UNIVERSITI PUTRA MALAYSIA

PLASMA VERY LOW DENSITY LIPOPROTEIN AND FAT DEPOSITION IN COMMERCIAL BROILER AND CROSSBRED VILLAGE CHICKENS

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By

TAN BEE KOON

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

October 2002

To my dearest daughter and son
Xuan Ni and Yan Shao

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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October 2002

Chairman: Dr. Loh Teck Chwen

Faculty: Agriculture

A study was conducted to determine the relationships between triacylglycerol (TAG) of plasma, very low density lipoprotein (VLDL), VLDL subfractions; postheparin plasma lipoprotein lipase (LPL) activity and fat deposition in two different breeds of chickens. The VLDL apolipoproteins of both breeds were also characterised.

Two breeds used were crossbred village chicken (AK) (Sasso crossed) and commercial broiler (CB) (*Avian*). One hundred and eighty day-old female and 180 day-old male birds from both breeds were used in this study. They were fed a conventional starter diet up to three weeks of age and a finisher diet until six weeks of age for CB and 12 weeks of age for AK. They were housed in six pens with 30 female and 30 male birds of each breed per pen. Three male and three female birds from each pen were slaughtered and the blood was collected. The VLDL was isolated and subfractionated by using Fast Protein Liquid Chromatography (FPLC).

Lipid compositions and types of apolipoproteins were determined. The LPL activity in the postheparin plasma was also measured by using non-esterified fatty acid kit.

The body weight (BW) and feed intake (FI) of CB were significantly ($P<0.01$) higher than that of AK but the feed conversion ratio was significantly ($P<0.01$) lower. Fat deposition of both breeds was positively correlated ($P<0.01$) with BW and FI.

Fast Protein Liquid Chromatography analysis showed the presence of two subfractions in VLDL. Subfraction 2 contained more apo E than subfraction 1 and believed to enhance the lipolysis process of VLDL TAG. The results also showed that CB had a significantly higher proportion of subfraction 2 ($P<0.01$), bigger VLDL particle size ($P<0.01$) and higher postheparin plasma LPL activity ($P<0.05$) than AK. All these factors lead to a higher fat deposition in CB ($P<0.01$) than that of AK. These results were further supported by the lower VLDL TAG concentration of CB ($P<0.01$). The CB, which had a higher LPL activity and proportion of subfraction 2, caused a faster catabolism of TAG and more fatty acids were released for fat deposition.

The AK and CB have almost similar types of apolipoproteins in both subfractions 1 and 2. The AK showed the presence of apo AI, AIV, D and E whereas the CB had apo AIV, D, E and H. The apo AIV and apo E were present in both subfractions of AK and CB.

Abstrak tesis yang dikemukakan kepada Senat Univeristi Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

PLASMA LIPOPROTIN KETUMPATAN PALING RENDAH DAN PENYIMPANAN LEMAK DALAM AYAM PEDAGING DAN AYAM KAMPUNG KACUKAN

Oleh

TAN BEE KOON

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Pengerusi: **Dr. Loh Teck Chwen**

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Satu kajian telah dijalankan untuk menentukan perhubungan antara triasilgliserol (TAG) dalam plasma, lipoprotin ketumpatan paling rendah (VLDL), subfraksi VLDL; aktiviti lipoprotin lipase (LPL) dalam posheparin plasma dan penyimpanan lemak dalam dua baka ayam. Ciri-ciri apolipoprotin VLDL mereka juga dikaji.

Dua baka ayam yang digunakan adalah ayam kampung kacukan (AK) (kacukan Sasso) dan ayam pedaging (CB) (*Avian*). Seratus lapan puluh ekor ayam jantan dan 180 ekor ayam betina yang berumur satu hari dari dua baka ini telah digunakan dalam kajian ini. Mereka dibagi makanan pemula konvensional sehingga berumur tiga minggu dan makanan akhiran sehingga berumur enam minggu untuk CB dan 12 minggu untuk AK. Mereka dipelihara dalam enam buah rumah dengan 30 ekor betina dan 30 ekor jantan ayam dari setiap baka dalam setiap rumah. Tiga ekor jantan dan tiga ekor betina ayam dari setiap rumah telah desembelih dan darah telah dikumpulkan. VLDL telah diasingkan dan disubfraksi dengan menggunakan 'Fast Protein Liquid Chromatography' (FPLC). Komposisi lipid dan jenis apolipoprotin dengan menggunakan 'Fast Protein Liquid Chromatography' (FPLC). Komposisi lipid dan jenis apolipoprotin telah ditentukan. Aktiviti LPL dalam posheparin plasma juga diukur dengan menggunakan 'non-esterified fatty acid kit'.

Berat badan and pengambilan makanan CB adalah lebih tinggi ($P<0.01$) daripada AK tetapi ratio penukaran makanan adalah lebih rendah ($P<0.01$). Penyimpanan lemak bagi dua baka ayam adalah berhubungan secara positif ($P<0.0.1$) dengan berat badan dan pengambilan makanan.

Analisis FPLC menunjukkan kehadiran dua subfraksi dalam VLDL. Subfraksi kedua mengandungi lebih banyak apo E yang dipercayai boleh meningkatkan proses lipolisis TAG VLDL. Keputusan juga menunjukkan CB mempunyai lebih tinggi bahagian subfraksi kedua ($P<0.01$), lebih besar size partikal VLDL ($P<0.01$) dan lebih tinggi aktiviti LPL dalam posheparin plasma ($P<0.05$) daripada AK. Semua ini menyebabkan lebih tinggi penyimpanan lemak oleh CB ($P<0.01$) daripada AK. Keputusan-keputusan ini disokongkan lagi oleh lebih rendah kepekatan VLDL TAG dari CB ($P<0.01$). Ayam pedaging yang mempunyai lebih tinggi aktiviti LPL dan bahagian subfraksi kedua, menyebabkan lebih pantas katabolisma TAG dan lebih banyak asid-asid lemak dilepaskan untuk penyimpanan lemak.

AK dan CB mempunyai jenis apolipoprotin yang hampir sama dalam kedua-dua subfraksi pertama dan kedua. AK menunjukkan kehadiran apo AI, AIV, D dan E manakala CB mempunyai apo AIV, D, E, dan H. Apo IV and apo E hadir dalam kedua-dua subfraksi AK dan CB.

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation and sincere thanks to my supervisor Dr. Loh Teck Chwen for his guidance, patience, constructive comments and invaluable advice throughout the study. Special thanks go to my co-supervisors Associate Professor Dr. Norhani binti Abdullah and Associate Professor Dr. Zulkifli bin Idrus who have given me invaluable advice and comments.

I wish to extend my special appreciation and sincere gratitude to Dr. Foo Hooi Ling from Faculty of Food Technology and Biotechnology, UPM for her helpful advice, discussion and assistance, which improved this study.

I am also thankful to Professor Dr. Ho Yin Wan for giving me permission to use the facilities in the Digestive Microflora Laboratory in the Institute of Bioscience. I also like to thank Associate Professor Dr. Liang Juan Boo from the Department of Animal Science who allowed me to use the facilities in the Nutrition Laboratory.

I like to express my sincere thanks to Mr. Ponnusamy Muniandy and Mr. Mazlan Hamzah for their assistance in the farm. Not forgetting, I would also like to thank all my friends who gave me their moral support and encouragement.

Finally, I wish to express my deepest gratitude to my family and my lovely husband Yee Ming for their constant support, patience and love.

I certify that an Examination Committee met on 12th October 2002 to conduct the final examination of Tan Bee Koon on her Master of Science thesis entitled "Plasma Very Low Density Lipoprotein and Fat Deposition in Commercial Broiler and Crossbred Village Chickens" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

TAN BEE KOON

Date: 18/11/2003

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACAT	Acyl-CoA cholesterol acyltransferase
AK	Village chicken
ANOVA	Analysis of variance
Apo	Apolipoprotein
ATP	Adenosine triphosphate
BW	Body weight
CB	Commercial broiler
CDP-	Cytidine diphospho-
CDP-DG	Cytidine-diphosphate diacylglycerol
CE	Cholesteryl ester
CETP	Cholesteryl ester transfer protein
CL	Citrate lyase
CTP	Cytidine triphosphate
CV	Column volume
DAG	Diacylglycerol
DHAP	Dihydroxyacetone phosphate
EDTA	Ethylenediamine tetra-acetic acid
FCE	Feed conversion efficiency
FCR	Feed conversion ratio
FI	Feed intake
FPLC	Fast protein liquid chromatography
HDL	High density lipoprotein
HMG-CoA	Hydroxy-3-methylglutaryl-CoA
IDL	Intermediate density lipoprotein
kD	Kilo dalton
LCAT	Lecithin:cholesterol acyltransferase
LDL	Low density lipoprotein
LPL	Lipoprotein lipase
LSD	Least significant difference
MAG	Monoacylglycerol
MARDI	Malaysian Agriculture Research and Development Institute
MD	Malate dehydrogenase
NADPH	Nicotinamide adenine dinucleotide phosphate, reduced form
NS	Not significant
PAGE	Polyacrylamide gel electrophoresis
PL	Phospholipid
RER	Rough endoplasmic reticulum
SD	Standard deviation
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SER	Smooth endoplasmic reticulum
TAG	Triacylglycerol
TEM	Transmission electron microscopy
TEMED	N'N'N'N'-Tetramethylethylenediamine
VLDL	Very low density lipoprotein

CHAPTER 1

INTRODUCTION

Excess body fat deposition in broiler is now the concern of both producers and consumers. Many studies in human have shown the relationship between high dietary fat intake to cardiovascular diseases and cancer. In the metabolism of very low density lipoprotein (VLDL), VLDL will be transformed into low density lipoprotein (LDL) after the VLDL hydrolysis. The LDL is the major cholesterol carrier in the blood. If there is too much LDL circulating in the blood, cholesterol may deposited in artery walls causing plaques and cardiovascular diseases. High body fat deposition in broiler chickens may represent an economic loss to producers, as it is inefficient in terms of energy metabolism and overall feed utilisation. Moreover, obesity in birds increases the incidence of reproductive failure and death due to heart failure (Zubair and Leeson, 1996).

Lipoprotein metabolism plays an important role in fattening of poultry. It involves the process of synthesis and secretion and catabolism intravascularly. These processes lead to lipid uptake and storage by adipose tissue (Hermier, 1997).

Very low density lipoprotein is one of the major lipoproteins transporting triacylglycerol (TAG) from the liver to extrahepatic tissues, such as the adipose tissue, heart and lung (Cryer, 1981). There have been many attempts to reduce excessive fatness in poultry that involves the control of VLDL metabolism. Plasma

VLDL concentration has been used as an indicator of fatness in broilers. It has been used to study the lipoprotein metabolism and body lipid content of live broilers. Lean and fat lines of chickens have been selected on the basis of their abdominal fat content or plasma VLDL concentration (Griffin *et al.*,1982a, 1989, 1991, 1992; Whitehead and Griffin, 1982, 1984; Whitehead, 1990; Whitehead *et al.*, 1984, 1990 and Griffin and Whitehead, 1982).

Previous studies have shown the importance of apolipoprotein in VLDL metabolism in human (Evans *et al.*, 1989, Elovson *et al.*, 1988, Young, 1990, Rustaeus *et al.*, 1999). Some of the functions of apolipoproteins in human have been identified (Sprecher *et al.*, 1984). However, there is limited information on the functions and characteristics of apolipoproteins in the VLDL of chickens.

In the present study, two breeds of chicken namely commercial broiler (*Avian*) (CB) and crossbred village chicken (Sasso crossed) (AK) were used. The CB is considered as fat line chicken and AK as lean line chicken. They were used in order to study their differences in VLDL metabolism.

Therefore, the specific objectives of the project were:

1. To determine the lipid composition of plasma, VLDL and VLDL subfractions.
2. To define the relationships between TAG of plasma, VLDL and VLDL subfractions lipids and fat deposition.
3. To study the lipoprotein lipase (LPL) activity in postheparin plasma.
4. To characterise VLDL apolipoproteins (apo) in AK and CB.

CHAPTER 2

LITERATURE REVIEW

2.1 Poultry Industry in Malaysia

The globalisation of the poultry industry has accelerated at an extraordinary pace since the mid of nineties. Consumption of poultry meat is expected to outpace population growth because of escalating per capita consumption (Raghavan, 2002). This is forecasted to an average of 1.5% annually over the next ten years by the US Food and Agriculture Policy Research Institute. Together with this, poultry production is expected to grow to 2.4% each year between 2000-2009.

Excessive fat deposition in broilers is widely recognised as one of the primary industry problems. The fat represents as a waste to consumers who are concerned about nutritional and health aspects of their food. Producers have recently started to trim some of the fat from broilers. This means a loss to the producers or a price increases (Cahaner, 1988).

Poultry is the most popular meat consumed in Malaysia due to its pricing and religious acceptability. In 2001, Malaysia consumed 383 million chickens and 5.72 billion eggs. Exports of chickens and eggs last year were 43 million and 622 million, respectively. (Source : Financial News, 11 September, 2002). The current per capita consumption for poultry is estimated to be 37.1kg (Source : Malaysia Agricultural Directory and Index 1999/2000).

The broiler production for the whole of Malaysia was about 452 million birds in 2001. The principal breeds of broiler are *Avian* (41%), *Arbor Acres* (26%), *Cobb* (17%) and *Shaver* (5%) (Source : Financial News, 11 Sept 2002).

The broiler industry in Malaysia has developed into a highly industrialised industry today through rapid technological, genetic and management changes. Although the development of this industry has been tremendous, the production of village chicken has been static (Ramlah, 1996).

Village chickens have been categorised into four distinct varieties base on plumage colour patterns: light brown, black, white and dark brown (cited from Ramlah, 1996). The traditional system of keeping village chicken is free range where the birds are allowed to scavenge for themselves during daytime, grazing weeds, grasses and picking up worms and insects. Sometimes they are given household leftovers.

Engku Azahan and Zainab (1980) had studied the growth performance of commercial broiler and village chickens raised under intensive and semi-intensive systems. The liveweights of those birds raised under intensive system were higher than those raised under semi-intensive system with the commercial broiler had higher body weight than village chicken. Under intensive system, the commercial broiler had an average body weight of 2600g at the age of 15 weeks while village chicken was 1100g. Under semi-intensive system, the body weight of commercial broiler was 1388g while AK was 525g at the age of 15 weeks. The better growth performance in birds raised intensively could be due to better nutrition.

2.2 Lipid

Lipid is a heterogeneous group of substances having the property of insolubility in water but solubility in non-polar solvents such as chloroform, hydrocarbons or alcohol (Lehninger *et al.*, 1993). Lipids play an important part in biological structures whose purpose is to provide barriers such as skin of animals that protect organisms against their environment. Lipid is also the principle form of stored energy in most organisms and TAG is the most important form of storage. Additionally, at the level of individual molecules, lipids participate as chemical messengers and are involved in the control of metabolism. For example, steroid hormones are carried in the bloodstream to target tissues, where they bind to specific receptor proteins and trigger changes in gene expression and metabolism.

2.2.1 Fatty Acid

Fatty acids are carboxylic acids with hydrocarbon chains of 4-36 carbons. Some fatty acid chains are fully saturated and unbranched, while others contain one or more double bonds (Figure 2.1). A few contain three carbon rings or hydroxyl groups (Lehninger *et al.*, 1993).

The length and degree of unsaturation of the hydrocarbon chain determine the physical properties of fatty acids. The longer the fatty acyl chain and the fewer the double bonds, the lower solubility in water. Unsaturated fatty acids and shorter fatty acyl chain fatty acids have lower melting points. Storage lipids in animals tend to have a preponderance of saturated and monounsaturated fatty acids. The biggest reservoir of fatty acids to supply long term energy is the adipose tissue. Fatty acids are mobilised from this tissue to meet the demands for energy at times when dietary energy is limiting (Gurr and Harwood, 1991).

Saturated fatty acid
Common name : Stearic acid   Systematic name : n-octadecanoic acid
$CH_3(CH_2)_{16}COOH$

Monounsaturated fatty acid
Common name : Oleic acid   Systematic name : cis-9-octadecenoic acid
$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$

Polyunsaturated fatty acid
Common name : α-Linolenic acid   Systematic name : cis-9,12,15-octadecatrienoic acid
$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$

Figure 2.1 : Classification of fatty acids (Lehninger *et al.*, 1993)

2.2.1.1 Biosynthesis of Fatty Acid (Lipogenesis)

Fatty acids are made initially by a combination of the action of acetyl-CoA carboxylase and fatty acid synthetase (Lehninger *et al.*, 1993). Acetyl-CoA carboxylase catalyses the formation of malonyl-CoA from acetyl-CoA. Acetyl-CoA is the starting compound involved in fatty acid synthesis. It may derive from the oxidative decarboxylation of pyruvate, the breakdown of exogenous or endogenous fatty acids or from catabolised amino acids (Nir *et al.*, 1988). Fatty acid synthetase is a multienzyme complex. It catalyses the formation of long chain fatty acids for example palmitate from acetyl-CoA and malonyl-CoA through four steps: condensation, reduction, dehydration and reduction again (Nir *et al.*, 1988; Lehninger *et al.*, 1993; Mayes, 2000).

The saturated acyl group produced during this set of reactions is recycled to become the substrate in another condensation with an activated malonyl group. With each passage through the cycle, two carbons extend the fatty acyl chain. When the chain length reaches 16, the product palmitic acid will leave the cycle (Figure 2.2) (Lehninger *et al*., 1993). However, in some animals, tissue products of 4C-14C are released. The uropygial gland fatty acid synthase from a number of birds produces medium branch chain fatty acids as products. These products are released by a specific hydrolase (Gurr and Harwood, 1991).

Fatty acids are transported between organs in animals either in the form of non-esterified fatty acids bound to serum albumin or as TAG associated with lipoproteins especially chylomicrons and VLDL. The TAG is hydrolysed on the outer surface of cells by LPL and fatty acids have been shown to enter liver, adipose and heart tissue cells (Gurr and Harwood, 1991).

In birds, lipogenesis is confined to the liver, where it is particularly important in providing lipids for egg formation. It has been shown that the main pathway for *de novo* synthesis of fatty acids occurred in the liver, with adipose tissue being chiefly a site of lipid storage (O'Hea and Leveille, 1969). Leveille *et al*. (1975) also indicated that tissues such as adipose tissue and skin may also contribute to fatty acid synthesis in the birds. Calabotta *et al*. (1985) provided further evidence that in chickens the skeleton is also an important site of lipogenesis.

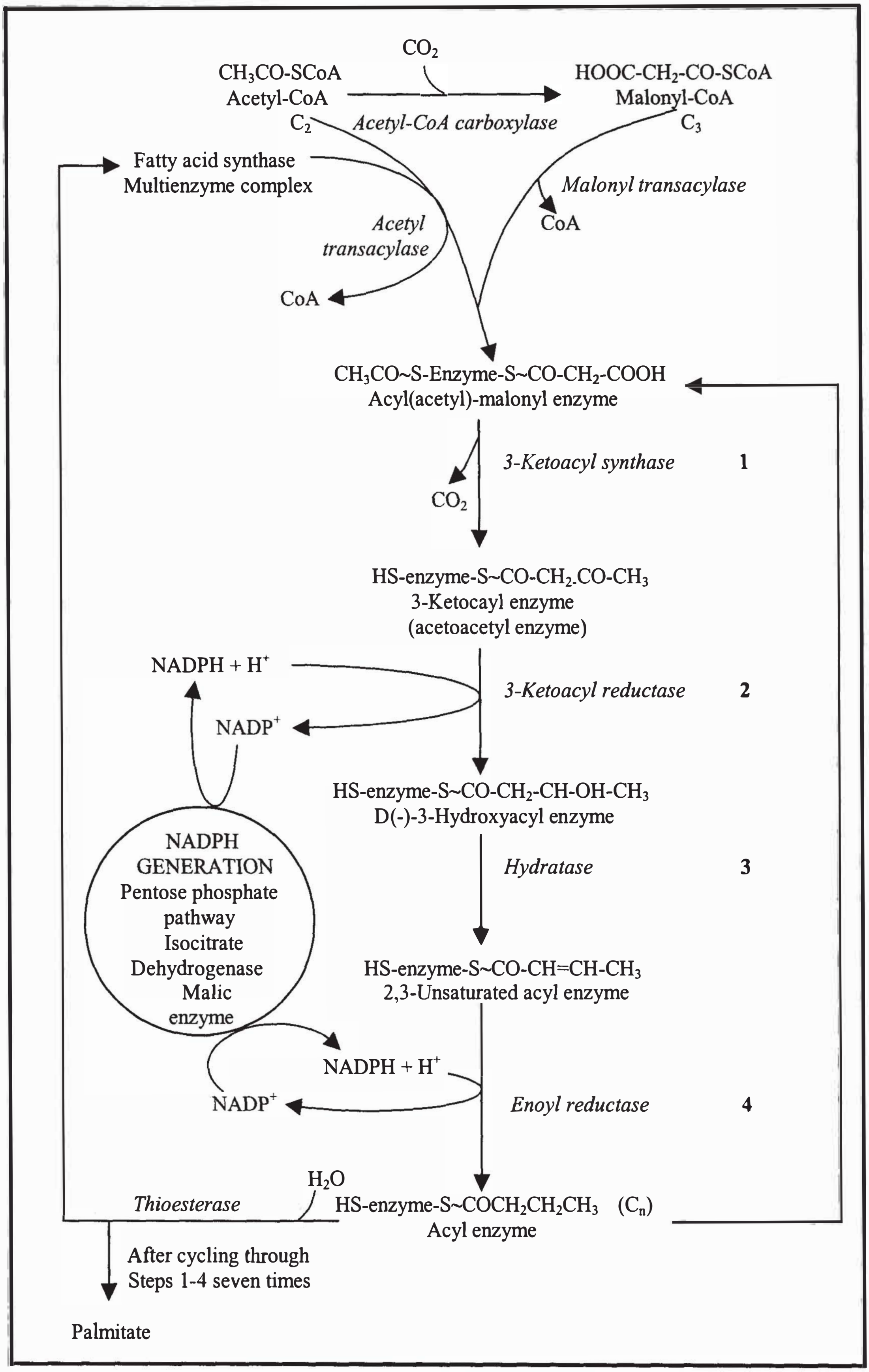

Figure 2.2 : Fatty acids biosynthesis (Mayes, 2000)