, OH 14128. Purity: palmitic acid, 97.2%; oleic acid, 65.4%; linoleic acid, 64.6%.

TABLE 2.2. Analyzed fatty acid composition of the experimental diets[1]

	Diet[3]		
Fatty acid[2]	PA	OA	LA
$C_{14:0}$	.67	1.94	.98
$C_{16:0}$	71.40	10.28	6.59
$C_{18:0}$	1.60	2.05	1.34
$C_{18:1(n-9)}$	7.72	52.37	24.06
$C_{18:2(n-6)}$	16.35	25.68	61.00
$C_{18:3(n-3)}$	1.54	2.44	1.81

[1]As a percentage of total methyl esters.
[2]Fatty acids are designated by the carbon chain length, number of double bonds and the position of the first double bond from the methyl end of the molecule.
[3]Diet PA = 97% basal mix plus 3% palmitic acid; Diet OA = 97% basal mix plus 3% oleic acid; Diet LA = 97% basal mix plus 3% linoleic acid.

TABLE 2.3. Effect of palmitic, oleic, and linoleic acids on the reproductive performance of Japanese quail hens from 8 to 32 wk of age (24-wk production period)

Measurement[1]	Diet[2] PA	OA	LA	SEM
Feed consumption[3]	28.40[a]	26.20[b]	26.40[b]	.08
Egg production, %	96.00[a]	93.20[b]	87.50[c]	.50
Egg weight, g	11.30[b]	11.30[b]	11.50[a]	.02
Estimated weight of egg produced[3]	10.85[a]	10.53[b]	10.06[c]	.05
Fertility, %	96.70	96.30	95.70	.40
Embryonic mortality				
7 days, %	11.80[b]	13.10[b]	18.40[a]	.80
18 days, %	10.10	10.40	10.30	.60
Total, %	21.90[b]	23.50[b]	28.70[a]	1.02
Hatchability, %	78.10[a]	76.50[a]	71.30[b]	1.02
Estimated number of quail chicks produced, chicks per hen per period	122.00[a]	115.00[b]	100.00[c]	2.00
Quail weight at hatch, g[4]	7.73[ab]	7.62[b]	7.89[a]	.08

[a-c]Means within a row with no common superscripts differ significantly ($P<.05$).

[1]Values represent overall means of 24-wk production period of 20 quail hens per diet.

[2]Diet PA=97% basal mix plus 3% palmitic acid; Diet OA=97% basal mix plus 3% oleic acid; Diet LA=97% basal mix plus 3% linoleic acid.

[3]Grams per hen per day.

[4]Overall means of 10 measurements of 80 quail chicks per diet. Each measurement was recorded every second hatch up to the 20th hatch.

TABLE 2.4. Effect of palmitic, oleic, and linoleic acids on the fatty acid composition of egg yolk lipids of Japanese quail[1].

	Diet[3]			
Fatty acid[2]	PA	OA	LA	SEM
$C_{14:0}$	.78[ab,4]	.71[b]	.86[a]	.04
$C_{16:0}$	30.63[b]	27.57[c]	33.84[a]	.49
$C_{16:1(n-7)}$	8.26[a]	6.98[b]	6.12[c]	.44
$C_{18:0}$	8.32[b]	8.29[b]	11.04[a]	.28
$C_{18:1(n-9)}$	43.00[b]	47.64[a]	31.84[c]	.66
$C_{18:2(n-6)}$	7.38[b]	7.13[b]	14.57[a]	.28
$C_{20:4(n-6)}$	1.63	1.68	1.73	.08
$C_{18:0}$:$C_{18:1(n-9)}$[5]	.19[b]	.17[b]	.35[a]	.02

[a-c]Means within a row with no common superscripts differ significantly ($P<.05$).
[1]As percentage of total methyl esters.
[2]Fatty acids are designed by the carbon chain length, number of double bonds, and the position of the first double bond from the methyl end of the molecule.
[3]Diet PA = 97% basal mix plus 3% palmitic acid; Diet OA = 97% basal mix plus 3% oleic acid; Diet LA = 97% basal mix plus 3% linoleic acid.
[4]Values are means of two pooled yolks of 10 quail per diet.
[5]Stearic to oleic acid ratio.

TABLE 2.5. Effect of palmitic, oleic, and linoleic acids on the weight, dry matter, total lipid, and cholesterol content of egg yolks of Japanese quail

Measurement[1]	Diet[2]			SEM
	PA	OA	LA	
Yolk weight, g	3.49	3.51	3.65	.07
Dry matter, %	54.10	54.62	54.42	.26
Total lipid, %	36.89	37.56	36.97	.45
Cholesterol, %[3]	12.90	13.20	12.80	.38

[1]Values are means of 20 individual yolks per diet.
[2]Diet PA = 97% basal mix plus 3% palmitic acid; Diet OA = 97% basal mix plus 3% oleic acid; Diet LA = 97% basal mix plus 3% linoleic acid.
[3]Percentage of total lipids.

TABLE 2.6. Effect of palmitic, oleic, and linoleic acids on the final body weight, liver weight, and total plasma phosphorus of Japanese quail[1]

	Diet[3]			
Measurement[2]	PA	OA	LA	SEM
Final body weight, g	209.40	218.70	209.00	5.90
Liver weight, g	6.42	6.42	6.97	.33
Liver weight, %BW	3.07	2.99	3.32	.15
Total plasma phoshorus, μg/mL	620.38[a]	554.61[ab]	511.26[b]	27.84

[a,b]Means within a row with no common letters differ significantly (P<.05).
[1]At 32 wk of age.
[2]Values are means of 16 observations per diet.
[3]Diet PA = 97% basal mix plus 3% palmitic acid; Diet OA = 97% basal mix plus 3% oleic acid; Diet LA = 97% basal mix plus 3% linoleic acid.

TABLE 2.7. Effect of palmitic, oleic, and linoleic acids on the major fatty acids of blood plasma lipids of Japanese quail[1].

	Diet[3]			
Fatty acid[2]	PA	OA	LA	SEM
$C_{16:0}$	31.25[a4]	26.54[b]	31.97[a]	.302
$C_{16:1(n-7)}$	6.23[a]	5.54[a]	4.14[b]	.395
$C_{18:0}$	9.62[b]	9.79[b]	12.24[a]	.419
$C_{18:1(n-9)}$	39.26[b]	44.90[a]	29.24[c]	.438
$C_{18:2(n-6)}$	7.94[b]	7.64[b]	16.65[a]	.237
$C_{18:3(n-3)}$	.28[a]	.22[b]	.31[a]	.020
$C_{20:4(n-6)}$	2.60	2.46	2.49	.168

[a-c]Means within a row with no common superscripts differ significantly ($P<.05$).

[1]As percentage of total methyl esters. Values do not add to 100% because minor percentages were not included.

[2]Fatty acids are designed by the carbon chain length, number of double bonds, and the position of the first double bond from the methyl end of the molecule.

[3]Diet PA = 97% basal mix plus 3% palmitic acid; Diet OA = 97% basal mix plus 3% oleic acid; Diet LA = 97% basal mix plus 3% linoleic acid.

[4]Values are means of eight observations per diet.

TABLE 2.8. Effect of palmitic, oleic, and linoleic acids on the major fatty acids of liver lipids of Japanese quail[1].

	Diet[3]			
Fatty acid[2]	PA	OA	LA	SEM
$C_{16:0}$	31.56[a4]	27.17[b]	32.11[a]	.378
$C_{16:1(n-7)}$	7.00	6.48	5.98	.439
$C_{18:0}$	9.18	9.23	10.96	.534
$C_{18:1(n-9)}$	38.84[b]	47.33[a]	34.13[c]	1.075
$C_{18:2(n-6)}$	6.06[b]	4.92[b]	12.22[a]	.473
$C_{18:3(n-3)}$	.18[a]	.12[b]	.18[a]	.019
$C_{20:4(n-6)}$	3.78[a]	2.28[b]	1.68[b]	.272

[a-c]Means within a row with no common superscripts differ significantly ($P<.05$).

[1]As percentage of total methyl esters. Values do not add to 100% because minor percentages were not included.

[2]Fatty acids are designed by the carbon chain length, number of double bonds, and the position of the first double bond from the methyl end of the molecule.

[3]Diet PA=97% basal mix plus 3% palmitic acid; Diet OA = 97% basal mix plus 3% oleic acid; Diet LA= 97% basal mix plus 3% linoleic acid.

[4]Values are means of eight observations per diet.

3. EFFECT OF FEEDING PALMITIC, OLEIC, AND LINOLEIC ACIDS TO JAPANESE QUAIL HENS (Coturnix coturnix japonica). 2. MATERNAL DIETS AND STAGE OF DEVELOPMENT ON THE LIPID METABOLISM OF QUAIL EMBRYOS[1]

C. VILCHEZ, S. P. TOUCHBURN, E. R. CHAVEZ, and C. W. CHAN

Department of Animal Science, Macdonald College of McGill University, and McGill Nutrition and Food Science Centre

Ste Anne de Bellevue, Québec, H9X 1C0, Canada

ABSTRACT An experiment was conducted to evaluate the effects of diets containing 3% of either palmitic acid (Diet PA), oleic acid (Diet OA) or linoleic acid (Diet LA) and three stages of development (Days 11, 13 and 15 of incubation) on 1) weights of yolk plus yolk sac membrane (TY), yolk sac membrane (YSM), yolk, liver, and extrahepatic tissue (ET); and 2) the fatty acid composition of phospholipid, triglyceride, and cholesterol ester fractions of YSM and ET of quail embryos. Embryos from birds fed Diet LA had the highest ($P<.05$) weights of TY and yolk followed by those from birds fed Diet OA and Diet PA. The weight of ET was the highest ($P<.05$) in embryos from birds fed fed Diet PA followed by those from birds

[1] As published, 1992 Poultry Science 71:1032-1042.

fed Diet LA and Diet OA. The weights of YSM and liver were not affected by the maternal diet ($P > .05$).The weight of TY decreased whereas the weights of liver and ET increased as incubation progressed ($P < .05$).The weight of YSM was maximum at Day 13 of incubation. In the phospholipid, triglyceride, and cholesterol ester fractions of both YSM and ET of embryos, the fatty acid present at the highest level, except palmitic acid, was the one added to the maternal diet. In phospholipids of both YSM and ET the oleic acid content decreased and linoleic acid increased as incubation progressed. In triglycerides and cholesterol esters of both YSM and ET, the levels of palmitic acid increased, but oleic and linoleic acids decreased with advancing embryonic development. The results of the present study indicate that embryos from birds fed Diet PA mobilize more yolk material and produce heavier ET than embryos from birds fed Diet OA and Diet LA. The fatty acid profiles of phospholipid, triglyceride, and cholesterol esters of embryonic tissues are consistently influenced by dietary fatty acid and the stage of development.

(Key words: quail, maternal diet, incubation, embryo, lipids)

INTRODUCTION

Lipid metabolism of chick embryos has been the subject of numerous studies (Donaldson, 1981; Noble, 1987b). It is recognized that most of the yolk lipid content is mobilized and absorbed into the embryonic tissue of the chick and that these events take place mainly in the last 7 days of incubation (Romanoff, 1960; Noble and

Moore, 1964). Egg yolk fatty acids were found to be altered slightly at 0, 8, 12, 16, and 20 days of incubation. It was observed, however, that the fatty acids of embryonic lipids were more saturated than those of the lipids of yolk from which the embryos developed (Donaldson, 1964). Noble and Moore (1967) reported that during the period of intense yolk uptake, there were increasing levels of cholesterol esters in the yolk sac membrane (YSM). Noble et al. (1984) found that the accumulation of cholesterol esters, mainly cholesterol oleate, that occurred within the liver during embryo development arose from synthesis in YSM. The latter suggests that cholesterol ester of oleic acid plays an important role in lipid transport and assimilation from the yolk during embryo development (Noble, 1987b).

In spite of the numerous studies on lipid metabolism of chick embryos, very few reports deal with the effect of the fatty acid composition of the maternal diet and stage of incubation on embryonic lipid metabolism. Studies with chick embryos (Donaldson, 1967) and turkey embryos (Couch et al., 1973) demonstrated that both the fatty acid composition of the maternal diet and the stage of development affect the fatty acid composition of the embryo.

Previous studies with quail hens showed that the fatty acid composition of diets significantly influenced the fatty acid composition of the egg yolk lipids (Vilchez et al., 1990a,b). On the other hand, it has also been suggested that the fatty acid composition of the egg yolk lipids affects the hatchability of fertile eggs in poultry

(Donaldson and Fites, 1970; Tullett, 1990; Vilchez et al., 1990a,b). However, the effect of yolk lipid composition on the fatty acid profile of the individual lipid classes during embryonic development has not been investigated.

The objectives of this experiment were to determine in Japanese quail the effects of maternal diet and stage of development on 1) changes in weights of yolk, YSM, and embryonic tissues; and 2) the changes in the fatty acid profiles of phospholipid, triglyceride, and cholesterol esters of the embryonic tissues and YSM during the last week of incubation.

MATERIALS AND METHODS

Sixty Japanese quail hens were housed in individual cages and fed diets containing 97% semipurified low-fat basal mix plus 3% of either palmitic acid (Diet PA), oleic acid (Diet OA), or linoleic acid (Diet LA). The composition and the fatty acid analyses of the diets were previously described (Vilchez et al., 1991). Briefly, the low-fat basal mix contained ground yellow corn (24%), corn starch (29.8%), isolated soybean protein (26%), alfalfa meal (10%), calcium carbonate (4.8%), calcium phosphate (3.6%), vitamin-mineral premix (1%), choline chloride (.3%), DL-Methionine (.3%) and sodium chloride (.2%). The proportions of palmitic, oleic and linoleic acids in Diets PA, OA, and LA were, respectively, 71.4%, 52.4%, and 61% of the total methyl esters.

Thirty-two eggs from each dietary group were removed from the incubator after 11, 13, and 15 days of incubation. The eggs were then broken and the yolk sac and its contents carefully excised from each embryo. The weights of both the yolk sac plus its contents (TY) and the embryo were recorded separately. An incision was made in the YSM, the liquid yolk removed, and the YSM repeatedly washed with .9% saline solution until the yolk material had been completely removed. The wet weight of the YSM was recorded. The weight of the yolk was obtained by substracting the weight of the YSM from the weight of TY. The liver was excised and its weight, and that of the extrahepatic tissue (ET) were recorded separately. Eight YSM and eight (ET) were pooled before analysis, resulting in four YSM and four ET samples per dietary treatment and stage of development.

The lipids from YSM and ET were extracted with chloroform:methanol (2:1, vol/vol) solution (Folch et al., 1957) modified by the addition of .01% butylated hydroxytoluene. Aliquots of the lipid extracts (35 to 45 mg) were fractionated into phospholipids, unesterified fatty acids, triglycerides and cholesterol esters on thin layer chromatoplates of silica gel GF (20 x 20 cm, .5 mm)[2] using a solvent system of hexane:diethyl ether:acetic acid (80:20:2, vol/vol/vol). Because the phospholipid and monoglyceride fractions were not well separated with this solvent system, the complete separation was achieved by developing the same plate (after being dried under nitrogen for 15 min) in the same direction using a solvent system of

[2]Analtech Inc., Newark, DE 19711.

acetone:acetic acid:water (100:2:1, vol/vol/vol) just up to 2 cm from the origin. The plates were then removed from the developing tank and dried again under nitrogen for about 15 min. Standards of each lipid class[3] were processed in the same way. Following visualization under iodine vapor, the areas corresponding to phospholipids, triglycerides, and cholesterol esters were scraped into culture tubes (16 x 125 mm), and the methyl esters of the fatty acids were prepared by a modification of the method reported by Sukhija and Palmquist (1988).

One milliliter of benzene and 3 mL of freshly prepared 5% methanolic HCl (prepared by slowly adding 10 mL of acetyl chloride to 100 mL of anhydrous methanol) were added to each of the tubes containing the silica gel GF with the lipid fraction scraped from the TLC. After being tightly capped, the culture tubes were swirled vigorously for 30 s at low speed so that the material remained 2 to 3 cm from the bottom. The tightly capped tubes were heated for 2 h in a water bath at 70 C. After the contents were cooled to room temperature, 5 mL of 6% K_2CO_3 were added, followed by .5 mL of benzene. The contents of the tube were swirled vigorously for 30 s and then centrifuged for 10 min at 400 x g. The benzene (upper) layer containing methyl esters was transfered with a Pasteur pipet to a 1 mL autosampler vial for analysis by gas-liquid chromatography (GLC).

Fatty acid analysis was done on a Hewlett-Packard 5711A gas-liquid

[3]Sigma Chemical Co., St. Louis, MO 63178-9916.

chromatograph fitted with automatic sampler 7671A, integrator 3380S, and FID detector.[4] Separation of fatty acid methyl esters was achieved using a Supelcowax 10 fused silica capillary column (30 m x .53 mm ID, 1.0 µm film).[5] Fatty acid methyl esters were identified by comparison of retention times with standards[6] and expressed as percentages of total methyl esters.

Statistical Analisis

The data were analyzed in a 3 x 3 factorial arrangement of treatments (three dietary treatments and three stages of development) and subjected to analysis of variance using the General Linear Models (GLM) procedure (SAS Institute, 1986). Significant differences of means were tested using Tukey's Studentized Range (HSD) test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Data on the effects of maternal diet and stage of development on the weights of TY, YSM, yolk, liver, and ET are presented in Table 3.1. Maternal diet

---

[4]Hewlett-Packard Co., Avondale, PA 19311.

[5]Supelco, Inc., Bellefonte, PA 16823.

[6]Nu-Ckek-Prep; Elysian, MN 56028.

significantly ($P<.05$) affected the weight of TY. The highest weight corresponded to the embryos from birds fed Diet LA, followed by those fed Diet OA, and Diet PA. Because there was no statistical difference ($P>.05$) in the weights of YSM among treatments, the difference observed must be due to the difference in the amount of yolk used by the embryo during development. The amount of yolk in the group fed Diet LA was significantly ($P<.05$) higher than that of the other treatments (Table 3.1). Feeding either Diet PA or Diet OA resulted in high levels of $C_{18:1(n-9)}$ in the YSM lipids as compared with those fed Diet LA (Table 3.2). However, dry matter, yolk weight, or the total lipid of the yolk of unincubated eggs was not altered (Vilchez et al., 1991). On the other hand, Noble et al. (1984) demonstrated the significance of the YSM in synthesizing cholesterol esters, particularly cholesterol oleate. Recently, Noble (1987) indicated the role of the cholesterol ester of oleic acid on lipid transport and assimilation from the yolk during embryonic development. Therefore, the low weight of TY observed with both Diet PA and Diet OA in the present study reflects a more rapid transport of yolk material to the embryos in these two groups than that observed with Diet LA. Less yolk was consumed during development by embryos from hens fed Diet LA.

A small but significant difference in he weights of TY between Diet PA and Diet OA was also observed (Table 3.1). Explanation for this difference is not readily apparent. It is possible that the level of $C_{18:1(n-9)}$ in the egg yolk is critical or that palmitic acid itself has some physiological effects that cannot be elucidated from this

experiment.

The weight of ET of the embryos was also affected by maternal diet (Table 3.1). The ET of embryos from birds fed Diet PA was heaviest followed by those fed Diet LA and Diet OA. This result would indicate that embryos from birds fed Diet PA deposited more ET than the other treatment groups. The heavy weight of ET in the group fed Diet PA may be the result of a better assimilation of the yolk material as discussed above. However, the low weight of ET of embryos of birds fed Diet OA can not be explained on the same basis. Other factors may be involved in this response. The low weight of ET of embryos from birds fed Diet LA is surprising because it was expected that embryos from birds fed an adequate or high level of $C_{18:2(n-6)}$ would be heavier than those fed a marginal or deficient level of this essential fatty acid (Menge and Richardson, 1968). To the authors' knowledge, this is the first report to indicate that the transfer of yolk material to the embryo and the weight of ET are influenced by the type of fatty acid fed to quail breeding hens.

The weights of TY and yolk significantly decreased ($P < .05$) as incubation progressed (Table 3.1). This was due to the increased assimilation of yolk material as development proceeded (Noble and Moore, 1967). Likewise, the weight of YSM significantly increased from Day 11 to Day 13, and decreased ($P < .05$) by Day 15 of incubation. It has been previously shown in chick embryos that the weight of YSM increased progressively during the first 16 days of incubation, but then decreased

markedly during the period between Day 16 and Day 21 (unpublished data). Noble and Shand (1985) reported that a large increase in total lipid content of YSM occurred between Day 15 and Day 17, but it declined between Day 17 and Day 19 of incubation.

The level of $C_{16:1(n-7)}$ in YSM phospholipids of embryos fed Diet PA was higher ($P<.05$) than in those fed Diet LA (Table 3.2). Feeding Diet OA produced YSM phospholipids with the highest and the lowest levels of $C_{18:1(n-9)}$ and $C_{16:0}$, respectively. On the contrary, embryos from birds fed Diet LA showed YSM phospholipids with the highest levels of $C_{16:0}$ and $C_{18:2(n-6)}$, but with the lowest levels of $C_{18:1(n-9)}$ and $C_{22:6(n-3)}$ as compared with the other two treatments.

The $C_{18:1(n-9)}$ content significantly decreased whereas the $C_{20:4(n-6)}$ increased from Day 11 to Day 15 of incubation (Table 3.2). The level of $C_{16:0}$ was higher at Day 11, but decreased by Day 13, and remained constant by Day 15. The $C_{18:2(n-6)}$ increased from Day 11 to Day 13 and remained constant by Day 15 of incubation. With exceptions of $C_{16:1(n-7)}$ and $C_{18:1(n-9)}$, the results of the present study agree with those reported in developing chick embryos (Noble and Moore, 1967). Noble and Moore (1967) found that $C_{16:1(n-7)}$ and $C_{18:1(n-9)}$ in YSM phospholipids increased in concentration as development proceeded. Analysis of variance showed significant interactions of maternal diet and embryonic development for $C_{16:0}$, $C_{18:1(n-9)}$, $C_{18:2(n-6)}$, and $C_{22:6(n-3)}$ in YSM phospholipids.

Embryos from birds fed Diet PA showed ET phospholipids with levels of $C_{16:0}$, $C_{16:1(n-7)}$, $C_{18:0}$, $C_{18:2(n-6)}$, $C_{20:4(n-6)}$, and $C_{22:6(n-3)}$ similar ($P > .05$) to those fed Diet OA (Table 3.3). However, these levels were either lower ($C_{16:0}$, $C_{18:0}$, $C_{18:2(n-6)}$) or higher ($C_{22:6(n-3)}$) when compared with those fed Diet LA. Feeding Diet OA produced ET phospholipids with the highest level of $C_{18:1(n-9)}$ ($P < .05$). This increase was accompanied by a significant decrease in the level of $C_{16:0}$. Similar observations were reported in chick embryos from hens fed a diet containing 10% of oleic acid (Donaldson, 1967). On the other hand, feeding Diet LA resulted in ET phospholipids with the lowest levels of $C_{18:1(n-9)}$ and $C_{22:6(n-3)}$. The level of $C_{20:4(n-6)}$ was higher in those fed Diet LA as compared with those fed Diet OA, but it was not different than in those fed Diet PA.

The levels of $C_{16:1(n-7)}$ and $C_{18:1(n-9)}$ decreased and the level of $C_{18:0}$ increased in ET phospholipids as incubation progressed (Table 3.3). The $C_{16:0}$ content was not different ($P > .05$) between Day 11 and Day 13 of incubation, but decreased by Day 15. The $C_{18:2(n-6)}$ content was lower ($P < .05$) at Day 11 than at Day 13, and this level did not change by Day 15 of incubation. Data on the fatty acid profiles of chick embryos (Noble and Moore, 1964), whole embryo phospholipids (Donaldson, 1967), and turkey embryo polar lipids (Couch et al., 1973) showed similar trends to those observed in the current experiment. Analysis of variance showed no significant interaction between maternal diet and embryonic developmet (Table 3.3).

For all diets and throughout development, the fatty acid composition of YSM phospholipids was different from that of ET phospholipids. The YSM phospholipids (Table 3.2) contained more $C_{16:1(n-7)}$, $C_{18:0}$, $C_{18:1(n-9)}$ and $C_{18:2(n-6)}$, and less $C_{16:0}$, $C_{20:4(n-6)}$ and $C_{22:6(n-3)}$ than did ET phospholipids (Table 3.3). A comparison between the fatty acid compositions of YSM phospholipids (Noble and Moore, 1967) and ET phospholipids (Noble and Moore, 1964) of developing chick embryos shows similar differences in fatty acid composition of the phospholipids between these two tissues, which agree with the results of the present study with developing quail embryos.

The fatty acid composition of YSM triglycerides of embryos from birds fed Diet PA showed the highest level of $C_{16:1(n-7)}$ (Table 3.4). The $C_{16:0}$ and $C_{18:1(n-9)}$ was the lowest and the highest ($P<.05$), respectively, in YSM triglycerides of embryos from birds fed Diet OA. The $C_{16:0}$, $C_{18:0}$, $C_{18:2(n-6)}$ and $C_{20:4(n-6)}$ were the highest ($P<.05$) in YSM triglycerides of embryos from birds fed Diet LA as compared with the other two groups.

The level of $C_{16:0}$ decreased and the level of $C_{18:2(n-6)}$ increased in YSM triglycerides from Day 11 to Day 15 of incubation (Table 3.4). There were no significant differences ($P>.05$) in the levels of $C_{16:1(n-7)}$ and $C_{18:1(n-9)}$ between Day 11 and Day 13, but the levels of these fatty acid decreased and increased, respectively, by Day 15 of incubation. The $C_{20:4(n-6)}$ content increased from Day 11 to Day 13 and remained constant by Day 15 of incubation. With the exception of $C_{20:4(n-6)}$, the fatty

acid composition of YSM triglycerides of developing quail embryos follows the pattern reported by Noble and Moore (1967) in YSM triglycerides of developing chick embryos.

Feeding Diet PA resulted in a significantly higher ($P<.05$) level of $C_{16:1(n-7)}$ in ET triglycerides than feeding either Diet OA or Diet LA (Table 3.5). The ET triglycerides of embryos fed Diet OA had the highest and lowest ($P<.05$) contents of $C_{18:1(n-9)}$ and $C_{16:0}$, respectively. Similar observations were reported in whole chick embryo triglycerides by Donaldson (1967). The ET triglycerides of embryos from birds fed Diet LA showed the highest ($P<.05$) levels of $C_{18:2(n-6)}$, $C_{18:0}$, and $C_{16:0}$ as compared to the other two dietary groups. The $C_{20:4(n-6)}$ content was not different ($P>.05$) between Diet OA and Diet LA, but both were higher than in those fed Diet PA.

The $C_{16:0}$ and $C_{22:6(n-3)}$ contents decreased whereas the $C_{18:2(n-6)}$ increased in ET triglycerides as development proceeded (Table 3.5). The levels of $C_{16:1(n-7)}$ and $C_{20:4(n-6)}$ were not different ($P>.05$) between Day 11 and Day 13 Of incubation, but they decreased by Day 15. The level of $C_{18:0}$ was different at Day 13 and Day 15, but both were not different than that at Day 11. With the exception of $C_{18:0}$, the fatty acid profiles of ET triglycerides of developing quail embryos agree with the results reported by Noble and Moore (1964) and Donaldson (1967) in triglycerides of ET and whole chick embryos, respectively. Analysis of variance indicated significant diet

by development interactions for $C_{16:1(n-7)}$, $C_{18:2(n-6)}$, and $C_{22:6(n-3)}$. Thus, $C_{16:1(n-7)}$ content in ET triglycerides decreased with increasing age of the embryo more rapidly when the hens were fed Diet PA than when they were fed Diet OA or Diet LA. In contrast, the $C_{18:2(n-6)}$ content increased more rapidly and the $C_{22:6(n-3)}$ decreased more rapidly when the hens were fed Diet LA than when they were fed Diet PA or Diet OA.

For all treatments, the fatty acid composition of YSM triglycerides (Table 3.4) showed considerably higher contents of $C_{18:1(n-9)}$, but lower levels of $C_{16:0}$, $C_{18:2(n-6)}$, and $C_{22:6(n-3)}$ than those observed in ET triglycerides (Table 3.5). The levels of $C_{16:1(n-7)}$ and $C_{18:0}$ tended to be similar in the triglyceride fraction of both tissues. Contrary to ET triglycerides, only negligible amounts of $C_{22:6(n-3)}$ were observed in YSM triglycerides of developing quail embryos. A similar observation was reported in chick embryos (Noble and Moore, 1967).

The $C_{16:1(n-7)}$ content was the highest ($P<.05$) in YSM cholesterol esters of embryos from birds fed Diet PA (Table 3.6). The highest level of $C_{18:1(n-9)}$ and the lowest levels of $C_{16:1(n-7)}$ and $C_{18:0}$ were observed in YSM cholesterol esters of embryos from birds fed Diet OA. Likewise, feeding Diet LA resulted in YSM cholesterol esters with the highest levels $C_{18:2(n-6)}$ and $C_{18:0}$, and the lowest level of $C_{18:1(n-9)}$.

The $C_{16:0}$ and $C_{18:0}$ contents decreased and the $C_{18:1(n-9)}$ content increased ($P<.05$) in YSM cholesterol esters as incubation progressed (Table 3.6). The level of $C_{18:2(n-6)}$ increased from Day 11 to Day 13, and remained constant by Day 15 of incubation. Noble and Moore (1967) also reported a significant decrease in $C_{16:0}$ and $C_{18:0}$ but the level of $C_{18:1(n-9)}$ increased significantly in YSM cholesterol esters in developing chick embryos. The ability of YSM to synthesize cholesterol esters, in particular cholesteryl oleate, in developing chick embryos was previously reported (Noble et al., 1984). Analysis of variance showed a significant diet by development interaction for $C_{18:2(n-6)}$ (Table 3.6).

The level of $C_{16:1(n-7)}$ was the highest in ET cholesterol esters of embryos from birds fed Diet PA (Table 3.7). Feeding Diet OA produced ET cholesterol esters with the highest ($P<.05$) level of $C_{18:1(n-9)}$, but the lowest levels of $C_{16:0}$ and $C_{18:0}$ compared to those in the other treatments. The ET cholesterol esters of embryos from birds fed Diet LA showed the highest ($P<.05$) levels of $C_{18:2(n-6)}$ and $C_{18:0}$, but the lowest ($P<.05$) level of $C_{18:1(n-9)}$. The $C_{20:4(n-6)}$ content was higher ($P<.05$) in ET cholesterol esters of embryos from birds fed Diet LA than in those fed Diet PA, but it was not different ($P>.05$) from those fed Diet OA.

The $C_{16:1(n-7)}$ and $C_{18:1(n-9)}$ contents significantly decreased ($P<.05$) as incubation progressed (Table 3.7). There were no significant differences ($P>.05$) in the levels of $C_{18:0}$ and $C_{20:4(n-6)}$ embryos of 11 and 13 days of incubation, but they were lower

($P < .05$) than those of 15 days of incubation. The $C_{18:2(n-6)}$ content decreased ($P < .05$) from Day 11 to Day 13, but remained constant by Day 15 of incubation. Significant increases in the $C_{18:0}$ and $C_{18:1(n-9)}$ contents as well as a decrease in the $C_{16:1(n-7)}$ level were reported by Noble and Moore (1964) for ET cholesterol esters in developing chick embryos. The patterns for $C_{16:0}$, $C_{18:2(n-6)}$ and $C_{20:4(n-6)}$ observed in the current study do not agree with those reported by Noble and Moore (1964). Analysis of variance showed significant diet by development interactions for $C_{16:0}$ and $C_{18:1(n-9)}$ (Table 3.7). Thus, the $C_{16:0}$ content in ET cholesterol esters increased more rapidly with increasing age of the embryo when the hens were fed Diet PA than when they were fed Diet OA or Diet LA. In contrast, the $C_{18:1(n-9)}$ content decreased less rapidly when the hens were fed Diet OA than when they were fed Diet PA or Diet LA.

At all stages of development and for all diets, the fatty acid composition of YSM cholesterol esters was considerably different from that of ET cholesterol esters. The YSM cholesterol esters (Table 3.6) contained more $C_{16:1(n-7)}$ and $C_{18:1(n-9)}$, and significantly less $C_{16:0}$ and $C_{18:2(n-6)}$ than did ET cholesterol esters (Table 3.7). With the exception of Day 15 of incubation, a similar pattern was observed in the $C_{18:0}$ content. Furthermore, a comparison of the data reported for YSM cholesterol esters (Noble and Moore, 1967) and ET cholesterol esters (Noble and Moore, 1964) in developing chick embryos also shows the difference in fatty acid profiles between YSM and ET as observed in the present study with developing quail embryos.

ACKNOWLEDGMENTS

This research was supported by the Ministère de l'Agriculture des Pêcheries et de l'Alimentation du Québec. The technical assistance of Suzanne Drouin is gratefully acknowledged.

TABLE 3.1. Effect of maternal diet (MD) and stage of development (DEV) on the weights of yolk plus yolk sac membrane (TY), yolk sac membrane (YSM), yolk, liver, and extrahepatic tissues (ET) of quail embryos.

MD[1]	DEV	TY	YSM	Yolk	Liver	ET
	days			(g)		
PA	11	2.78[2]	.74	2.04	.061	2.71
OA	11	2.88	.71	2.17	.057	2.58
LA	11	2.98	.70	2.28	.058	2.59
PA	13	2.46	.87	1.59	.107	4.31
OA	13	2.71	.83	1.88	.098	4.05
LA	13	2.82	.78	2.04	.105	3.95
PA	15	1.74	.57	1.17	.149	6.24
OA	15	1.83	.60	1.23	.144	5.76
LA	15	1.96	.61	1.35	.150	6.14
mean ± SEM		2.46 ±.06	.71±.02	1.75 ±.06	.103±.003	4.26 ±.07
Source of variation(df)				Probabilities		
MD (2)		<.01	.156	<.01	.031	<.01
DEV (2)		<.01	<.01	<.01	<.01	<.01
MD by DEV (4)		.647	.025	.2	.717	<.01
				Combined effects of MD and DEV		
MD						
PA		2.33[c]	.73	1.60[c]	.106	4.42[a]
OA		2.47[b]	.71	1.75[b]	.100	4.13[c]
LA		2.59[a]	.70	1.89[a]	.104	4.23[b]
DEV						
11		2.88[a]	.72[b]	2.16[a]	.059[c]	2.63[c]
13		2.66[b]	.83[a]	1.83[b]	.103[b]	4.10[b]
15		1.84[c]	.59[c]	1.25[c]	.148[a]	6.05[a]

[a-c]Means within a column and variable with no common superscripts differ significantly ($p<.05$).

[1]Diets containing 97% basal mix plus 3% of: Diet PA, palmitic acid; Diet OA, oleic acid; and Diet LA, linoleic acid.

[2]Values are means of 32 observations.

TABLE 3.2. Major phospholipid fatty acids of yolk sac membrane of quail embryos as affected by maternal diet (MD) and stage of developement (DEV)

MD[1]	DEV	Fatty acid						
		$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:4}$	$C_{22:6}$
	days	(% of total methyl esters)						
PA	11	26.6[2]	3.0	19.8	32.9	9.0	4.8	.93
OA	11	26.5	2.4	20.0	35.7	8.3	3.6	.82
LA	11	30.7	2.3	21.7	26.7	11.1	3.7	.44
PA	13	24.5	2.8	19.6	31.5	9.7	6.1	1.22
OA	13	21.4	2.1	21.0	32.7	10.1	7.2	1.44
LA	13	25.5	2.2	20.1	25.2	14.8	5.2	.90
PA	15	24.4	2.4	23.0	29.8	9.2	8.1	.74
OA	15	20.8	2.0	23.1	32.3	8.8	7.6	.82
LA	15	24.7	1.7	23.9	21.0	15.1	7.6	.70
mean ± SEM		25.0 ± .41	2.3 ± .14	21.4 ± .55	29.8 ± .40	10.7 ± .29	6.0 ± .61	.89 ± .06
Source of variation (df)		Probabilities						
MD (2)		<.01	<.01	.066	<.01	<.01	.21	<.01
DEV (2)		<.01	<.01	<.01	<.01	<.01	<.01	<.01
MD by DEV (4)		<.01	.904	.224	<.01	<.01	.263	<.01
		Combined effects of MD and DEV						
MD								
PA		25.1[b]	2.7[a]	20.8	31.4[b]	9.3[b]	6.3	1.0
OA		22.9[c]	2.2[b]	21.3	33.6[a]	9.1[b]	6.2	1.0[a]
LA		27.0[a]	2.1[b]	21.9	24.3[c]	13.7[a]	5.5	.7[b]
DEV								
11		27.9[a]	2.6[a]	20.5[b]	31.8[a]	9.5[b]	4.0[c]	.7[b]
13		23.8[b]	2.4[a]	20.2[b]	29.8[b]	11.5[a]	6.2[b]	1.2[a]
15		23.3[b]	2.0[b]	23.3[a]	27.7[c]	11.0[a]	7.8[a]	.8[b]

[a-c]Means within a column and variable with no common superscripts differ significantly ($P<.05$).

[1] Diets containing 97% basal mix plus 3% of: Diet PA, palmitic acid: Diet OA, oleic acid; and Diet LA, linoleic acid.

[2] Values are means of four observations (eight pooled yolk sac membranes per observation).

TABLE 3.3. Major phospholipid fatty acids of extrahepatic tissue of quail embryos as affected by maternal diet (MD) and stage of developement (DEV)

MD[1]	DEV	Fatty acid						
		$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:4}$	$C_{22:6}$
	days	(% of total methyl esters)						
PA	11	27.7[2]	2.5	15.0	20.8	5.0	13.5	5.8
OA	11	27.0	2.4	15.0	21.6	5.7	13.4	6.6
LA	11	27.8	2.2	16.0	17.4	8.71	14.5	3.8
PA	13	27.5	1.6	18.5	18.8	6.8	13.5	5.42
OA	13	26.6	1.6	18.0	19.9	6.7	13.3	5.64
LA	13	28.4	1.6	18.4	15.4	9.6	13.7	3.5
PA	15	26.0	1.3	18.8	18.2	6.7	12.8	6.4
OA	15	26.0	1.3	18.8	18.7	6.3	12.7	7.0
LA	15	27.6	1.2	19.8	14.1	8.7	13.0	4.2
mean ± SEM		27.2 ± .33	1.7 ± .07	17.6 ± .27	18.3 ± .22	7.1 ± .32	13.4± .30	5.4 ± .28
Source of variation (df)		Probabilities						
MD (2)		<.01	0.061	<.01	<.01	<.01	.034	<.01
DEV (2)		<.01	<.01	<.01	<.01	<.01	<.01	<.01
MD by DEV (4)		.179	.395	.302	.388	.156	.612	.625
		Combined effects of MD and DEV						
MD								
PA		27.0[b]	1.8[a]	17.5[b]	19.2[b]	6.2[b]	13.3[ab]	5.9[a]
OA		26.5[b]	1.7[ab]	17.3[b]	20.1[a]	6.2[b]	13.1[b]	6.4[a]
LA		27.9[a]	1.6[b]	18.1[a]	15.6[c]	9.0[a]	13.8[a]	3.8[b]
DEV								
11		27.5[a]	2.4[a]	15.4[c]	19.9[a]	6.5[b]	13.8[a]	5.3[ab]
13		27.5[a]	1.6[b]	18.3[b]	18.0[b]	7.7[a]	13.6[a]	4.9[b]
15		26.5[b]	1.2[c]	19.2[a]	16.9[c]	7.2[a]	12.8[b]	5.7[a]

[a-c]Means within a column and variable with no common superscripts differ significantly (P<.05).

[1]Diets containing 97% basal mix plus 3% of: Diet PA, palmitic acid; Diet OA, oleic acid; and Diet LA, linoleic acid.

[2]Values are means of four observations (eight pooled extrahepatic tissues per observation).

TABLE 3.4. Major triglyceride fatty acids of yolk sac membrane of quail embryos as affected by maternal diet (MD) and stage of developement (DEV)

MD[1]	DEV	Fatty acid					
		$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:4}$
	(days)	(% of total methyl esters)					
PA	11	30.9[2]	6.8	6.7	48.6	5.0	.21
OA	11	28.0	5.3	6.6	53.5	4.8	.18
LA	11	36.0	5.3	9.3	37.6	9.7	.20
PA	13	29.2	6.3	6.6	50.3	5.6	.26
OA	13	26.6	5.4	6.2	54.1	5.6	.23
LA	13	33.5	5.6	8.1	37.9	12.0	.34
PA	15	27.2	5.7	7.3	51.8	6.1	.22
OA	15	24.2	4.1	6.9	57.0	5.8	.21
LA	15	31.2	5.0	8.8	37.3	15.2	.31
mean ± SEM		29.6 ± .38	5.5 ± .22	7.4 ± .29	47.6 ± .60	7.8 ± .32	.24± .03
Source of variation (df)		Probabilities					
MD (2)		<.01	<.01	<.01	<.01	<.01	<.01
DEV (2)		<.01	<.01	<.01	<.01	<.01	<.01
MD by DEV (4)		.505	.090	.310	.013	<.01	<.01
		Combined effects of MD and DEV					
MD							
PA		29.1[b]	6.2[a]	6.9[b]	50.2[b]	5.6[b]	.23[b]
OA		26.3[c]	4.9[b]	6.6[b]	54.9[a]	5.4[b]	.21[b]
LA		33.6[a]	5.3[b]	8.8[a]	37.6[c]	12.3[a]	.28[a]
DEV							
11		31.6[a]	5.8[a]	7.5[ab]	46.6[b]	6.5[c]	.20[b]
13		29.8[b]	5.8[a]	7.0[b]	47.4[b]	7.7[b]	.27[a]
15		27.5[c]	4.9[b]	7.7[a]	48.7[a]	9.0[a]	.25[a]

[a-c]Means within a column and variable with no common superscripts differ significantly (P<.05).

[1]Diets containing 97% basal mix plus 3% of: Diet PA, palmitic acid; Diet OA, oleic acid; and Diet LA, linoleic acid.

[2]Values are means of four observations (eight pooled yolk sac membranes per observation).

TABLE 3.5. Major triglyceride fatty acids of extrahepatic tissue of quail embryos as affected by maternal diet (MD) and stage of developement (DEV)

MD[1]	DEV	Fatty acid						
		$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:4}$	$C_{22:6}$
	days	(% of total methyl esters)						
PA	11	34.1[2]	6.9	6.2	43.4	5.5	.75	.46
OA	11	31.6	5.2	7.1	46.6	5.5	.78	.31
LA	11	38.9	5.2	8.3	32.1	11.2	.72	.76
PA	13	31.8	6.4	6.55	45.9	5.7	.64	.27
OA	13	28.9	5.4	6.1	49.7	6.4	.80	.39
LA	13	36.0	5.6	7.9	34.0	13.0	.81	.19
PA	15	30.9	5.6	7.4	46.3	7.0	.56	.17
OA	15	27.6	4.7	7.3	50.6	6.8	.67	.21
LA	15	34.1	5.1	8.5	34.0	15.1	.66	.10
mean ± SEM		32.7 ± .25	5.6 ± .19	7.2 ± .27	42.5 ± .36	8.5 ± .20	.71 ± .03	.32 ± .04
Source of variation (df)		Probabilities						
MD (2)		<.01	0.061	<.01	<.01	<.01	.034	.179
DEV (2)		<.01	<.01	<.01	<.01	<.01	<.01	<.01
MD by DEV (4)		.075	.025	.130	.119	<.01	.056	<.01
		Combined effects of MD and DEV						
MD								
PA		32.3[b]	6.3[a]	6.7[b]	45.2[b]	6.1[b]	.65[b]	.30
OA		29.4[c]	5.1[b]	6.9[b]	49.0[a]	6.2[b]	.75[a]	.30
LA		36.3[a]	5.3[b]	8.2[a]	33.4[c]	13.1[a]	.73[a]	.35
DEV								
11		34.9[a]	5.8[a]	7.2[ab]	40.7[b]	7.4[c]	.75[a]	.51[a]
13		32.2[b]	5.8[a]	6.8[b]	42.2[a]	8.4[b]	.75[a]	.28[b]
15		30.9[c]	5.1[b]	7.7[a]	43.6[a]	9.7[a]	.63[b]	.16[c]

[a-c]Means within a column and variable with no common superscripts differ significantly (P<.05).

[1]Diets containing 97% basal mix plus 3% of: Diet PA, palmitic acid; Diet OA, oleic acid; and Diet LA, linoleic acid.

[2]Values are means of four observations (eight pooled extrahepatic tissues per observation).

TABLE 3.6. Major cholesterol ester fatty acids of yolk sac membrane of quail embryos as affected by maternal diet (MD) and stage of developement (DEV)

MD[1]	DEV	Fatty acid				
		$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$
	days	(% of total methyl esters)				
PA	11	15.7[2]	4.2	8.5	63.9	6.7
OA	11	12.8	3.2	7.7	69.3	5.6
LA	11	15.8	3.5	10.4	58.2	11.1
PA	13	11.5	4.2	6.8	69.0	6.2
OA	13	9.6	3.2	6.2	72.3	6.7
LA	13	12.4	3.7	8.2	59.5	13.8
PA	15	11.4	3.9	6.4	71.0	6.6
OA	15	8.4	3.0	5.7	76.0	6.2
LA	15	12.4	3.5	7.2	62.9	14.8
mean ± SEM		12.2 ± .49	3.6 ± .17	7.4 ± .29	66.9 ± .80	8.6 ± .52
Source of variation (df)		Probabilities				
MD (2)		<.01	<.01	<.01	<.01	<.01
DEV (2)		<.01	.261	<.01	<.01	<.01
MD by DEV (4)		.534	.962	.323	.218	<.01
		Combined effects of MD and DEV				
MD						
PA		12.8[a]	4.1[a]	7.2[b]	68.0[b]	6.5[b]
OA		10.3[b]	3.2[c]	6.5[c]	72.5[a]	6.2[b]
LA		12.9[a]	3.4[b]	8.6[a]	60.2[c]	13.2[a]
DEV						
11		14.8[a]	3.7	8.9[a]	63.8[c]	7.8[b]
13		11.2[b]	3.7	7.1[b]	66.9[b]	8.9[a]
15		10.1[c]	3.5	6.4[c]	70.0[a]	9.2[a]

[a-c]Means within a column and variable with no common superscripts differ significantly (P<.05).

[1]Diets containing 97% basal mix plus 3% of: Diet PA, palmitic acid; Diet OA, oleic acid; and Diet LA, linoleic acid.

[2]Values are means of four observations (eight pooled yolk sac membrane per observation).

TABLE 3.7. Major cholesterol ester fatty acids of extrahepatic tissue of quail embryos as affected by maternal diet (MD) and stage of developement (DEV)

MD[1]	DEV	Fatty acid					
		$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:4}$
	days	(% of total methyl esters)					
PA	11	15.4[2]	3.8	4.6	57.2	13.1	3.5
OA	11	14.3	3.3	4.2	59.6	12.2	3.4
LA	11	17.6	3.5	5.6	46.8	20.4	4.1
PA	13	27.5	3.1	5.0	47.6	10.0	3.1
OA	13	26.6	1.9	3.8	51.4	9.9	3.4
LA	13	28.4	2.8	6.0	41.0	17.1	3.5
PA	15	27.8	1.9	8.3	44.6	10.8	5.0
OA	15	21.7	1.7	7.7	50.2	10.0	4.9
LA	15	24.4	1.6	8.8	37.3	16.2	5.4
mean ± SEM		22.6 ± .66	2.6 ± .22	6.0 ± .31	48.4 ± .73	13.3± .38	4.0± .23
Source of variation (df)		Probabilities					
MD (2)		<.01	<.01	<.01	<.01	<.01	.049
DEV (2)		<.01	<.01	<.01	<.01	<.01	<.01
MD by DEV (4)		<.01	.156	.360	.047	.074	.764
		Combined effects of MD and DEV					
MD							
PA		23.6[a]	2.9[a]	6.0[b]	49.8[b]	11.3[b]	3.9[b]
OA		20.9[b]	2.3[b]	5.2[c]	53.7[a]	10.7[b]	4.0[ab]
LA		23.4[a]	2.6[ab]	6.8[a]	41.7[c]	17.9[a]	4.3[a]
DEV							
11		15.8[c]	3.5[a]	4.8[b]	54.5[a]	15.2[a]	3.8[b]
13		27.5[a]	2.6[b]	4.9[b]	46.7[b]	12.3[b]	3.3[b]
15		24.6[b]	1.7[c]	8.3[a]	44.0[c]	12.3[b]	5.1[a]

[a-c]Means within a column and variable with no common superscripts differ significantly (P<.05).

[1]Diets containing 97% basal mix plus 3% of; Diet PA, palmitic acid; OA oleic acid; and Diet LA, linoleic acid.

[2]Values are means of four observations (eight pooled extrahepatic tissue per observation).

4. RESEARCH NOTE: EGGSHEL QUALITY IN JAPANESE QUAIL FED DIFFERENT FATTY ACIDS[1]

C. VILCHEZ, S. P. TOUCHBURN, E. R. CHAVEZ, AND P. C. LAGUE

Department of Animal Science, Macdonald Campus of McGill University and McGill Nutrition and Food Science Centre, Sainte. Anne de Bellevue, Québec, H9X 1C0, Canada.

ABSTRACT The purpose of the present study was to determine the effects of diets containing 3% of palmitic acid (Diet PA), oleic acid (Diet OA), or linoleic acid (Diet LA) on shell quality of eggs of Japanese quail. Each diet was fed to 10 hens maintained individually in wire quail laying cages. There was no difference ($P > .05$) in feed consumption between hens fed Diets OA and LA, but hens of both groups consumed less ($P < .05$) feed than those fed Diet PA. Egg weight, shell weight and thickness of shell plus membrane were not influenced by dietary treatment ($P > .05$). However, specific gravity of eggs from hens fed Diet OA was significantly higher ($P < .05$) than those fed either Diet PA or Diet LA. Comparison of these data with hatchability data obtained in a previous experiment conducted under similar conditions suggests that the differences in hatchability would not be explained by differences in eggshell quality.

---

[1] As accepted for publication. 1992 Poultry Sci. 71:In press.

(**Key words**: quail, fatty acids, shell quality, egg specific gravity)

INTRODUCTION

The influence of eggshell quality on hatchability of avian embryos has been reported by several researchers (McDaniel et al., 1979; Brake et al., 1989; Peebles and Marks, 1991). The eggshell is the major determinant of respiratory gas exchange of the developing avian embryo (Rahn et al., 1979) and its porosity is essential for embryonic metabolism and development (Burton and Tullett, 1983). Hamilton (1982) indicated that egg specific gravity (SG), which estimates eggshell thickness (Harms et al., 1990), is one of the most widely used methods to assess shell quality. Reductions in SG have been associated with depressed hatchability; however, in eggs with SG higher than 1.080, there appears to be little relationship between SG and hatchability (McDaniel et al., 1979).

Among the factors influencing eggshell quality (Wolford and Tanaka, 1970; Washburn, 1982), dietary components were the most intensively investigated. Studies on calcium (Hamilton and Cipera, 1981), protein and energy (Roland, 1980), vitamin D_3 (cholecalciferol, Wyatt et al., 1990), vitamin C (Bell and Marion, 1990), and phosphorus (Roland, 1990), among others, were conducted to determine their effects on eggshell quality. Brake et al. (1989) found that feeding 5% of poultry fat to broiler breeders resulted in a significantly higher SG and shell weight in one of two

experiments when compared with eggs from a control group that did not receive supplemental fat.

It was reported recently (Vilchez et al., 1991) that hatchability of fertile eggs from Japanese quail hens fed a low-fat basal mix plus 3% of linoleic acid was lower than in those fed the same basal mix plus 3% of either palmitic or oleic acids. Therefore, this study was undertaken to evaluate the effects of dietary fatty acids on shell quality and to determine whether the differences in hatchability observed in the groups fed these three fatty acids (Vilchez et al., 1991) were associated with changes in eggshell quality.

MATERIALS AND METHODS

Thirty individually caged Japanese quail hens 5.5 mo in lay were used in this study. The animals were divided into three groups of 10 birds each. Each group was fed a diet containing 97% semipurified, low-fat basal mix plus 3% of either palmitic acid (Diet PA), oleic acid (Diet OA) or linoleic acid (Diet LA). The composition of the low-fat basal mix, the analyzed fatty acid composition of the experimental diets, and the husbandry of the animals were similar to those previously described (Vilchez et al., 1991). The animals used in this study were those maintained for metabolic studies in quail embryos, and they were on the experimental diets for 6 mo by the time the present experiment was conducted.

Eggs from each hen were collected daily at 0800 h during 6 to 7 consecutive days (total of 50 eggs per dietary treatment; five eggs per hen). Each egg was identified, weighed and its SG determined immediately after collection. The SG was determined using a series of saline solutions adjusted to a range of 1.056 to 1.084 g per mL in .004 g per mL increments. After the collection period, all eggs were broken, the contents removed and the shells rinsed thoroughly with warm running tap water, dried at 70 C overnight, cooled, and weighed individually. The thickness of the shell plus membrane was measured at the equatorial region to .001 mm accuracy with a digital outside micrometer.[2] The statistical model for this experiment was: $Y_{ijk} = u + T_i + H(T)_{j:i} + D_k + e_{ijk}$; where: Y_{ijk} = individual observation; u = overall mean; T = dietary treatment (i = 1, 2, 3); H = hens ($J_1 = 10, j_2 = 10, j_3 = 10$); D = day of collection (k = 1 to 5); e = the random error. In this model, T and H were considered fixed and random effects, respectively. Analysis of variance of the data was performed using the General Linear Models (GLM) procedure of SAS software (SAS Institute, 1986). Means for dietary treatments were separated by Tukey's studentized range (HSD) test using $H(T)_{j:i}$ as experimental error (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

There was no significant difference ($P > .05$) in feed consumption between

[2]Mitutoyo Manufacturing Co., Ltd., Tokyo.

quail hens fed Diet OA and Diet LA, but hens of both groups consumed significantly less ($P < .05$) feed than those fed Diet PA (Table 4.1). The greater feed consumption in birds fed Diet PA may be due to an apparent lower energy content of this diet because of the low absorbability of palmitic acid (Garrett and Young, 1975) as demonstrated by the analysis of the feces of quail fed Diet PA for total fat and its fatty acid composition (Vilchez et al., 1991). With the exception of the SG, dietary treatment had no significant effect ($P > .05$) on the weight, shell weight and thickness of shell plus membrane of the eggs collected during this trial (Table 4.1). Thus, the SG of eggs from quail fed Diet PA and Diet LA were similar ($P > .05$), but both were lower ($P < .05$) than those fed Diet OA.

Because there is no information, as far as the authors are aware, on the effects of dietary fats or fatty acids on eggshell quality of Japanese quail, the results of the present study can only be discussed in relation to data on chicken eggs. The addition of 5% of poultry fat to the diet of broiler breeders improved both SG and shell weight in one of two experiments (Brake et al., 1989). They explained that the higher SG and shell weight in one of their trials was probably due to a slightly higher intake of calcium. The greater SG of eggs from quail fed Diet OA observed in this study can not be explained by the difference in feed consumption since this was lower than Diet PA and similar to Diet LA. However, there is evidence in chickens that SG increases as egg weight decreases (Roland, 1990; Frost et al., 1990). Although the differences were not statistically significant, the mean egg weight was the lowest while the shell

weight and thickeness of shell plus membrane were the highest in eggs from hens fed Diet OA. These may have accounted for the higher SG in this group (Table 4.1). The results of the present investigation suggest that feeding either palmitic or linoleic acids had the same effect on eggshell quality , which would not have been expected because of the interference of saturated fatty acids, like palmitic acid, with mineral metabolism (Gardiner and Whitehead, 1976). Atteh and Leeson (1985) found no significant differences in eggshell minerals in laying hens fed diets supplemented with 8% of palmitic acid, oleic acid or a 50:50 mixture of palmitic and oleic acids.

The data presented in this study demonstrate that feeding oleic acid to quail hens resulted in eggs with SG values higher than those fed either palmitic or linoleic acids. Eggshell quality did not differ between groups fed either palmitic or linoleic acids. Mean hatchability values from a 24-wk period in a previous experiment (Vilchez et al., 1991) are included in Table 4.1. The present study employed the same type and source of quail, feed ingredients and diets, so that comparison of these data appears justified. This comparison suggests that differences in hatchability would not be explained by differences in shell quality, particularly in egg SG.

ACKNOWLEDGMENT

The technical assistance of Stéphane Adam is greatly appreciated.

TABLE 4.1. Effect of palmitic, oleic, and linoleic acids on feed consumption, and on egg weight, specific gravity, and thickness of shell plus membrane of eggs of Japanese quail

	Diet[1]			
Measurement	PA	OA	LA	SEM
Feed consumption, g[2]	27.85[a]	26.18[b]	26.56[b]	.320
Egg weight, g[3]	12.04	11.55	12.02	.286
Shell weight, g[3]	.883	.886	.844	.025
Thickness of shell plus membrane, mm[3]	.178	.184	.176	.004
Specific gravity[3]	1.066[b]	1.072[a]	1.065[b]	.001
Hatchability, %[4]	78.10[a]	76.50[a]	71.30[b]	1.020

[a,b] Means within a row with no common superscripts differ significantly ($P<.05$).

[1] Diet PA = 97% basal mix plus 3% palmitic acid; Diet OA = 97% basal mix plus 3% oleic acid; Diet LA = 97% basal mix plus 3% linoleic acid.

[2] grams per hen per day. Values are means of 10 hens per dietary treatment.

[3] Values are means of individual measurements of 50 eggs per dietary treatment.

[4] Data from Vilchez et al. (1991).

5. METABOLISM OF [1-^{14}C]-PALMITATE, -OLEATE, AND -LINOLEATE IN INTACT QUAIL EMBRYOS AS AFFECTED BY MATERNAL DIET

ABSTRACT The present study was contucted to determine the effects of fatty acid composition of egg yolk of Japanese quail fed a semipurified low-fat basal mix plus 3% of palmitic acid (Diet PA), oleic acid (Diet OA), or linoleic acid (Diet LA) on 1) the utilization of [1-^{14}C]-palmitic, -oleic and -linoleic acids by developing quail embryos, and 2) the distribution of the radioactivity of the labeled fatty acids in the lipid classes of both yolk plus yolk sac membrane (TY) and embryo lipids. Twenty fertile eggs per dietary treatment and per labeled fatty acid were injected with .05 uCi of each labeled fatty acid, and incubated for 15 days. Maternal diet did not influence ($P > .05$) the apparent oxidation of the labeled fatty acids, but Diet PA promoted more ($P < .05$) retention of radioactivity in embryo lipids. Among labeled fatty acids, [1-^{14}C]-oleic acid showed a high rate of disappearance ($P < .05$) whereas [1-^{14}C]-linoleic acid was less mobilized from the yolk to the embryo. The results also indicate that there was a preferential incorporation of labeled fatty acids into a particular lipid class.

(**Key words**: quail, maternal diet, embryo, fatty acid, oxidation)

INTRODUCTION

Almost the entire lipid content of the yolk is metabolized and absorbed into the chick embryo during the last 7 days of its 21-day period of incubation (Noble and Moore, 1964). During this period intensive fatty acid desaturation and elongation in embryonic tissues also occur in order to provide adequate amounts of polyunsaturated fatty acids (PUFA) necessary for the developing embryo (Bordoni et al., 1986). Quantitative and qualitative measurements of these changes have been obtained by studies involving radiolabeled fatty acids (Miyamoto et al., 1967; Noble and Shand, 1985).

Thus, when [1-^{14}C]-linoleic acid was injected into the yolk sac of 10-day old chicken embryos, arachidonic and docosatetraenoic acids were found to be the major radiactive PUFA (Miyamoto et al., 1967). In contrast, with [1-^{14}C]-linolenic acid, eicosapentaenoic, docosapentaenoic and docosahexaenoic acids were found to be the major PUFA in the embryonic brain. All these fatty acids were associated with the phospholipid fraction of the total lipid. In the same study (Miyamoto et al., 1967) it was reported that with the injection of either [1-^{14}C]-acetate, -myristate, -palmitate or -steareate, the radiactivity was found mainly in the saturated fatty acids of the brain phospholipids and was localized primarily in palmitic or stearic acids. Another study carried out with chicken embryos demonstrated that the rates of oxidation of [1-^{14}C]-palmitic and -oleic acids by intact embryos and embryo-liver homogenates

were similar, suggesting that there is no selective oxidation of unsaturated fatty acids by the chick embryos (Donaldson and Mueller, 1971).

In general, it is suggested that dietary inclusion of unsaturated fatty acids inhibits desaturation and the long-chain fatty acids inhibit elongation (Donaldson, 1968). In fact, recently Vilchez et al (1992) reported that the yolk sac membrane and extrahepatic tissues of quail embryos from hens fed a diet containing 3% of linoleic acid showed significantly lower levels of palmitoleic and oleic acids than embryos from hens fed 3% of palmitic acid. Likewise, experiments with whole quail embryo homogenates Donaldson (1968) observed a reduced relative incorporation of [1-^{14}C]-acetate into unsaturated long-chain fatty acids when hens were fed a diet high in unsaturated fatty acids. Further, relative acetate incorporation into C_{18} fatty acids in embryo tissues decreases as the dietary level of C_{18} fatty acids increases.

The present study was undertaken to determine the effects of fatty acid composition of egg yolk of Japanese quail fed a semi-purified low-fat basal mix plus 3% of either palmitic, oleic or linoleic acids on 1) the utilization of [1-^{14}C]-palmitic, -oleic and -linoleic acids by developing quail embryos, and 2) the distribution of the radioactivity of the labeled fatty acids in the lipid classes of both yolk plus yolk sac membrane (TY) and embryo lipids.

MATERIALS AND METHODS

Thirty 32-wk old female Japanese quail were placed individually in wire laying cages. The animals were divided into three groups of 10 birds each. Each group was fed one of the experimental diets containing 97% of semipurified low-fat basal mix plus 3% of palmitic acid (Diet PA), oleic acid (Diet OA) or linoleic acid (Diet LA). In addition, fifteen 32-wk old male Japanese quail were also placed individually in wire quail laying cages in the same facility and fed a commercial turkey grower diet. The composition of the experimental diets and the husbandry of the animals were reported earlier (Vilchez et al., 1991).

Twenty unincubated fertile eggs from birds receiving each experimental diet were injected with the [1-^{14}C]-labeled fatty acids following a modification of the procedure reported by Donaldson and Mueller (1971). Each egg received .05 uCi of either [1-^{14}C]-palmitic acid (8.4 mCi/mmol), -oleic acid (56 mCi/mmol), or -linoleic acid (50 mCi/mmol)[1] bound to fatty acid-free bovine serum albumin.[2] The injection was made directly into the yolk and the shell puncture was sealed with plastic cement.

---

[1]New England Nuclear, NEN; Du Pont Canada Inc., Ontario.

[2]Sigma Chemical Co., St. Louis, MO 63178.

After 15 days of incubation,[3] the eggs were removed, opened and the TY were removed from the embryos. Lipids from both whole TY and embryo were extracted using separatory funnels (60 and 250 mL capacity, respectively) with a chloroform:methanol (2:1; v/v) solution (Folch et al., 1957).

One-mL and five-mL aliquots of the TY and embryo lipid extracts, respectively, were taken into plastic counting mini vials.[4] Twenty uL of 5N HCL were added to each vial and evaporated to dryness on a water bath under a stream of nitrogen. Five mL of emulsifier/scintillator UNIVERSOL (ICN) were added to each vial containing the samples and the radioactivity was counted in a liquid-scintillation LS5801 spectrometer.[5] Total lipids from eight unincubated, [1-^{14}C] labeled fatty acid-injected egg yolks were extracted and processed as indicated above.

Aliquots of both TY and embryo lipid extracts were fractionated into lipid classes by a slight modification of the procedure reported by Vilchez et al. (1992). In the present study, .25 mm instead of .5 mm thin layer chromoplates of silica gel GF (20 x 20 cm)[6] were used. Bands corresponding to each lipid class (phospholipid, monoglyceride, diglyceride, free fatty acid, triglyceride and cholesterol ester) were

---

[3]Roll-X incubator; Lyon Electric Co., Inc., CA 92011.

[4]ICN, Biomedicals Inc., Cleveland, OH 14128.

[5]Beckman Intruments, Inc., Fullerton, CA 92634.

[6]Analtech Inc., Newark, DE 19711.

scraped into counting vials. Five mL of emulsifier/ scintillator UNIVERSOL (ICN) were added to each vial and the radioactivity counted as above.

Statistical Analysis

The data were analyzed on a 3 x 3 factorial arrangement in a complete randomized design with three maternal diets (Diets PA, OA and LA) and three [1-^{14}C]-labeled fatty acids (palmitic, oleic and linoleic acids). Analyses of variance were performed on data using the general linear models (GLM) procedure (SAS Institute, 1986). Significant differences of means were tested using Tukey's Studentized Range (HSD) test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Maternal diet showed a significant effect ($P<.05$) on incorporation of labeled fatty acid into embryo lipids after 15 days of incubation ; however, no significant differences ($P>.05$) in either the percentage of ^{14}C dose remaining in TY or the apparent oxidation of the labeled fatty acids were observed (Table 5.1). Diet PA promoted the highest ($P<.05$) retention of radioactivity in embryo lipids followed by Diet OA and Diet LA. An explanation of this difference is not readily apparent. It may be possible that the yolk composition per se, in which the embryo develops, is responsible for this result.

There was a significant difference ($P < .05$) in apparent oxidation among the three labeled fatty acids used in this study (Table 5.1). The apparent oxidations of both [1-^{14}C]-palmitic acid and [1-^{14}C]-linoleic acid were similar ($P > .05$), but lower ($P < .05$) than those observed with [1-^{14}C]-oleic acid. This difference may be explained by the statement of Bordoni et al. (1986) that oleic acid is considered to be the fatty acid mainly used for energy. More recently, Vilchez et al. (1992) reported that the content of oleic acid significantly decreases in phospholipid, triglyceride and cholesterol ester fractions of quail embryo lipids as incubation progresses, regardless of the maternal diet.

The results of the present study differ from those reported by Donaldson and Mueller (1971) who found no difference between incorporation of [1-^{14}C]-palmitic acid and [1-^{14}C]-oleic acid into chick embryo lipids after 20 days of incubation. In addition, they did not include [1-^{14}C]-linoleic acid in their study. The difference between the two reports may be due to species difference or to methodology followed during lipid extraction. Data in Table 1 also show that after 15 days of incubation, significantly more ($P < .05$)[1-^{14}C]-linoleic acid remained in the TY lipids than [1-^{14}C]-palmitic acid or [1-^{14}C]-oleic acid. This observation would indicate that a selective uptake of fatty acids from the yolk had occurred. It is in conflict with the reports by Donaldson (1964, 1967) that there is no selective uptake of saturated acids from yolk and by Bordoni et al. (1986) that lipid resorption from the yolk sac does not favor any fatty acid.

Data on the distribution of the radioactivity of the labeled fatty acids in the TY lipid classes are presented in Table 5.2. The maternal diet had a significant effect ($P < .05$) on the distribution of the radioactivity only in the phospholipid and diglyceride fractions. Phospholipid and diglycerides of TY lipids from quail fed Diet OA showed the highest ($P < .05$) radioactivity compared to those fed either Diet PA or Diet LA. The reason for this difference is not clear. There is evidence in rats, however, which indicates that the amount of labeled unsaturated fatty acids, mainly [1-^{14}C]-arachidonic acid, was incorporated into the phospholipid fraction of liver lipids to a greater extent when the animals were fed an oleic acid-rich diet than when rats were fed partially hydrogenated soybean oil or a mixture of linseed oil:coconut oil:cocoa butter (Zevenbergen and Houtsmuller, 1989). The latter observation would indicate that the type of fat or fatty acid in the diet does affect to some degree the amount of radioactivity that is incorporated into a particular lipid class as observed in the present study (Table 5.2).

[1-^{14}C]-palmitic acid was found to be preferentially incorporated into the triglyceride fraction of TY lipids. In contrast, [1^{14}C]-oleic acid and [1-^{14}C]-linoleic acid were incorporated into both triglyceride and cholesterol ester fractions of TY lipids (Table 5.2). The amount of radioactivity from [1-^{14}C]-oleic acid in the cholesterol ester fraction was the highest ($P < .05$) followed by [1-^{14}C]-linoleic and -palmitic acids. The differences may be due to the fact that acyl-CoA:cholesterol acyltransferase esterifies cholesterol preferentially with unsaturated long-chain fatty acids (Ochoa et

al., 1990). In addition, Noble et al. (1984) reported that although oleic acid is one of the major fatty acids in all lipid classes of yolk sac membrane, its concentration in the cholesterol ester constituted about 70 to 80% of all long-chain fatty acids associated with the cholesterol ester fraction. The same figure was observed by Vilchez et al. (1992) in the yolk sac membrane of developing quail embryos.

It is also interesting to note that there was no significant difference ($P > .05$) in the amount of radiactivity deposited into the phospholipids when [1-^{14}C]-palmitic or [1-^{14}C]-linoleic acids were injected ; however, they were higher ($P < .05$)than those observed when [1-^{14}C]-oleic acid was injected. Lands (1979) and Blomstrand and Svensson (1983) indicated that both oleic and linoleic acids can occupy the 1- and 2-positions of phospholipids, and they compete with each other for incorporation into phospholipids. This may explain the low linoleic acid in phospholipids of yolk sac membrane of quail embryos from quail hens fed an oleic acid-rich diet (Vilchez et al., 1992). In the present study, however, the comparison among labeled fatty acids was made across maternal diets; therefore, the low radioactivity in the phospholipid fraction when [1-^{14}C]-oleic acid was injected may be due to a preferential esterification with either [1-^{14}C]-palmitic or -linoleic acids rather than with -oleic acid (Table 5.2).

Significant interactions between maternal diet and labeled fatty acid were observed for phospholipid, monoglyceride and cholesterol ester fractions of TY lipids

(Table 5.2). Thus, less radioactivity from [1-^{14}C]-palmitic acid was present in phospholipids when hens were fed Diet PA than when they were fed Diet OA or Diet LA. In contrast, more radioactivity from [1-^{14}C]-linoleic acid was observed in phospholipids when hens were fed Diet PA or Diet OA than when they were fed Diet LA. In the case of monoglycerides, the highest level of labeled fatty acid incorporation occurred when the fatty acid injected corresponded with that of the maternal diet. On the other hand, when [1-^{14}C]-oleic acid was injected, the cholesterol ester fraction of embryos from hens fed Diet PA showed a higher incorporation of radioactivity than in those fed Diets OA and LA. It should be emphasized that the incorporation of labeled oleic acid was the highest level among the three labeled fatty acids injected regardless of maternal diet.

The effects of maternal diet and labeled fatty acid on the distribution of the radioactivity in embryo lipid classes are presented in Table 5.3. The amount of radioactivity in each lipid class was not significantly affected ($P > .05$) by the type of fatty acid fed to quail hens, but it was influenced by the type of labeled fatty acid injected into the yolk. Thus, [1-^{14}C]-linoleic acid was preferentially incorporated into the phospholipid fraction of embryo lipids followed by -palmitic acid and -oleic acid. More radioactivity from [1-^{14}C]-palmitic acid was present in the triglyceride fraction than from -oleic and -linoleic acids. In contrast, more radioactivity from [1-^{14}C]-oleic acid was present in the cholesterol ester fraction than from -palmitic and -linoleic acids.

The distribution of radioactivity in the phospholipid, triglyceride and cholesterol ester fractions of the embryo lipids (Table 5.3) follows the pattern observed in the same lipid classes of TY lipids (Table 5.2). However, the percentages of radioactivity in the phospholipid and cholesterol ester fractions of embryo lipids are higher and lower, respectively, than those observed in the same lipid classes of TY lipids. It is also important to indicate that the relative distribution of the radioactivity in the diglyceride fraction of embryo lipids was higher than those observed in the diglyceride fraction of TY lipids (Table 5.2).

In conclusion, the results of the present study indicate that maternal diet did not influence the apparent oxidation of labeled fatty acids, but Diet PA promoted more retention of radioactivity in embryo lipids. Among labeled fatty acids, [1-^{14}C]-oleic acid showed a high rate of disappearance, presumably via oxidation whereas [1-^{14}C]-linoleic acid was less mobilized from the yolk to the embryo. The data also show that there is a preferential incorporation of a particular fatty acid into a particular lipid class.

TABLE 5.1. Oxidation of 1-^{14}C-palmitate, -oleate, and -linoleate by quail embryos from hens fed diets containing different fatty acids.

Maternal diet (MD)[2]	% of ^{14}C dose remaining[1]		Apparent % oxidation[3]
	Yolk lipid	Embryo lipids	
	1-^{14}C-palmitate		
PA	13.2[4]	12.7	74.1
OA	14.1	11.4	75.5
LA	14.0	10.8	75.2
	1-^{14}C-oleate		
PA	14.1	10.4	75.5
OA	12.3	9.6	78.1
LA	15.0	8.1	76.6
	1-^{14}C-linoleate		
PA	14.8	12.5	72.7
OA	15.6	11.2	73.2
LA	16.0	9.8	74.7
mean ± SEM	14.3±.38	10.7±.48	75.1±.55
Source of variation (df)	Probabilities		
RI[5](2)	.04	< .01	< .01
MD(2)	NS	< .01	NS
RI by MD(4)	NS	NS	NS
	Combined effects of RI and MD		
RI			
^{14}C-PA	13.8^{b}	11.6^{a}	74.6^{b}
^{14}C-OA	13.8^{b}	9.5^{b}	76.7^{a}
^{14}C-LA	15.5^{a}	11.2^{a}	73.4^{b}
MD			
PA	14.0	11.9^{a}	74.1
OA	14.0	10.7^{b}	75.3
LA	15.0	9.7^{c}	75.4

$^{a-c}$Means within a column and variable with different superscripts differ significantly (P<.05).
[1]As percentage of the original dose remaining after 15 days of incubation.
[2]97% low-fat basal mix plus 3% of either palmitic acid (Diet PA), oleic acid (Diet OA) or linoleic acid (Diet LA).
[3]Calculated by subtracting the sum of yolk lipid and embryo lipids from 100.
[4]Values are means of eight observations per dietary treatment.
[5][1-^{14}C]-labeled fatty acid.

TABLE 5.2. Distribution of radioactivity from 1-^{14}C-palmitate, -oleate, and -linoleate in the yolk plus yolk sac lipid classes of quail embryos from hens fed diets containing different fatty acids.

Maternal diet (MD)[2]	Lipid classes[1] (%)					
	PL	MG	DG	FFA	TG	CE
	1-^{14}C-palmitate					
PA	9.38[3]	1.82	1.49	1.18	81.51	4.51
OA	16.71	1.40	2.34	1.61	71.76	6.20
LA	10.45	1.65	1.19	1.64	77.84	7.59
	1-^{14}C-oleate					
PA	5.52	2.25	2.39	1.91	46.90	41.41
OA	5.52	4.29	3.50	1.90	50.29	34.84
LA	6.39	2.85	3.05	1.59	52.82	33.26
	1-^{14}C-linoleate					
PA	14.52	3.06	4.89	1.51	55.34	20.68
OA	13.26	3.24	5.50	1.66	56.56	19.74
LA	9.64	4.39	3.34	1.10	58.05	23.34
mean ± SEM	10.15±1.34	2.77 ±.36	3.08 ±.48	1.57 ±.09	61.23±4.18	21.29 ±4.46
Source of variation (df)	Probabilities					
RI[4](2)	<.01	<.01	<.01	<.01	<.01	<.01
MD(2)	<.01	.20	.03	.55	.27	.50
RI by MD(4)	<.01S	.02	.42	.67	.09	.04
	Combined effects of RI and MD					
RI						
^{14}C-PA	12.18[a]	1.62[b]	1.56[c]	1.48[a]	77.04[a]	6.12[c]
^{14}C-OA	5.81[b]	3.13[a]	2.98[b]	1.58[a]	50.00[c]	36.50[a]
^{14}C-LA	12.48[a]	3.56[a]	4.58[a]	1.42[a]	56.71[b]	21.25[b]
MD						
PA	9.81[b]	2.38	2.92[ab]	1.53	61.25	22.11
OA	11.83[a]	2.98	3.78	1.72	59.65	20.26
LA	8.82[b]	2.96	2.52	1.44	62.86	21.40

[a-c]Means within a column and variable with different superscripts differ significantly (P<.05).

[1]PL = phospholipid; MG = monoglyceride; DG = diglyceride; FFA = free fatty acid; TG = triglyceride; CE = Cholesterol ester.

[2]97% low-fat basal mix plus 3% of either palmitic acid (Diet PA), oleic acid (Diet OA) or linoleic acid (Diet LA).

[3]Values are means of eight observations per dietary treatment.

[4][1-^{14}C]-labeled fatty acid.

TABLE 5.3. Distribution of radioactivity from 1-^{14}C-palmitate, -oleate, and -linoleate in embryo lipid classes of quail embryos from hens fed diets containing different fatty acids.

Maternal diet (MD)[2]	Lipid classes[1] (%)					
	PL	MG	DG	FFA	TG	CE
	1-^{14}C-palmitate					
PA	24.60[3]	3.01	4.04	2.60	61.81	3.91
OA	26.62	3.20	3.99	3.10	58.22	4.84
LA	29.39	1.71	4.29	4.10	56.76	3.70
	1-^{14}C-oleate					
PA	20.20	2.44	9.56	2.34	54.08	11.34
OA	20.89	2.40	8.72	2.91	53.01	12.00
LA	21.26	1.95	9.64	2.11	52.66	12.44
	1-^{14}C-linoleate					
PA	37.08	2.90	9.11	3.62	40.00	7.19
OA	38.19	2.38	9.62	4.34	38.68	7.08
LA	31.78	3.38	8.38	3.66	44.24	8.50
mean ± SEM	27.78 ±2.27	2.60 ±.19	7.48 ±.86	3.20 ±.26	51.05 ±2.73	7.89 ±1.14
Source of variation (df)	Probabilities					
RI[4](2)	<.01	.07	<.01	<.01	<.01	<.01
MD(2)	.69	.25	.94	.37	.56	.37
RI by MD(4)	.08	<.01	.54	.35	.34	.38
	Combined effects of RI and MD					
RI						
^{14}C-PA	26.87^{b}	2.64^{ab}	4.14^{b}	3.27^{ab}	58.93^{a}	4.15^{c}
^{14}C-OA	20.78^{c}	2.26^{b}	9.28^{a}	2.45^{b}	53.25^{b}	11.92^{a}
^{14}C-LA	35.68^{a}	2.88^{a}	9.04^{a}	3.88^{a}	40.86^{c}	7.59^{b}
MD						
PA	27.29	2.78	7.57	2.85	51.93	7.48
OA	28.57	2.66	7.45	3.45	49.87	7.97
LA	27.48	2.34	7.40	3.29	51.22	8.21

[a-c]Means within a column and variable with different superscripts differ significantly (P<.05).
[1]PL=Phospholipids; MG=Monoglycerides; DG=Diglycerides; FFA=Free fatty acids; TG=Triglycerides; CE=Cholesterol esters.
[2]97% low-fat basal mix plus 3% of either palmitic acid (Diet PA), oleic acid (Diet OA) or linoleic acid (Diet LA).
[3]Values are means of eight observations per dietary treatment.
[4][1-^{14}C]-labeled fatty acid.

IV. GENERAL DISCUSSION

The inclusion of palmitic acid in the diets of quail hens consistently resulted in an increased feed consumption as compared with the inclusion of linoleic acid (Experiment 1) and with the inclusion of either oleic or linoleic acids (Experiment 2). A similar observation was reported earlier (Vilchez et al., 1990b). It was suggested that the greater feed consumption in birds fed palmitic acid may be related to an apparent low energy value of the feed because of the low absorbalility of the palmitic acid (Hurwitz et al., 1973; Garrett and Young, 1975), and is thought to be utilized less efficiently when it is the major component of the fat in the diet (Atteh and Leeson, 1985). The results of the analyses of quail feces for the total fat content and its fatty acid composition (Experiment 2) support the above statements.

A high egg production rate was observed in quail hens fed palmitic acid in Experiments 1 and 2. This result may be associated with the highest total plasma phosphorus in birds fed palmitic acid as compared with those fed either oleic or linoleic acids. Total plasma phosphorus has been reported to be a good measure of the plasma VLDL fraction (Bacon et al., 1982) which is the yolk lipid precursor (Bacon, 1981). The significance of saturated fatty acids, unlike the PUFA, in promoting lipid storage and lipoprotein synthesis was documented earlier (Beynen and Katan, 1985). Furthermore, dietary linoleic acid has been reported to reduce concentrations of VLDL (Grundy, 1975).

The importance of linoleic acid in increasing average egg weight has been documented in chicken (Balnave, 1971; Whitehead, 1981; Scragg et al., 1987). In the present study, average egg weight of quail fed linoleic acid was heavier than those of hens fed either palmitic or oleic acids (Experiment 2); however, the positive effect of linoleic acid was not manifested in Experiments 1 and 4, in which average egg weight was similar to those receiving diets containing either palmitic acid (Experiment 1) or palmitic and oleic acids (Experiment 4). The differences in response observed among experiments may be due to differences in the duration of each experiment.

An estimation of weight of egg produced, egg output in g per hen per day, clearly indicates that quail hens fed palmitic acid had the highest egg output followed by those fed oleic and linoleic acids (Experiment 2). Although egg output was not reported in Experiment 1, observation of the data show agreement with the results of Experiment 2.

Hatchability of fertile eggs was significantly influenced by the type of fatty acid fed to the quail hen. Feeding palmitic acid consistently resulted in higher hatchability as compared with those fed linoleic acid (Experiments 1 and 2). However, when oleic acid was included in the diet of quail hens, hatchability of fertile eggs was similar to those observed when hens were fed palmitic acid, but higher than those fed linoleic acid (Experiment 2).

The increased livability of embryos from birds fed palmitic acid or oleic acid may be due to the high levels of oleic acid present in their yolk lipids as compared with those fed linoleic acid (Experiments 1 and 2). The significance of oleic acid in eggs on quail embryo survival was stated by Donaldson and Fites (1970). It was also reported that hatchability was adversely affected when the ratio of stearic to oleic acid exceeded .25 (Tullett, 1990). In the present study, the fatty acid composition of yolk lipids of (Experiments 1 and 2) showed that yolk lipids of birds fed either palmitic or oleic acids had a decreased stearic to oleic acid ratio than yolk lipids from quail hens fed linoleic acid.

The high levels of oleic acid in yolk lipids of hens fed either palmitic or oleic acids, together with the ability of the yolk sac membrane to synthesize cholesterol esters of oleic acid (Noble, 1987b) may have enhanced the lipid transport and assimilation from the yolk during embryo development, resulting in an improved hatchability. In this regard, data of Experiment 3 support the latter suggestion because it was found that embryos from birds fed either palmitic or oleic acids mobilized more yolk material than those fed linoleic acid . Further, it was found that labeled oleic acid is preferentially esterified in the cholesterol ester fraction of yolk sac membrane lipids; moreover, it appears that labeled oleic acid is utilized to a great extent by the quail embryo during its development (Experiment 5).

The fatty acid composition of the lipid classes of both YSM and ET as

affected by maternal diet and stage of development were investigated in Experiment 3. The fatty acid composition of the yolk lipids consistently influenced the fatty acid profile of each lipid class in both YSM and ET. Thus, the high levels of palmitoleic, oleic and linoleic acids observed in egg yolks from hens fed, respectively, Diets PA, OA and LA (Experiment 2) were also reflected in the fatty acid composition of each lipid class of the tissues in question. It is also important to note that the inclusion of linoleic acid in the diets of quail hens resulted in a significant increase in the synthesis of saturated fatty acids such as palmitic and stearic acids, and the decreased synthesis of palmitoleic and oleic acids. This pattern was observed in each lipid class reported in Experiment 3, as well as in the fatty acid profiles of egg yolk lipids (Experiments 1 and 2), plasma and liver lipids (Experiment 2).

The stage of development also had a significant effect on the fatty acid profile of lipid classes of both YSM and ET (Experiment 3). With the exception of the cholesterol ester fraction of ET, the level of palmitic acid decreased and that of linoleic acid increased in the lipid classes of both YSM and ET from Day 11 to Day 15 of incubation. The level of oleic acid, however, followed a different pattern depending on the lipid fraction. Thus, as incubation progressed, its level decreased in phospholipids of both YSM and ET. The opposite pattern was observed in the triglyceride fraction of both tissues. On the other hand, the level of oleic acid in cholesterol esters increased in YSM and decreased in ET from Day 11 to Day 15 of incubation. This would reflect the statement by Noble et al. (1984) that the formation

of the cholesterol ester of oleic acid is important in the transport across the YSM but that the ester is rapidly separated in the embryo liver.

Overall, the positive response of palmitic acid in improving the reproductive performance of Japanese quail observed in this investigation suggests that this saturated, non-essential fatty acid, has a physiological role for reproduction that needs further investigated.

V. CONCLUSIONS

From the results of these experiments, in which a semipurified low-fat (1.3% fat containing .7 to .8% linoleic acid) basal mix were used, the following conclusions can be drawn:

The level of .7 to .8% of linoleic acid in the diet of quail breeder hens appears to be sufficient for overall reproductive performance.

Feeding palmitic acid to quail hens results in a high rate of egg production and a high level of egg mass output.

Quail hens fed an oleic acid-rich diet produced eggs with high specific gravity.

Feeding palmitic acid promoted a high concentration of lipoproteins, measured as total plasma phosphorus.

Fertility is not affected by the three fatty acid included in the diet of quail hens in this study.

Feeding palmitic acid resulted in a high hatchability of fertile eggs and high estimated number of quail chicks produced per hen.

Feeding linoleic acid to quail hens resulted in increased gross weight of eggs and chicks at hatch, but they were similar to those fed a palmitic acid-rich diet.

The percentages of dry matter, total lipid and cholesterol of eggs were not affected by the type of fatty acid fed to quail hens.

Adult body weight, liver weight and liver weight expressed as percentage of body weight were not influenced by the type of fatty acid included in the quail diet.

Embryos from quail hens fed a diet containing palmitic acid mobilized more yolk material and produced the heaviest extrahepatic tissue by Day 15 of incubation.

The weight of yolk sac membrane of quail embryos was maximun at Day 13 of incubation.

High levels of oleic and linoleic acids were found in egg yolk, plasma and liver lipids from birds fed diets containing oleic and linoleic acids, respectively. However, a high level of palmitoleic acid was observed when birds were fed palmitic acid.

In phospholipid, triglyceride and cholesterol ester fractions of yolk sac membrane and extrahepatic tissues of quail embryos, the fatty acid compositions corresponded to those observed in the yolk lipids.

In phospholipids of both yolk sac membrane and extrahepatic tissues of quail embryos, the oleic acid decreased while linoleic acid increased as incubation progressed.

In triglyceride and cholesterol esters of yolk sac membrane of quail embryos, the level of palmitic acid decreased, but oleic and linoleic acids increased with advancing embryonic development.

Preferential mobilization of labeled fatty acids from the yolk to the embryo and preferential oxidation of these fatty acids by the embryo occurred during development.

VI. LITERATURE CITED

Abad, C., M. A. Bosch, A. M. Municio, and A Rivera, 1976. Age differences in the positional distribution of phosphoglycerides and molecular species of choline phosphoglycerides during development of the chick embryo liver. Biochem. Biophys. Acta. :431:62-74.

Allen, N. K., and U.K. Young, 1980. Studies on the amino acid and protein requirements of laying Japanese quail (Coturnix coturnix japonica). Poultry Sci. 59:2029-2037.

Atteh, J. O., and S. Leeson, 1985. Response of laying hens to dietary saturated and unsaturated fatty acids in the presence of varying dietary calcium levels. Poultry Sci. 64:520-528.

Bacon, W. L., 1981. Metabolism of lipid labeled very low density lipoprotein from laying turkey hens in laying turkey hens and immature turkeys. Poultry Sci. 60:1525-1536.

Bacon, W. L., K. E. Nestor, D.W. Long, and D. H. Ohm, 1982. Divergent selection for body weight and yolk precursor in Coturnix coturnix japonica. 2. Correlated responses in plasma lipoproteins and lipids. Poultry Sci. 61:161-165.

Balnave, D., 1968. Studies on changes in egg size immediately following supplementation of a layers' diet of low-fat content with corn oil. North Irel. Minist. Agric. Rec. Agric. Res. 17:141-145.

Balnave, D., 1971a. The contribution of absorbed linoleic acid to the metabolism of the mature laying hen. Comp. Biochem. Physiol. 40A:1097-1105.

Balnave, D., 1971b. Response of laying hens to dietary supplementation with energetically equivalent amount of maize starch and maize oil. J. Sci. Food. Agric. 22:125-128.

Balnave, D., and S.T.C. Weatherup, 1974. The necessity of supplementing laying hen diets with linoleic acid. Br. Poult. Sci. 15:325-331.

Balnave, D., 1982. Egg weight and production responses of laying hens fed rice pollard. J. Sci. Food Agric. 33:231-236.

Bell, D. E., and J. E. Marion, 1990. Vitamin C in laying hen diets. Poultry Sci.

69:1900-1904.

Berdanier, C. D., 1988. Role of membrane lipids in metabolic regulation. Nutr. Rev. 46:145- 149.

Beynen, A. C., and M. B. Katan, 1985. Why do polyunsaturated fatty acids lower serum cholesterol? Am. J. Clin. Nutr. 42:560-563.

Bitman, J., and D. L. Wood, 1980. Cholesterol and cholesterol esters of eggs from various avian species. Poultry Sci. 59:2014-2023.

Bitman, J., D. L. Wood, H. F. Tyrrell, D. E. Bauman, C. J. Peel, A.C.G. Brown, and P.J. Reynolds, 1984. Blood and milk lipid responses induced by growth hormone administration in lactating cows. J. Dairy Sci. 67:2873-2880.

Blomstrand, R., and L. Svensson, 1983. The effects of partially hydrogenated marine oils on the mitochondrial functions and membrane phospholipid fatty acids in rat heart. Lipids. 18:151-170.

Bordoni, A., M. Cocchi, R. Lodi, E. Turchetto, and F. Ruggeri, 1986. Metabolism and distribution of linoleic acid and a-linolenic acid derivatives in chick embryo development. Prog. Lipid Res. 25:407-411.

Brake, J., J. D. Garlich, and G. R. Baughman, 1989. Effects of lighting program during the growing period and dietary fat during the laying period on broiler breeder performance. Poultry Sci. 68:1185-1192.

Bray, D. J., 1967. The effect of restricted caloric intake upon the egg weight response to dietary corn oil. Poultry Sci. 46:476-484.

Budowski, P., N. R. Bottino, and R. Reiser, 1961. Lipid transport in the laying hen and the incubating egg. Arch. Biochem. Biophys. 93:483-490.

Burton, F. G., and N. A. Halls, 1983. A comparison of the effects of eggshell porosity on the respiration and growth of domestic fowl, duck, and turkey embryos. Comp. Biochem. Physiol. 75A:167-174.

Calvert, C.C., 1967. Studies on hatchability of fertile eggs from hens receiving a linoleic acid deficient diet. Poultry Sci. 46:967-973.

Calvert, C. C., 1969. The performance of adult female Japanese quail on linoleic acid deficient diets. Poultry Sci. 48:975-978.

Chapman, M. J., 1980. Animal lipoproteins:chemistry, structure and comparative

aspects. J. Lipid Res. 21:789-853.

Chen, P. H., 1964. The effect of added dietary fats on the lipid composition of the hen's egg yolk. M. Sc. Thesis, McGill University.

Christie, W. W., and J. H. Moore, 1972. The lipid components of the plasma, liver and ovarian follicles in the domestic chicken (Gallus gallus). Comp. Biochem. Physiol. 41B:287-295.

Chung, R. A., J. C. Rogler, and W. J. Stadelman, 1965. The effect of dietary cholesterol and different dietary fats on cholesterol content and lipid composition of egg yolk and various body tissues. Poultry Sci. 44:221-228.

Clandinin, M. T., 1978. The role of dietary lon chain fatty acids in mitochondrial structure and function. Effects on rat cardiac mitochondrial respiration. J. Nutr. 108:273-281.

Clandinin, M. T., C. J. Field, K. Hargreaves, L. Morson, and E. Zsigmond, 1985. Role of diet fat in subcellular structure and function. Can. J. Physiol. Pharmacol. 63:546-556.

Clandinin, M. T., S. Cheema, C. J. Field, M. L. Garg, J. Venkatraman, and T. R. Clandinin, 1991. Dietary fat: exogenous determination of membrane structure and cell function. FASEB J. 5:2761-2769.

Cooper, J. B., and B. D. Barnet, 1968. Response of turkey hens to dietary linoleic acid fed as corn oil. Poultry Sci. 47:671-677.

Couch, J. R., T. M. Ferguson, and B. M. Cornett, 1973. Egg yolk lipids and maternal diet in the nutrition of turkey embryo. Lipids 8:682-689.

Creger, C. R., R. L. Atkinson, and J. R. Couch, 1970. Fat levels in the diets of turkey breeders. Nutr. Rep. Int. 2:1-8.

Dashti, N., J. L. Kelley, R. H. Thayer, and J. A. Ontko, 1983. Concurrent inductions of avian hepatic lipogenesis, plasma lipids, and plasma apolipoprotein B by estrogen. J. Lipid Res. 24:368-380.

De Henau, P., S. P Touchburn, C. W. Chan, and R. B. Buckland, 1985. Effect of dietary energy, protein and fat levels on reproductive performance of turkey hens. Poultry Sci. 64: (Suppl. 1):88 (Abst.).

Donaldson, W. E., 1964. Fatty acid composition of chick embryo and egg yolk at various stages of incubation. Poultry Sci. 43:784-785.

Donaldson, W. E., 1967. Lipid composition of chick embryo and yolk as affected by stage of incubation and maternal diet. Poultry Sci. 46:693-697.

Donaldson, W. E., 1968. Influence of dietary fatty acid composition on in vitro acetate incorpration into saturated versus unsaturated and C_{16} versus C_{18} fatty acids by Coturnix quail. Comp. Biochem. Physiol. 27:419428.

Donaldson, W. E., and B. L. Fites, 1970. Embryo mortality in quail induced by cyclopropene fatty acids: Reduction by maternal diets high in unsaturated fatty acids. J. Nutr. 100:605-610.

Donaldson, W. E., and N. S. Mueller, 1971. Synthesis of unsaturated fatty acids in chick embryo liver. Can. J. Biochem. 49:563-567.

Donaldson, W. E., N. S. Mueller, and J. V. Mason, 1971. Intercellular localization of fatty acid synthesis in chick embryo liver and stimuf synthesis by exogenous glucose. Biochem. Biophys. Acta. 248:34-40.

Donaldson, W. E., 1981. Lipid metabolism in chick embryos. Poultry Sci. 60:1964-1970.

Ewards, D. G., and T. R. Morris, 1967. The effect of maiz and maiz oil on eggweight. Br. Poult. Sci. 8:163-168.

Evans, R. J., C. J. Flegal, C. A. Foerder, D. H. Bauer, M. Lavigne, 1977. The influence of crude cottonseed oil in the feed on the blood and egg yolk lipoproteins of laying hens. Poultry Sci. 56:468-479.

Folch, J., M. Lees, and G. H. Sloane-Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.

Freeman, B. M., and M. A. Vince, 1974. Developing of the avian embryo. Chapman and Hall Ltd., London, Engl.

Frost, T. J., D. A. Roland, SR., and G. G. Untawale, 1990. Influence of vitamin D_3, 1α-hydroxyvitamin D_3, and 1,25-dihydroxyvitamin D_3 on eggshell uality, tibia strength, and various production parameters in commercial laying hens. Poultry Sci. 69:2008-2016.

Gardiner, E.E., and C. C. Whitehead, 1976. Effect of dietary palmitic acid on broiler chicks fed on various concentrations of calcium. Br. Poult. Sci. 17:241-244.

Garrett, R. L., and R. J. Young, 1975. Effect of micelle formation on the absorption of neutral fat and fatty acids by the chicken. J. Nutr. 105:827-838.

Gilbert, A., S. Touchburn, C. Vilchez, and C. Chan, 1991. Addition of fat to diets of female or male broiler breeder chickens improves reproduction. Poultry Sci. 70(Supp. 1):44 (Abst.).

Gildersleeve, R. P., D. Sugg, C. R. Parkhurst and D. I. McRee, 1987. Egg production in four generations of paired Japanese quail. Poultry Sci. 66:227-230.

Ginsberg, B. H., J. Jabour, and A. A. Spector, 1982. Effect of alterations in membrane lipid unsaturation on the properties of the insulin receptor of Ehrlich ascites cells. Biochem. Biophys. Acta. 690:157-164.

Gormal l, D. A., and A. Kuksis, 1973. Alteration of lipid composition of plasma lipoproteins during deposition of egg yolk. J. Lipid Res. 14:197-205.

Griffin, H., G. Grant, and M. Perry, 1982. Hydrolysis of plasma triacylglycerol rich lipoproteins from immature and laying hens (Gallus domesticus) by lipoprotein lipase in vitro. Biochem. J. 206:647-654.

Griffin, H. D., M. M. Perry, and A. B. Gilbert, 1984. Yolk formation, in: The Physiology and Biochemistry of the Domestic Fowl. Vol. 5. pp. 345-380. (London, Academic Press).

Grizzle, J. M., R. A. Voitle, and R. H. Harms. Evaluation of destillers dried grains with solubles in diets of turkey hens. Poultry Sci. 61:1363-1366.

Grundy, S. M., 1975. Effects of polyunsaturated fats on lipid metabolism in particular with hypertriglyceridemia. J. Clin. Inv. 55:269-282.

Grundy, S. M., 1989. Monounsaturated fatty acids and cholesterol metabolism: Implications for dietary recommendations. J. Nutr. 119:529-533.

Grundy, S. M., and M. A. Denke, 1990. Dietary influences on serum lipids and lipoproteins. J. Lipid Res. 31:1149-1172.

Guenter, W., D. B. Bragg, and P. A. Kondra, 1971. Effect of dietary linoleic acid on fatty acid composition of egg yolk, liver and adipose tissue. Poultry Sci. 50:845-850.

Hamilton, R. M. G., and J. D. Cipera, 1981. Effects of dietary calcium levels during the brooding, rearing, and early laying period on feed intake, egg production, and shell quality of White Leghorn hens. Poultry Sci.

60:349-357.

Hamilton, R. M. G., 1982. Methods and factors that affect the measurement of eggshell quality. Poultry Sci. 61:2022-2039.

Hammer, C. T., and E. D. Wills, 1979. The effect of dietary fats on the composition of the liver endoplasmic reticulum and oxidative drug metabolism. Br. J. Nutr. 41:465-475.

Harms, R. H., and H. R. Wilson, 1983. Improved performance of turkey hens from the addition of dietary fat. Poultry Sci. 62:2283-2284.

Harms, R. H., R. E. Buresh and H. R. Wilson, 1984. The influence of the grower diet and fat in the layer diet on performance of turkey hens. Poultry Sci. 63:1634-1637.

Harms, R.H., A. F. Rossi, D. R. Sloan, R. D. Miles, and R. B. Christmas, 1990. A method for estimating shell weight and correcting specific gravity for egg weight in eggshell quality studies. Poultry Sci. 69:48-52.

Hebert, J. A., J. Perez-Buriel, and L.F. Berrio, 1987. Effects of various dietary oils and cholesterol on yolk weight and egg cholesterol in the laying hen. Nutr. Rep. Int. 6:1123-1128.

Heuck, C. C., A. Nothhelfer, H. Raetzer, and G. Schlierf, 1977. Microdetermination of cholesterol in serum lipoproteins. J. Lipid Res. 18:259-263.

Hidalgo, C., M. De la Puente, and M. E. Gonzales, 1986. Role of lipids in sarcoplasmic reticulum: A higher lipid content is required to sustain phosphoenzyme decomposition than phosphoenzyme formation. Arch. Biochem. Biophys. 247:365-371.

Hillyard, L. A., H. M. White, and S. A. Pangburn, 1972. Characterization of apoliproteins in chicken serum and egg yolk. Biochemistry. 11:511-518.

Hood, R. L., 1990. Effect of diet and substrate on the in vitro measurements of cholesterol and fatty acid synthesis in hepatic tissue of Japanese quail. Poultry Sci. 69:647-651.

Horani, F., and J. L. Sell, 1977. Effect of feed grade animal fat on laying hen performance and on metabolizable energy of rations. Poultry Sci. 56:1972-1980.

Hornstra, G., 1972. Digestibility, efficiency and other metabolic effects of dietary

rapeseed oil in rats. Nutr. Metab. 14:282-29

Hoyle, C. M., and J. D. Garlich, 1987. Effect of a high fat diet fed prior or at sexual maturity on egg weight. Poultry Sci. 66:1202-1207.

Huyghebaert, G., 1988. Response of laying hens to different types and levels of fat in the presence of varying calcium levels. In:Proceedings of XVIII World's Poultry Congress, Japan, pp. 890-892.

Innis, S. M., and M. T. Clandinin, 1981. Dynamic modulation of mitochondrial membrane physical properties and ATPase activity by diet lipid. Biochem J. 198:167-175.

Jackson, N., H. R. Kirkpatrick, and R. B. Fulton, 1969. An experimental study of the utilization, by the laying hen, of dietary energy partially supplied as animal fat. Br. Poult. Sci. 10:115-126.

Jensen, L. S., J. B. Allred, R. E. Fry, and J. McGinnis, 1958. Evidence for unidentified factor necessary for maximum egg weight in chickens. J. Nutr. 65:214-233.

Jensen, L. S., and J. V. Shutze, 1963. Essential fatty acid deficiency in the laying hen. Poultry Sci. 42:1014-1019.

Kruski, A. W., and K. A. Narayan, 1972. The effect of dietary supplementation of cholesterol and its subsequent withdrawal on the liver lipids and serum lipoproteins of chickens. Lipids. 7:742-749.

Lands, P., 1979. Geometric and Positional Fatty Acid Isomers. Emken, E. A. and

Dutton, H. J., eds). Am. Oil Chem. Soc., Champaign, IL.

Lepage, G., and C. C. Roy, 1986. Direct transesterification of all classes of lipids in one-step reaction. J. Lipid Res. 27:114-120.

Legendre, S., L. Letellier, and E. Schechter, 1980. Influence of lipids with branched-chain fatty acids on the physical, morphological and functional properties of Escherichia coli cytoplasmic membrane. Biochem. Biophys. Acta. 602:491-505.

Lindsey, S., J. Benattar, A. Pronczuk, and K. C. Hayes, 1990. Dietary palmitic acid (16:0) enhances high density lipoprotein cholesterol and low density lipoprotein receptor mRNA abundance in Hamsters. Proc. Soc. Exp. Biol. Med. 195:262-269.

Machlin, L. J., R. S. Gordon, J. Marr, and W. W. Pope, 1962. Effect of dietary fat on the fatty acid composition of eggs and tissues of the hen. Poultry Sci. 41:1340-1343.

Marcel, Y. L., and C. Vezina, 1973. A method for the determination of the initial rate of reaction of lecithin: Cholesterol acetyltransferase in human plasma. Biochim. Biophys. Acta. 306:497-504.

March, B. E., and C. MacMillan, 1990. Linoleic acid as a mediator of egg size. Poultry Sci. 69:634-639.

Marion, J. E., and H. M. Edwards, Jr., 1962. The response of fat deficient laying hens to corn oil supplementation. Poultry Sci. 41:1785-1792.

Marion, J. E., and H. M. Edwards, Jr., 1964. The response of laying hens to dietary oils and purified fatty acids. Poultry Sci. 43:911-918.

Marks, H. L., 1975. Relationships of embryonic development to egg weight, hatch weight, and growth in Japanese quail. Growth. 42: 129-140.

McDaniel, G. R., D. A. Roland, SR., and M. A. Coleman, 1979. The effect of eggshell quality on hatchability and embryonic mortality. Poultry Sci. 58:10-13.

Menge, H., C. C. Calvert, and C. A. Denton, 1965a. Further studies on the effect of linoleic acid on reproduction in the hen. J. Nutr. 86:115-119.

Menge, H., C. C. Calvert, and C. A. Denton, 1965b. Influence of dietary oils on reproduction in the hen. J. Nutr. 87:365-370.

Menge, H., and G. V. Richardson, 1968. The influence of a linoleic-acid-deficient maternal diet on growth of progeny. Poultry Sci. 47:542-547.

Miller, K. W., and M. D. Lane, 1984. Estradiol-induced alteration of very-low-density lipoprotein assembly. Possible competition among apoproteins for incorporation into nascent very-low-density lipoprotein. J. Biol. Chem. 259:15277-15286.

Miyamoto, K., L. M. Stephanides, and J. Bernsohn, 1967. Incorporation of [1-^{14}C]linoleate and linolenate into polyunsaturated fatty acids of phospholipids of the embryonic chick brain. J. Neur. 14:227-237.

Morrison, W. R., and L. M. Smith, 1974. Preparation of fatty acid methyl esters and

dimethylacetals from lipids with boron fluoride-methanol. J. Lipid Res. 5:600-608.

Morson, L. A., and M. T. Clandinin, 1986. Diets varying in linoleic acid and linolenic acid content alter liver plasma membrane lipid composition and glucagon-stimulated adenylate cyclase activity. J. Nutr. 116:2355-2362.

Murty, N. L., and R. Reiser, 1961. Influence of graded levels of dietary linoleic and linolenic acids on the fatty acid composition of hens' eggs. J. Nutr. 75:287-294.

Nakagawa, Y., K. Waku, and Y. Ishima, 1982. Changes in the composition of fatty chains of diacyl, alkylacyl and alkenylacyl ethanolamine and choline phosphoglycerides during the development of chick heart ventricular cells. Biochem. Biophys. Acta. 712:667-676.

Narahari, D., K. A. Mujeer, A. Thangavel, N. Ramamuathy, S. Viswanathan, B. Mohan, B. Muruganandan, and V. Sundararasu, 1988. Traits influencing the hatching performance of Japanese quail eggs. Br. Poult. Sci. 29:102-112.

Neelands, P. J., and M. T. Clandinin, 1983. Diet fat influences liver plasma-membrane lipid composition and glucagon-stimulated adenylate cyclase activity. Biochem. J. 212:573-583.

National research Council, 1984. Nutrient Requirements of Poultry. 8th. ed. National Academy of Sciences, Washington, DC.

Noble, R. C., and J. H. Moore, 1964. Studies on the lipid metabolism of the chick embryo. Can. J. Biochem. 42:1729-1741.

Noble, R. C., and J. H. Moore, 1967a. The partition of lipids between the yolk and yolk-sac membrane during the development of the chick embryo. Can. J. Biochem. 45:949-958.

Noble, R. C., and J. H. Moore, 1967b. The transport of phospholipids from the yolk to the yolk sac membrane during the development of the chick embryo. Can. J. Biochem. 45:1125-1133.

Noble, R. C., K. Connor, and W. K. Smith, 1984. The synthesis and accumulation of cholesterol esters by the developing embryo of the domestic fowl. Poultry Sci. 63:558-564.

Noble, R. C., and J. H. Shand, 1985. Unsaturated fatty acid compositional changes

and desaturation during the embryonic development of the chicken (Gallus domesticus). Lipids 20:278-282.

Noble, R. C., 1987a. Egg lipids. In: Egg Quality: Current Problems and Recent Advances. (Eds. Wells, R. G. and Belyavin, C. G.). Butterworth, UK. pp. 159-177.

Noble, R. C., 1987b. Lipid metabolism in the chick embryo: Some recent ideas. J. Exp. Zool.(Suppl. 1):65-73.

Ochoa, B., A. Gee, B. Jackson, and K. G. Suckling, 1990. Regulation of cholesteryl ester metabolism in the hamster liver. Biochem. Biophys. Acta. 1044:133-138.

Patterson, R., J. S. Youngner, W. O. Weigle, and F. J. Dixon, 1962. The metabolism of serum proteins in the hen and chick and secretion of serum proteins by the ovary of the hen. J. Gen. Physiol. 45:501-513.

Peebles, E. D., and H. L. Marks, 1991. Effects of selection for growth and selection diet on eggshell quality and embryonic development in Japanese quail. Poultry Sci. 70:1474-1480.

Perry, M. M., and A. B. Gilbert, 1979. Yolk transport in the ovarian follicle of the hen (Gallus domesticus): lipoprotein-like particles at the periphery of the oocyte in the rapid grow phase. J. Cell Sci. 39:257-262.

Potter. L. M., J. R. Shelton, and G. B. Meyer, 1978. Added fat, isopran and rofenaid in diets of breeder turkeys. Poultry Sci. 57:485-488.

Pourreza, J. K. Smith, and P. Dun, 1988. Responses of layers to lipid supplementationof feeds inadequate in protein and sulfur amino acids. In: Proceedings of XVIII World's Poultry Congress, Japan, pp. 938-940.

Rahn, H., A. Ar, and C. V. Pagenelli, 1979. How birds breathe. Sci. Am. 240:46-55.

Romanoff, A. L., 1960. The Avian Embryo. MacMillan, New York, NY.

Renner, R., S. M. Innis, and M. T. Clandinin, 1979. Effects of high and low erucicacid rapeseed oils on energy metabolism and mitochondrial function of the chick. J. Nutr. 109:378-387.

Roca, P., F. Sainz, M. Gonzales, and M. Alemany, 1984. Structure and composition of the eggs from several avian species. Comp. Biochem. Physiol. 77A:307-310.

Roland, D. A., SR., 1980. Egg shell quality. I. Effect of dietary manipulations of protein, amino acids, energy, and calcium in aged hens on egg weight, shell weight, shell quality, and egg production. Poultry Sci. 59:2038-2046.

Roland, D. A., SR., 1990. The relationship of dietary phosphorus and sodium aminosilicate to the performance of commercial Leghorns. Poultry Sci. 69:105-112.

Rosebrough, R. W., E. Geis, P. James, H. Ota, and J. Whitehead, 1980. Effect of dietary energy substitution of reproductive performance, feed efficiency and lipogenic enzyme activity in Large White turkey hens. Poultry Sci. 59:1485-1492.

Sakurai, H., 1981. Influence of dietary levels of protein and energy on nitrogen and energy balance for egg production of Japanese quail. Japan. Poult. Sci. 18:185-192.

Santoma, G., 1989. Nutrition of domestic quail. Pages 221-253 in: Proc. 7th Eur. Symposium on Poultry Nutrition, June 19-21, 1989. IRTA, ed. World's Poultry Science Asssociation, Barcelona, Spain.

SAS Institute, 1986. SAS User's Guide: Statistics, Version 6 Edition. SAS Institute, Inc., Cary, N.C.

Schneider, W. J., R. Carroll, D. L. Severson, and J. Nimpf, 1990. Apolipoprotein VLDL-II inhibits lipolysis of triglyceride-rich lipoproteins in the laying hen. J. Lipid Res. 31:507-513.

Scragg, R. H., N. B. Logan, and N. Geddes, 1987. Response of egg weight to the inclusion of various fats in layer diets. Br. Poult. Sci. 28:15-21.

Sell, J. L., S. H. Choo, and P. A. Kondra, 1968. Fatty acid composition of egg yolk and adipose tissue as influenced by dietary fat and strain of hen. Poultry Sci. 47:1246-1302.

Sell, J. L., G. Tenesaca, and G. L. Bales, 1979. Influence of dietary fat on energy utilization by laying hens. Poultry Sci. 58:900-905.

Sell, J. L., C. R. Angel, and F. Escribano, 1987. Influence of supplemental fat on weights of eggs and yolks. Poultry Sci. 66 (Supp. 1):174 (Abst.).

Shanawany, M. M., 1984. Interrelationship between egg weight, parenteral age and embryonic development. Br. Poult. Sci. 25:449-455.

Shanon, D. W. F., and C. C. Whitehead, 1974. Lack of response in egg weight or output to increasing levels of linoleic acid in practical layers' diets. J. Sci. Food Agric. 25:553-561.

Shapira, N., I. Nir, and P. Budowski, 1978. Response of lipogenic enzymes to intensity and duration of overfeeding in the adult Japanese quail (Coturnix coturnix japonica). Br. Poult. Sci. 39:289-295.

Shutze, J. V., L. S. Jensen, and J. McGinnis, 1962. Accelerated increase in egg weight of young pullets fed practical diets supplemented with corn oil. Poultry Sci. 41:1846-1851.

Shutze, J. V., and L. S. Jensen, 1963. Influence of linoleic acid on egg weight. Poultry Sci. 42:921-924.

Siek, T. J., and R. W. Newburgh, 1965. Origin of the phospholipids in chick embryo development. J. Lipid Res. 6:556-564.

Sim, J. S., D. B. Bragg, and G.C. Hodgson, 1973. Effect of dietary animal tallow and vegetable oil on fatty acid composition of egg yolk, adipose tissue and liver of laying hen. Poultry Sci. 52:51-57.

Solomonson, L. P., V. A. Liepkalns, and A. A. Spector, 1976. Changes in (Na + K)-ATPase activity of Ehrlich ascites tumor cells produced by alteration of membrane fatty acid composition. Biochemistry. 15:892-897.

Spector, A. A., and M. A. Yorek, 1985. Membrane lipid composition and cellular function. J. Lipid Res. 26:1015-1035.

Steel, R. G. D., and J. H. Torrie, 1980. Principles and Procedures of Statistics. A Biomedical Approach. 2nd ed. McGraw-Hill Book Co., New York, NY.

Strong, C. F., Jr., K.E. Nestor, and W. L. Bacon, 1978. Inheritance of egg production, egg weight, body weight and certain plasma constituents in Coturnix. Poultry Sci. 57:1-9.

Sukhija, P. S., and D. L. Palmquist, 1988. Rapid method for the determination of total fatty acid content and composition of feedstuffs and feces. J. Agric. Food Chem. 36:1202-1206.

Thomsen, K. V., 1966. The influence of coconut fat and soybean oil meals on the fatty acid composition of hens' eggs. Acta Agric. Scand. 16:194-198.

Thomson, A.B.R., M. Keelan, and M. T. Clandinin, 1987. Onset and persistense of

changes in intestinal transport following dietary fat manipulation. Lipids. 22:22-27.

Thomson, A.B.R., M. Keelan, M. Garg, and M. T. Clandinin, 1989. Evidence for critical-period programming of intestinal transport function:variation in the dietary ratio of polyunsaturated to saturated fatty acids alters ontogeny of the rat intestine. Biochem Biophys. Acta. 1001:302-315.

Tullett, S. G., 1990. Science and the art of incubation. Poultry Sci. 69:1-15.

Vance, D. E., and J. E. Vance, 1985. Biochemistry of Lipid and Membrane. Benjamin/Cummings Publishing Co., Menlo Park, CA.

Vilchez, C., S. T. Touchburn, E. R. Chavez, and C. W. Chan, 1990. The influence of different dietary fats on the reproductive performance of turkey hens. Can. J. Anim. Sci. 70:679-684.

Vilchez, C., S. P. Touchburn, E. R. Chavez, and C. W. Chan, 1990a. The influence of supplemental corn oil and free fatty acids on the reproductive performance of Japanese quail (Coturnix coturnix japonica). Poultry Sci. 69:1533-1538.

Vilchez, C., S. P. Touchburn, E. R. Chavez, and C. W. Chan, 1990b. Dietary palmitic and linoleic acids and reproduction of Japanese quail (Coturnix (coturnix japonica). Poultry Sci. 69:1922-1930.

Vilchez, C., S. P. Touchburn, E. R. Chavez, and C. W. Chan, 1991. Effect of feeding palmitic, oleic, and linoleic acids to hens of Japanese quail(Coturnix coturnix japonica). 1. Reproductive performance and tissue fatty acids. Poultry Sci. 70:2484-2493.

Vilchez, C., S. P. Touchburn, E. R. Chavez, and C. W. Chan, 1992. Effect of feeding palmitic, oleic, and linoleic acids to hens of Japanese quail (Coturnix coturnix japonica). 2. Maternal diets and stage of development on the lipid metabolism of quail embryos. Poultry Sci. 71:1032-1042.

Washburn, K. W., and D. F. Nix, 1974. A rapid technique for extraction of yolk cholesterol. Poultry Sci. 53:1118-1122.

Washburn, K. W., 1982. Incidence, cause, and prevention of egg shell breakage in commercial production. Poultry Sci. 61:2005-2012.

Whitehead, C. C., 1981. The response of egg weight to the inclusion of different amounts of vegetable oil and linoleic acid in the diet of laying hens Br.

Poult. Sci. 22:525-532.

Whitehead, C. C., and K. M. Herron, 1988. Fatty acid requirements of breeding turkeys. Br. Poult. Sci. 29:761-768.

Wilson, W. O., U. K. Abbot, and H. Abplanalp, 1961. Evaluation of Coturnix (Japanese quail) as pilot animal for poultry. Poultry Sci. 40:651-657.

Wyatt, C. L., L. S. Jensen, and G. N. Rowland, III, 1990. Effect of cimetidine on eggshell quality and plasma 25-hydroxycholecalciferol in laying hens. Poultry Sci. 69:1892-1899.

Wolford, J. H., and K. Tanaka, 1970. Factors influencing egg shell quality. A review.World's Poultry Sci. J. 26:763-780.

Yamane, T., K. Ono, and T. Tanaka, 1980. Energy requirement of laying Japanese quail. Br. Poult. Sci. 21:451-455.

Yannakopoulos, A. L., and A. S. Tserveni-Gousi, 1987. Effect of breeder quail age and egg weight on chick weight. Poultry Sci. 66:1558-1560.

Yu, M. M., and J. S. Sim, 1987. Biological incorporation of n-3 polyunsaturated fatty acids into chicken eggs. Poultry Sci. 66 (Suppl. 1):195 (Abst.).

Zevenbergen, J. L., and U.M.T. Houtsmuller, 1989. Effect of dietary fats on linoleic acid metabolism. A radiolabel study in rats. Biochem. Biophys. Acta. 1002:312-323.