INFLUENCE OF SELECTED ESSENTIAL AMINO ACID AND FATTY ACID SUPPLEMENTATION ON GROWTH PERFORMANCE AND IMMUNE RESPONSE OF BROILER CHICKENS CHALLENGED WITH INFECTIOUS BURSAL DISEASE VIRUS

ELHAM MAROUFYAN

FP 2012 64
INFLUENCE OF SELECTED ESSENTIAL AMINO ACID AND FATTY ACID SUPPLEMENTATION ON GROWTH PERFORMANCE AND IMMUNE RESPONSE OF BROILER CHICKENS CHALLENGED WITH INFECTIOUS BURSAL DISEASE VIRUS

ELHAM MAROUFYAN

DOCTOR OF PHILOSOPHY
UNIVERSITI PUTRA MALAYSIA

2012
INFLUENCE OF SELECTED ESSENTIAL AMINO ACID AND FATTY ACID SUPPLEMENTATION ON GROWTH PERFORMANCE AND IMMUNE RESPONSE OF BROILER CHICKENS CHALLENGED WITH INFECTIOUS BURSAL DISEASE VIRUS

By

ELHAM MAROYFAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

April 2012
THIS THESIS IS DEDICATED TO

MY PARENTS

WITH

LOVE AND GRATITUDE
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

INFLUENCE OF SELECTED ESSENTIAL AMINO ACID AND FATTY ACID SUPPLEMENTATION ON GROWTH PERFORMANCE AND IMMUNE RESPONSE OF BROILER CHICKENS CHALLENGED WITH INFECTIOUS BURSAL DISEASE VIRUS

By

ELHAM MAROUFYAN

April 2012

Chairman: Associate Professor Azhar Kasim, PhD
Faculty: Agriculture

Products of poultry are now understood to be vulnerable to contagious diseases and are indicated to affect global industries. According to the research-based evidences shows that infectious bursal diseases (IBD) play a major role in economic losses due to reduced production efficiency, mortality and also the increased usage of antibiotics and chemicals to fight against infections which is a main concern for human health. Therefore, minimizing its impact is an important policy with different strategically approaches for success in the poultry industry. Nutrition is known as a strategy to control immunodeficiency. It is suggested that essential amino acids and fatty acids as immunostimulants are important for animal health. A series of experiments were conducted to examine the effects of essential amino acids and fatty acids as growth and health promotes in broiler chickens challenged by IBD virus.
Experiment I was conducted to examine the effects of dietary methionine and threonine levels higher than the National Research Council (NRC) recommendation on growth performance and immune responses of broiler chickens challenged with IBDV. A total of 450 day-old male broiler chicks (Cobb) were housed in 45 pens of 10 birds each until day 42. The dietary treatments were three levels of methionine in the form of DL-methionine; at recommended (M1), double (M2) and triple (M3) NRC levels and three levels of threonine in the form of L-threonine at recommended (T1), double (T2) and triple (T3) NRC levels. There was significant decline in body weight and feed intake in birds subjected to the highest level of threonine and methionine but highest antibody titers and also the lowest lesion score were obtained in broilers receiving M2T3 and M3T3. Performance and immune responses of chickens fed with two folds of methionine (M2T1) were significantly (P<0.05) better than other treatment. The results obtained in the present study indicated that threonine and methionine requirements of broilers based on the recommendations of NRC did not give the maximum response.

Experiment II was conducted to examine the effects of tuna fish oil and sunflower oil as sources of n-3 and n-6 PUFA on growth performances, fatty acid profiles and immune responses to IBDV challenged broiler chickens. Commencing from day one, five replicate pens of 15 one-day-Cobb male chicks each were assigned to one of the five dietary treatments, giving a total of 25 pens for 42 days. The dietary treatments were: 1) Basal diet (NRC) (C), 2) Basal diet containing 5.5% tuna oil + 0.5% sunflower oil (VL), 3) Basal diet containing 4% tuna oil + 2% sunflower oil (L), 4) Basal diet containing 2.5% Tuna oil + 3.5% sunflower oil (H) and 5) Basal diet containing 1.5% tuna oil + 4.5% sunflower oil (VH). The lowest feed
conversion ratio of 1.94 (P<0.05) was observed in birds treated with H group. The birds fed VH group had lowest level of Interferon-gamma (IFN-γ) at 2 d post challenge ((P<0.05). The increase in n-3 PUFA levels significantly (P<0.05) tended to enhance Interlukin-2 (IL-2) as well as antibody titers production in IBDV challenged broiler chickens. Therefore, an ideal fatty acid profile should be maintained in the diet to improve the broiler chickens’ immune system.

Experiment III was conducted to examine the response of IBDV challenged broiler chickens receiving different dietary ratios of n-6 to n-3 PUFA and supplementation of methionine on performance and immunity. A total of 350 one-day-old male broiler chicks (Cobb 500) were assigned to one of the six dietary treatments, giving a total of 35 cages. There were three ratio of n-6: n-3 PUFA [(45 (N1), 1.1 (N2) and 4.19 (N3)] and two levels of methionine in the form of DL-methionine [NRC (M1), 2 times NRC (M2)]. The birds aged 0-21 days were fed diets containing graded concentrations of methionine (0.25 and 0.80% of diet) similarly graded concentrations of methionine (0.23% and 0.74% of diet) to birds aged 22-42 days. Therefore, six dietary treatments were compared: 1) Basal diet based on NRC recommendation (M1N1), 2) Basal diet containing methionine 2 fold higher than NRC (M2N1), 3) Basal diet containing 5.5% tuna oil + 0.5% sunflower oil (M1N2), 4) Basal diet containing 2.5% tuna oil + 3.5% sunflower oil (M1N3), 5) Combination of diet 2 and 3 (M2N2) and 6) Combination of diet 2 and 4 (M2N3). A second control group served as IBDV-unchallenged group (CON) in this study. Pre-challenge performance data indicates that body weight gain and feed conversion ratios significantly affected by the interaction between the methionine levels and n-6: n-3 PUFA ratios. A mean body weight gain of 1411 g was highest at weeks 4 in
birds treated with M2N3 group with an improved feed conversion ratio of 1.48 at pre-challenged period. However, there was no any interaction between methionine and n-6: n-3 PUFA in broilers subjected to immunological challenge (P < 0.05). An antibody titer of 4.15 ng/ml and 4.04 ng/ml was significantly (P < 0.05) higher in birds fed a low ratio of n-6: n-3 PUFA (N2) than the other groups at 7 and 14 days after challenge. When methionine × n-6: n-3 PUFA ratio interaction was significant, the IL-2 level of M1N2 and INF-γ level of M2N1 were higher than other groups on 2 days and 7 days post-challenged, respectively. On d 28, serum cholesterol levels were significantly lowered (2.5mmol/L) in the birds treated with N2M1 (P<0.05). A significant improvement (P<0.05) in lesion score was observed in N2, N3 group on 7 days post-challenged and M2 group on 14 days post-challenged (P<0.05). Therefore, this study emphasizes those changes induced by the amount of n-6: n-3 fatty acid ratio may be supplied to the human diet as a result of consuming a portion of fat-modified chicken meat.

In conclusion, current studies demonstrated that increasing the dietary methionine level up to two-fold of NRC (1994) standards is required to achieve adequate growth, feed conversion ratio and optimal immunity in IBD challenged broiler chicken. Dietary n-3 PUFA enrichment may improve the immune response and IBD resistance, but the optimum performance does not coincide with the optimum immune response. It seems that dietary n-3 PUFA modulates the broiler chicken performance and immune response in a dose-dependent manner. Thus, a moderate level of dietary n-3 PUFA enrichment may help to put together the efficiency of performance and relative immune response enhancement in broiler chickens. Further, although there was no interaction between high level of methionine and
ratio of n-6:n-3 PUFA for performance parameters, humeral immunity and lesion score of bursa in broilers subjected to immunological challenge, the individual roles of n-6:n-3 PUFA ratio and methionine on bursa lesion reduction and/or antibody production are documented. Therefore, a balanced intake of both n-6 and n-3 fatty acid combined with methionine supplementation have the potential to promote performance and improve the broiler chicken immune system. Moreover, supplementation of fish oil in broiler diet may be considered as a functional practice to produce n-3 PUFA enriched meat with optimum n-6:n-3 PUFA ratios with regards to human health.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGARUH ASID AMINO PERLU TERPILIH DAN SUPLEMEN ASID LEMAK TERHADAP PRESTASI PERTUMBUHAN DAN RESPON IMUN PADA AYAM PEDAGING DICABAR DENGAN VIRUS PENYAKIT JANGKITAN BURSA

Oleh

ELHAM MAROUFYAN

April 2012

Pengerusi : Profesor Madya Azhar Kasim, PhD

Fakulti : Pertanian

Produk poltri kini difahami boleh terdedah kepada penyakit berjangkit dan menjadi penunjuk kesan terhadap penjejasan industri global. Banyak bukti penyelidikan menunjukkan bahawa penyakit berjangkit bursal (IBD) memainkan peranan sebagai penentu dalam kerugian ekonomi akibat daripada pengurangan pengeluaran yang efisien dan kadar kematian serta peningkatan penggunaan antibiotik dan bahan kimia untuk melawan jangkitan yang menjadi kebimbangan utama bagi kesihatan manusia. Oleh itu, meminimumkan kesannya adalah polisi penting dengan pendekatan strategik yang berbeza bagi menjayakan industri poltri. Pemakanan dikenali sebagai strategi untuk mengawal kekurangan immunisasi. Asid amino perlu dan asid lemak telah disarankan sebagai perangsang imuniti yang penting untuk
kesihatan haiwan. Satu siri eksperimen telah dijalankan untuk mengkaji kesan asid amino perlu dan asid lemak sebagai penggalak pertumbuhan dan kesihatan dalam ayam pedaging yang dicabar dengan virus IBD.

Eksperimen I telah dijalankan untuk memeriksa kesan diet dengan aras metionin dan treonin yang lebih tinggi daripada cadangan NRC ke atas prestasi pertumbuhan dan respon imun ayam pedaging dicabar dengan IBDV. Sejumlah 450 ayam pedaging (Cobb) yang baru menetas telah diletakkan di dalam 45 sangkar di mana setiap sangkar mengandungi 10 ekor ayam sehingga berusia 42 hari. Rawatan diet ialah tiga aras metionin dalam bentuk DL-metionin; seperti cadangan (M1), dua kali ganda (M2) dan tiga kali ganda (M3) aras NRC dan tiga aras treonin dalam bentuk L-treonin seperti cadangan (T1), dua kali ganda (T2) dan tiga kali ganda (T3) aras NRC. Terdapat penurunan signifikan terhadap berat badan dan pengambilan makanan pada ayam yang tertakluk kepada aras treonin dan metionin yang tertinggi tetapi titer antibodi tertinggi dan juga skor lesi terendah diperolehi dalam ayam pedaging yang menerima rawatan M2T3 dan M3T3. Prestasi dan respon imun ayam pedaging yang diberi dua kali ganda lebih tinggi metionin (M2T1) adalah ketara lebih baik berbanding daripada rawatan lain. Keputusan yang diperolehi daripada kajian ini menunjukkan bahawa keperluan treonin dan metionin oleh ayam pedaging seperti cadangan NRC adalah tidak memberi respon yang maksimum.

Eksperimen II telah dijalankan untuk memeriksa kesan minyak ikan tuna dan minyak bunga matahari sebagai sumber n-3 dan n-6 PUFA terhadap prestasi pertumbuhan, profil asid lemak dan respon imun pada ayam pedaging digab di cabar IBD. Bermula dari hari pertama, lima replikasi sangkar dengan setiapnya 15 ekor anak ayam jantan Cobb telah diberi salah satu daripada lima rawatan diet, iaitu keseluruhan sangkar ialah 25 sangkar selama 42 hari. Rawatan diet tersebut adalah:
1) Diet basis (NRC) (C), 2) Diet basis mengandungi 5.5% minyak tuna + 0.5% minyak bunga matahari (VL), 3) Diet basis mengandungi 4% minyak tuna + 2% minyak bunga matahari (L), 4) Diet basis mengandungi 2.5% minyak tuna + 3.5% minyak bunga matahari (H) dan 5) Diet basis mengandungi 1.5% minyak tuna + 4.5% minyak bunga matahari (VH). Nisbah penukaran makanan terendah sebanyak 1.94 (P <0.05) dapat dilihat dalam ayam yang dirawat dengan kumpulan H. Ayam yang diberi makan dengan kumpulan VH mempunyai aras Interferon-gama (IFN-γ) terendah selepas 2 hari dicabar (P <0.05). Peningkatan aras n-3 PUFA adalah signifikan (P <0.05) cenderung untuk meningkatkan Interlukin-2 (IL-2) serta produksi titer antibodi pada ayam pedaging dicabar IBDV. Oleh itu, profil asid lemak yang ideal perlu dikekalkan dalam diet bagi meningkatkan sistem imun ayam pedaging.

Eksperimen III telah dijalankan untuk memeriksa respon ayam pedaging yang dicabar IBDV di samping menerima nisbah diet berbeza iaitu PUFA n-6 kepada n-3 dan suplemen metionin terhadap prestasi dan imuniti. Sebanyak 350 anak ayam jantan pedaging (Cobb 500) telah dibahagikan kepada salah satu daripada enam rawatan diet, iaitu sejumlah 35 sangkar. Terdapat tiga nisbah PUFA n-6: n-3 [(45 (N1), 1.1 (N2) dan 4.19 (N3)] dan dua aras metionin dalam bentuk DL-metionin [NRC (M1), 2 kali ganda NRC (M2)]. Ayam yang berumur 0-21 hari telah diberi makan diet yang mengandungi metionin pada nilai kepekatan (0.25 dan 0.80% dari pada diet) dan sama keadaannya dengan ayam yang berumur 22-42 hari diberi metionin pada nilai kepekatan (0.23 dan 0.74% daripada diet). Oleh kerana itu, enam rawatan diet telah dibandingkan: 1) Diet basis berdasarkan cadangan NRC (M1N1), 2) Diet basis mangandungi metionin 2 kali ganda lebih banyak daripada NRC (M2N1), 3) Diet basis mengandungi 5.5% minyak tuna + 0.5% minyak bunga
matahari (M1N2), 4) Diet basis mengandungi 2.5% minyak tuna + 3.5% minyak bunga matahari (M1N3), 5) Kombinasi antara diet 2 dan 3 (M2N2) dan 6) Kombinasi antara diet 2 dan 4 (M2N3). Kumpulan kawalan kedua bertindak sebagai kumpulan tidak dicabar IBDV (CON) dalam kajian ini. Data prestasi sebelum dicabar menunjukkan peolehan berat badan dan nisbah penukaran makanan secara signifikan (p < 0.05) dipengaruhi oleh interaksi antara aras metionin dan nisbah n-6: n-3 PUFA. Purata berat badan ayam ialah 1411g adalah paling tinggi pada minggu ke 4 yang diberi rawatan M2N3 dengan peningkatkan nisbah penukaran makanan 1.48 pada waktu sebelum dicabar. Walau bagaimanapun, tiada interaksi didapati antara metionin dengan n-6: n-3 PUFA pada ayam pedaging yang menghadapi cabaran imunologi (P > 0.05). Titer antibodi 4.15 ng/ml dan 4.04 ng/ml adalah signifikasi (P < 0.05) lebih tinggi dalam ayam yang diberi makan pada nisbah rendah n-6: n-3 PUFA (N2) berbanding daripada kumpulan lain pada hari ke 7 dan 14 selepas dicabar. Semasa interaksi antara metionin x nisbah PUFA n-6: n-3 adalah signifikan (P < 0.05), aras IL-2 daripada M1N2 dan aras INF- dan aras M2N1 adalah lebih tinggi daripada kumpulan lain masing-masing pada hari ke 2 dan 7 selepas dicabar. Pada hari ke 28, ayam yang dirawat N2M1 mempunyai aras serum kolestrol (2.5mmol/L) yang signifikan lebih rendah (P < 0.05). Pencapaian signifikan (P < 0.05) dalam skor lesi telah dilihat pada kumpulan N2, N3 selepas 7 hari dicabar dan kumpulan M2 selepas 14 hari dicabar (P < 0.05). Oleh demikian, kajian ini menekankan bahawa perubahan tersebut didorong oleh jumlah nisbah asid lemak n-6: n-3 yang dibekalkan kepada diet manusia sebagai kesan terhadap pengambilan daging ayam yang lemaknya telah terubah- suai.

Kesimpulannya, kajian terkini membuktikan bahawa peningkatan aras diet metionin kepada dua kali ganda daripada keperluan NRC (1994) dalam diet adalah diperlukan
untuk mencapai pertumbuhan yang cukup, nisbah petukaran makanan dan imuniti optima dalam ayam pedaging dicabar IBD. Pengkayaan diet n-3 PUFA mampu meningkatkan respon imun dan pertahanan IBD, namun prestasi optima tidak setara dengan respon imun optima. Diet n-3 PUFA mengubah prestasi ayam pedaging dan respon imun dengan kebergantungan terhadap dos. Oleh sebab itu, aras sederhana n-3 PUFA yang diperkaya dalam diet boleh membantu meningkatkan prestasi yang efisyen dan peningkatan relatif respon imun dalam ayam pedaging. Walaupun tiada interaksi anatara aras tinggi metionin dengan nisbah n-6:n-3 PUFA untuk parameter prestasi, imuniti humeral dan skor lesi bursa ayam pedaging yang dikaitkan dengan cabaran imunologi, peranan individu pada nisbah n-6:n-3 PUFA dan metionin pada pengurangan lesi bursa dan / atau penghasilan antibodi telah didokumenkan. Oleh demikian, pengambilan kedua-dua asid lemak n-6 dan n-3 yang seimbang dengan kombinasi suplemen metionin mempunyai potensi untuk menggalakkan prestasi dan memperbaiki sistem imun ayam pedaging. Tambahan pula, suplemen minyak ikan dalam diet ayam pedaging mungkin boleh dipertimbangkan sebagai amalan berfungsi untuk menghasilkan daging diperkaya PUFA n-3 dengan nisbah PUFA n-6:n-3 yang optima demi kesihatan manusia.
ACKNOWLEDGEMENTS

An effort such as this dissertation is only made possible through the immeasurable support, mentorship, time and patience of many individuals. I am indebted to all who have helped along the way and made the current work a reality.

First and foremost, I would like to express my honest appreciation to my supervisory committee, who were involved in my training towards obtaining this degree. I am most grateful to Assoc. Prof. Dr. Azhar Kasim, chairman of my supervisory committee for his patience, tireless support, willingness to help, encouragement, kindness and guidance throughout the research and during the preparation of the thesis. I am very much indebted to the members of my supervisory committee namely Professor Dr. Mohd Hair Bejo and Professor Loh Teck Chwen for their generous financial support, encouragement, constructive discussion, excellent advice, comments and suggestions throughout the project.

I wish to express my sincere gratitude to the Deans of the Faculty of Agriculture Faculty of Veterinary Medicine and Universiti Putra Malaysia for the use of their facilities and the unlimited assistance from their staff during the course of this study.

I will always cherish help from farm technician, Mr Mazlan Hamzah, Ponnusamy Muniandy, Azam Azman and Khairulnizam Kamarudin during the experimental period.
I want to express my huge thanks to fellow students have given lots of help in my research namely Seyed Reza Hashemi, AbdoReza Soleimani, Mahdi Ebrahimi, Arash Javanmard and specially Tang Siew Ching for thesis abstract translation to Bahasa Malaysia. I would also like to thank my Heavenly Father Mohammed Ali and Mother Esmat for providing so many opportunities for both my physical and spiritual growth. They have strengthened me during the times when I thought I could go no further. Without them, none of this would have been possible.
I certify that a Thesis Examination Committee has met on 17 April 2012 to conduct the final examination of Elham Maroufyan on her thesis entitled "Selected Essential Amino Acid and Fatty Acid on Growth Performance and Immune Response of Broiler Chickens Infected with Infectious Bursal Disease Virus" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Doctor of Philosophy (Ph.D).

Members of the Thesis Examination Committee were as follows:

Zulkifli Idrus, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Abdul Razak Alimon, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Mohd Ali Rajion, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

Gene M. Pesti, PhD
Professor
Faculty of Agriculture
Universiti of Georgia
(Independent Examiner)

BUJANG KIM HUAT, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:
This thesis was submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

**Azhar Kasim, Ph.D**  
Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Chairman)

**Mohd Hair Bejo, Ph.D**  
Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Member)

**Loh Teck Chwen, Ph.D**  
Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Member)

-----------------------------------------------------------------------

**BUJANG BIN KIM HUAT, PhD**  
Professor/Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:
DECLARATION

I declare that the thesis is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously, and it is not concurrently submitted for any other degree at Universiti Putra Malaysia or at any other institution.

Elham Maroufyan

Date: 17 April 20122
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>TABLE OF CONTENTS</th>
<th>page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>Viii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>Xiii</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>Xv</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>Xvii</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>Xviii</td>
</tr>
<tr>
<td>LIST OF TABLE</td>
<td>Xxiii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>Xxvii</td>
</tr>
<tr>
<td>LIST OF ABBRIVATION</td>
<td>Xxviii</td>
</tr>
</tbody>
</table>

## CHAPTER

### 1. INTRODUCTION

1.1 General Introduction                                                1  
1.2 Statement of the Research Purposes                                  2  
1.3 Statement of Research Problems                                     5  
1.4 General research Objectives                                         6  
1.5 Hypothesis                                                         7  

### 2. LITERATURE AND REVIEW

2.1 Introduction                                                        8  
2.2 Infectious Bursal Disease (IBD)                                     9  
2.2.1 Pathogenesis of IBD                                              12  
2.2.2 Epidemiology and mode of transmission                            14  
2.2.3 Clinical signs                                                   15  
2.2.4 Immunosuppression                                                16  
2.2.5 Gross lesions                                                    17  
2.2.6 Diagnosis                                                        18  
2.2.6.1 Virus isolation                                                19  
2.2.6.2 Serology                                                       20  
2.2.7 Prevention                                                       22  
2.3 The Avian Immune System                                             23  
2.4 Interrelationship of Nutrition and Immunity                        24  
2.5 Amino Acid and Immune Function                                      25  
2.5.1 Methionine                                                       32  
2.5.2 Methionine metabolism                                            35  
2.5.3 Threonine                                                        37  
2.5.4 Threonine metabolism                                             39  
2.6 Essential Fatty Acid                                                41  
2.6.1 Dietary lipids                                                   44  
2.6.1.1 Palm oil                                                       44  
2.6.1.2 Fish oil                                                       45  
2.6.1.3 Sunflower oil                                                  46  
2.6.2 Fatty acid metabolism and Immune Function                       47  
2.6.2.1 Lipid rafts, T cell signaling and Fatty acids                  47  
2.6.2.2 Fatty acids and antigen presentation                            48  
2.6.2.3 Fatty acid-derived mediators                                   48
3. **EFFECTS OF METHIONINE AND THREONINE SUPPLEMENTATION ON GROWTH PERFORMANCE AND IMMUNE RESPONSE OF BROILER CHICKENS CHALLENGED WITH INFECTIOUS BURSAL DISEASE VIRUS**

3.1. **Introduction**

3.2. **Materials and Methods**
   - 3.2.1. Birds and housing environment
   - 3.2.2. Experimental design
   - 3.2.3. Chemical analysis
   - 3.2.4. Chicken vaccination
   - 3.2.5. Challenge protocol
   - 3.2.6. Performance parameters
   - 3.2.7. Lymphoid Organ weight
   - 3.2.8. Serum metabolites
   - 3.2.9. Serology and immune responses
   - 3.2.10. Histopathological changes and bursal lesion score
   - 3.2.11. Statistical analysis

3.3. **Results**
   - 3.3.1. Body weight
   - 3.3.2. Feed intake
   - 3.3.3. Feed conversion rate
   - 3.3.4. Mortality
   - 3.3.5. ND and IBD antibody titer levels
   - 3.3.6. Lymphoid organ weight
   - 3.3.7. Lesion score of bursa Fabricious
   - 3.3.8. Serum proteins

3.4. **Discussion**
   - 3.4.1. Growth performance traits
   - 3.4.2. Serum antibody responses
   - 3.4.3. Histopathology of IBDV-induced lesions in bursa
   - 3.4.4. Serum biochemical parameters

3.2. **Conclusion**

---

xix
4. EFFECT OF N-6 TO N-3 RATIO ON PERFORMANCE AND IMMUNE RESPONSES OF BROILER CHICKENS CHALLENGED WITH INFECTIOUS BURSAL DISEASE VIRUS

4.1 Introduction

4.2 Materials and Methods
  4.2.1 Birds and housing environment
  4.2.2 Experimental design
  4.2.3 Challenge protocol
  4.2.4 Performance parameters
  4.2.5 IL2 and INF-
  4.2.6 Fatty acid composition analysis
  4.2.7 Blood parameters, antibody titers, organ weight and histopathological examination
  4.2.8 Statistical analysis

4.3 Results
  4.3.1 Growth performance
  4.3.2 Mortality rate
  4.3.3 IL-2 and INF- serum levels
  4.3.4 Serum antibody titer
  4.3.5 Blood parameters
  4.3.6 Lymphoid organ weight
  4.3.7 Histopathological lesion scores
  4.3.8 Fatty acid composition of oil and diets
  4.3.9 Plasma fatty acid
  4.3.10 Fatty acid profile of breast meat

4.4 Discussion
  4.4.1 Comparison of growth, feed intake, and feed efficiency
  4.4.2 Cell mediate immunity (CMI) response
  4.4.3 Antibody responses
  4.4.4 Changes in the fatty acid composition

4.5 Conclusion
EFFECT OF METHIONINE AND ESSENTIAL FATTY ACID SUPPLEMENTATION ON PERFORMANCE AND IMMUNE RESPONSES OF BROILER CHICKENS CHALLENGED WITH INFECTIOUS BURSAL DISEASE VIRUS

5.1 Introduction

5.2 Materials and Methods
5.2.1 Birds and housing environment
5.2.2 Experimental design
5.2.3 Chemical analysis
5.2.3.1 Determination of dry matter
5.2.3.2 Determination of ash content
5.2.3.3 Determination of crude protein content
5.2.3.4 Determination of crude fiber
5.2.3.5 Determination of crude fat
5.2.3.6 Amino acid composition of diets
5.2.4 Challenge protocol
5.2.5 Performance parameters
5.2.6 Lymphoid Organ weight
5.2.7 Serum metabolites, liver function test, heterophils to lymphocytes ratio and total white blood cell count
5.2.8 Serological analysis
5.2.9 IL-2 and INF-γ assay
5.2.10 Fatty acid analysis
5.2.11 Peroxide value (PV) of oil
5.2.12 Histopathological changes and bursal lesion score
5.2.13 Statistical analysis

5.3 Results
5.3.1 Growth characteristics
5.3.2 Mortality rate
5.3.3 Serum antibody titer to IBDV
5.3.4 Cytokine production
5.3.5 Lesion scores
5.3.6 Liver Function Test (LFT)
5.3.7 Serum metabolites
5.3.8 Serum Cholesterol and triglyceride concentration
5.3.9 Lymphoid organ weight
5.3.10 Fatty acid composition of dietary feeds, oils and peroxide value
5.3.11 Plasma fatty acid profile
5.3.12 Breast meat fatty acid profile

5.4 Discussion
5.4.1 Performance parameters
5.4.2 Humoral responses to IBD
5.4.3 Chickens IL-2 and INF-ELIZA assay
5.4.4 Histopathological changes in bursa of Fabricious
5.4.5 Assessment of biochemical functions in serum
5.4.6 Feed and plasma, breast meat fatty acids determination

5.5 Conclusion
6 GENERAL DISCUSSION 174
7 SUMMARY AND CONCLUSION 182
REFERENCES 185
APPENDICES 219
BIODATA OF STUDENT 226
LIST OF PUBLICATIONS 227