SHORT TERM DIETARY MODIFICATIONS ALTER MAMMARY LIPOGENIC GENE EXPRESSION IN MID LACTATING DAIRY COW USING NOVEL NON-INVASIVE RNA EXTRACTION TECHNIQUE

Thesis presented by

ALZAHRAA MUHAMMAD ABDELRASOUL ABDELATTY
B.V. Sc.; 2006, Fac. Vet. Med., Cairo University
M. V. Sc., 2011, Fac. Vet. Med., Cairo University
Nutrition and Clinical Nutrition

For the degree of

Doctor of Philosophy
(Nutrition and Clinical Nutrition)

Under supervision of

Dr. Fathy Farouk Mohamed
Professor of Nutrition & Clinical Nutrition
Faculty of Veterinary Medicine
Cairo University

Dr. Richard Arthur Erdman
Professor of Avian &Animal Sciences
Faculty of Agriculture &Life Science
University of Maryland

Dr. Mohamed Ahmed Tony
Professor of Nutrition &Clinical Nutrition
Faculty of Veterinary Medicine
Cairo University

Dr. Beverly Teter
Professor of Avian & Animal Sciences
Faculty of Agriculture & Life Science
University of Maryland

2016

Approval sheet

The examining committee approve Vet. Alzahraa Muhammad Abdelrasoul Abdelatty Hassanin for the PhD degree in Veterinary Medical Science (Nutrition and Clinical Nutrition).

Examining and Judgment Committee:

Professor. Dr. Al-Said Muhammad Ibrahim Hegazy
Professor of Nutrition and Clinical Nutrition
Vice president for graduate student affairs- Kafr Alshaikh University

Professor Dr. Ramadan Abdel-Monteleb Albanna
Professor of Nutrition and Clinical Nutrition- Head of department
Cairo University

Professor Dr. Richard Arthur Erdman- Supervisor
Professor of Animal and Avian Nutrition
University of Maryland- USA

Professor Dr. Fathy Farouk Muhammad-Supervisor
Professor of Nutrition and Clinical Nutrition
Former dean-Cairo University

Supervision Sheet

Dr. Fathy Farouk Mohamed

Professor of Nutrition and Clinical Nutrition

Faculty of Veterinary Medicine

Cairo University

Dr. Mohamed Ahmed Tony

Professor of Nutrition and Clinical Nutrition

Faculty of Veterinary Medicine

Cairo University

Dr. Richard Arthur Erdman

Professor of Avian and Animal sciences

Faculty of Agriculture and Life Science

University of Maryland

Dr. Beverly Teter

Professor of Avian and Animal sciences

Faculty of Agriculture and Life Science

University of Maryland

GENERAL ABSTRACT

Milk fat is the most variable component of milk, it is easily changed by nutritional and environmental factors rendering it a central point of interest for the daily market and scientists. Dietary manipulation can have a profound influence on milk fat synthesis and its fatty acid profile. However, our basic understanding of how dietary manipulations regulates mammary lipogenesis is limited by the use of mammary biopsy to obtain representative tissue sample for gene expression analysis, as repeated biopsies over short time interval from the same animal is not possible.

Therefore, the general objective of this dissertation was to measure the sequential changes in milk fat concentration, milk fatty acid profile and mammary lipogenic gene expression that occur during the transition from normal to dietary induced either elevated or depressed milk fat concentration, using new non-invasive alternative technique by extracting RNA from cytosolic crest trapped in large fat globules. Two experiments were conducted: 1) feed restriction that induce negative energy balance, body fat mobilization, and elevated milk fat concentration, 2) dietary induced milk fat depression by the use of dietary CLA that resulted in depression in milk fat concentration.

In the first study ten multiparous Holstein cows were used in completely randomized repeated measure design, cows were blocked by parity and milk yield, ca. 36.4 kg/d milk and 84 (±17) days in milk at the start of the experiment. All cows were fed ad- libitum the basal control diet for the first 14 days of the experiment, and data recorded during this period were used as covariate in the statistical analysis. This was followed by 4 days of feed restriction to only 60 percent of the estimated ad libitum intake for 5 cows and the other 5 cows continue receiving their ad libitum intake of their basal diet. Then at day 19 to day 20 all cows were fed ad libitum the same basal diet to follow up for any carry over effect of feed restriction.

During the experiment, blood serum NEFA, milk composition, milk fatty acid, and mammary lipogenic gene expression were measured. Feed restriction resulted in increased serum NEFA, milk fat

percent and preformed fatty acids, and downregulation of several mammary lipogenic genes, and caused a decrease in milk yield and *de novo* synthesized fatty acids.

In the second study milk fat depression was induced by the use of CLA supplement. Ten multiparous Holstein cows averaging 39.9 kg milk and 100 (±11) days in milk at the start of the experiment were used in completely randomized design. At the first 18 days of the experiment all cows were fed the basal control diet. Data recorded during this period were used as covariate in the statistical analysis. From day 19 to day 24, cows were split into two equal groups, one group continue on the basal diet and the other group received the basal diet topdressed with CLA supplement, at dose of 200g/d. At day 25 the CLA supplementation was stopped and all cows received the basal control diet and data recorded at day 25 to 27 were used to investigate for carry over effect. Milk yield was recorded during the whole experiment period plus extra 4 days, to follow up the complete recovery of milk yield. Top dressing the diet with CLA supplement for 6 consecutive days resulted in moderate reduction in milk fat percentage and yield, associated with moderate reduction in *de novo* synthesized fatty acids, and increase in milk yield. Due to limitation of the invasive mammary biopsy technique, the effect of short term dietary manipulation of mammary lipogenic gene expression was difficult to be investigated. From these two experimental models we conclude that extraction of RNA from cytosolic crest trapped in milk fat globule during its formation, can be successfully used as non-invasive alternative to mammary biopsy, and reflects the changes in mammary lipogenic gene expression in mammary epithelial cells. Thus, this approach can be used successfully to study the very short dietary changes to measure the mammary gene expression. We were able to continuously monitor the gene expression in both experiments, since the sampling method requires only that we milk each cow.

Key words: Dairy cow, Cytosolic crescent, CLA, Feed restriction, Nutrition

DEDICATION

بشكر رب العالمين علي توفيقي لإخراج ذلك العمل.....

……..To my beloved family

ACKNOWLEDGEMENTS

First, I am grateful to the almighty Allah for providing guidance and wisdom to accomplish this great work.

I would like to express my sincere gratitude to my committee members for the great co-operation and guidance to get this great work done. All of this would not have been possible without the support from my family. The highest recognition goes to my mother and siblings.

I will forever be thankful for the best mentor ever **Dr. Richard Erdman** for accepting the co-supervision of my thesis and provide me with ease all what I need to get all my work done, you were really ideal mentor, you gave me confidence, taught me the basics of research and working hard on improving my writing and presentation skills. I am so proud of being one of your lab team, and i am lucky to meet you in the path of my life, and learn a lot of wisdom.

I appreciate your visit to Egypt to attend and act as a judge in my PhD defend. And also, I am thankful to your lovely wife **Fary Samy** for her hospitality and giving me the family feelings, and treating me as a family member.

I extend my sincere appreciation to my lab mate **Dr. Marie Iwaniuk** for helping me during initial stages of dissertation, getting acquainted with the lab atmosphere, thank you for your help and every day driving back and forth to the farm, and sharing with me your experience in SAS programing and constituting great lab team, having an answer for every question.

I would like to extend special sincere thanks to **Dr. Fathy Farouk** for supporting me and accepting with flexibility the joint supervision of my dissertation with Dr. Erdman. You were really supportive and helpful to get me through my pre-doctor exams with ease and get the traveling paperwork done in a heartbeat. I am thankful for your visit to me in USA and follow up the lab work and for your continuous support and follow-up during my whole PhD course. I appreciate your help and guidance after my return back to Egypt.

I am also thankful to **Dr.Mohamed Tony** my committee member for his support and guidance during my masters and PhD.

I am grateful to **Dr. Beverly Teter** my committee member for teaching me the concepts of gas chromatography, fatty acid analysis and also for all those insightful discussions which were immensely beneficial in shaping up my dissertation.

I also express deep thank to **Dr**. **Pierluigi Delmonte** for helping me with the fatty acid analysis, in my two experiments, at FDA, I would not finish the fatty acid analysis and GC work without your help and team work spirit. Would also like to thank **Dr. Anil Kadegowda** and **Dr. Latisha** for being great friend and helping me in fatty acid analysis, get me oriented with the fatty acid analysis lab.

I am also grateful to **Dr. Miriam Garcia** for being wonderful friend, and sharing with me her experience in molecular biology technique and blood serum NEFA analysis. Also, I would like to transfer my thanks to **Dr. Kasy Moyse** lab, **Dr. Orlando** lab (**Ian** and **Tyler**).

I also would like to acknowledge the help we received from CMREC farm crew; **Michael Dwyer**, **Brian Spellman, and Tim**. I really appreciate your efforts towards my farm studies.

Many thanks to my new lab mate **Tracy Chatterton** for getting me into the farm in the early morning, help with me in sample collection.

I express special thanks to my friends, **Kate Pinter, Hyun Su, Tao Ye, and Alessandro Maria** for constant support and motivation over the years.

I would like to acknowledge the funding from Egyptian mission sector, to cover the expenses of my joint supervision program.

TABLE OF CONTENT

General abstract……. i

Dedication…… iii

Acknowledgements……. iv

Table of contents…… vii

List of tables…… ix

List of figures…… x

List of abbreviations…… xi

Chapter 1: GENERAL INTRODUCTION……. 1

Chapter 2: LITERATURE REVIEW……. 3

I. Bovine milk fat synthesis and factors regulate it……. 3

a) Bovine milk fat synthesis…… 3

De novo fatty acid synthesis…….. 4

Preformed fatty acid (uptake by the mammary gland)…….. 5

Glycerol-3-phosphate pathway (Triglyceride synthesis)…….. 6

Milk lipid droplet formation and cytosolic crest ……. 7

b) Factors affecting milk fat synthesis……. 9

c) Nutritional regulation of milk fat synthesis……..... 10

1. Limited energy intake ……..... 11

2. Milk fat depression (MFD, low-fat milk syndrome)…….. 12

II. Sources of RNA for mammary gene expression study…….. 17

Chapter 3: EXPERIMENT 1……. 22

Abstract…….. 23

Introduction…… 25

Materials And Methods……. 28

Results…… 35

Discussion……... 40

Conclusion ……. 52
Chapter 4: EXPERIMENT 2……. 67
Abstract…….. 68
Introduction……..... 70
Materials And Methods…….. 72
Results…… 79
Discussion…….. 84
Conclusion ……. 96
Chapter 5: a. SUMMARY ……...…… 106
b. REFERENCES…….. 108

List of tables

Table 1.1. Ingredient composition of the basal diet (% DM basis) 53

Table 1.2. Chemical composition of the basal diet (% DM basis) 54

Table 1.3. Features of primers used for RT- qPCR analysis 55

Table 1.4. Feed intake, milk yield, and milk composition in cow fed ad- libitum (Ad-Lib) or 60% of ad libitum (RES) 56

Table 1.5. Milk fatty acid composition 57

Table 1.6. Effect of feed restriction on selected mammary lipogenic gene expression 58

Table 2.1. Ingredient composition of the basal diet (% DM basis) 97

Table 2.2. Chemical composition of the basal diet (% DM basis) 98

Table 2.3. Features of primers used for RT-qPCR analysis 99

Table 2.4. Feed intake, milk yield, and milk composition in cow fed Control (CON) or CLA-containing diet (TRT) 100

Table 2.5. Milk fatty acid composition [%FAME] in cow fed Control (CON) or CLA-containing diet (TRT) 101

Table 2.6. Effect of CLA on selected mammary lipogenic gene expression 102

List of figures

Figure 1.1. DMI during the EXP and POST periods for cows fed either ad libitum or restricted to 60% of ad-libitum 59

Figure 1.2. NEFA during the EXP and POST periods for cows fed either ad libitum or restricted to 60% of ad-libitum 60

Figure 1.3. milk yield over time during the EXP and POST periods for cows fed either ad libitum or restricted to 60% of ad-libitum 61

Figure 1.4. milk fat percent over time during the EXP and POST periods for cows fed either ad libitum or restricted to 60% of ad-libitum 62

Figure 1.5. LPL gene expression during the EXP and POST periods for cows fed either ad libitum or restricted to 60% of ad-libitum 63

Figure 1.6. BTN1A1 gene expression during the EXP and POST periods for cows fed either ad libitum or restricted to 60% of ad-libitum 64

Figure 1.7. GPAM gene expression during the EXP and POST periods for cows fed either ad libitum or restricted to 60% of ad-libitum 65

Figure 1.8. SREPF1 gene expression during the EXP and POST periods for cows fed either ad libitum or restricted to 60% of ad-libitum 66

Figure 2.1. milk yield during the EXP, POST, and RECOVERY periods for cows fed either control or CLA treated diet 103

Figure 2.2. Milk fat yield during the EXP and POST periods for cows fed either control or CLA treated diet 104

Figure 2.3. milk fat percentage during the EXP and POST periods for cows fed either control or CLA treated diet 105

List of abbreviations

ACAC	Acetyl-coenzyme A carboxylase alpha
ACTB	Beta-actin
AGPAT	I-acylglycerol-3-phosphate O-acyltransferase 6
CLA	Conjugated linoleic acid
DIM	Days in milk
DMI	Dry matter intake
EXP	Experimental Period
FA	Fatty acids
FABE	Fatty acid butyl ester
FABP3	Fatty acid-binding protein 3
FAME	Fatty acid methyl ester
FASN	Fatty acid synthase
FCM	Fat corrected milk
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GPAM	Glycerol-3-phosphate acyltransferase, mitochondrial
LPL	Lipoprotein lipase
MFD	Milk fat depression
MEC	Mammary epithelial cells
MUFA	Monounsaturated fatty acid
NE_L	Net energy of lactation
NEFA	Non-esterified fatty acid
NRC	National research counsel
RES	Restricted cow
PPARγ(PPARG)	Peroxisome proliferator activated receptor gamma
PUFA	Polyunsaturated fatty acid
RPS9	Ribosomal protein S9
RPS15	Ribosomal protein S15
RQI	RNA Quality Indicator
SCD 1	Stearoyl -CoA desaturase (Delta-9-desaturase)
SFA	saturated fatty acids
SREBF1	Sterol regulatory-element binding transcription factor I
UXT	Ubiquitously-expressed transcript

Chapter 1: General Introduction

The rapid changes in dairy market, and increase the social awareness of the effect of dairy products on coronary heart disease (Jenkins and McGuire, 2006) **i**ncreased the interest of researchers to explore the mammary lipogenesis and how the diet alters the milk fat synthesis and even control the milk fatty acid profile. Feeding high concentrate, low forage diet (Piperova et al., 2000), or diets rich in polyunsaturated fatty acids (Kairenius et al., 2015), alter the milk fatty acid profile and depress milk fat production. Conversely, limiting energy intake stimulate mobilization of body fat depot and increase milk fat secretion in addition to changing fatty acid profile (Guinard-Flament, et al. (2007). Therefore, the nutritional regimen of the dairy cow controls the milk fat production and fatty acid composition. However, the effect of dietary manipulation on mammary lipogenic gene expression is limited by the mammary biopsy technique, as due to its invasiveness, does not allow repeated sampling from the same animal over short time interval (Khatun et al., 2013). One potential alternative is the cytoplasmic crescent found in milk fat globules. During the secretion of milk fat globules mammary epithelial cell cytosol becomes trapped between the emerging milk fat globule and the apical cell membrane that envelopes the milk fat globule when secreted into the mammary alveoli (Huston and Patton, 1990). It has been shown to contain all of the proteins and organelles present in the mammary epithelial cell except for the nucleus (Huston and Patton, 1990). One study examined several alternatives to mammary biopsy to study the changes in mammary lipogenic genes (Ca´novas et al., 2014), they find that milk fat globules are excellent representative to mammary epithelial cells. Two major conditions related to mammary lipogenic genes are not well