UNIVERSITI PUTRA MALAYSIA

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

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By

ELHAM MAROUFYAN

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

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April 2012

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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-) 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- 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 (2.5mmo/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

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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 efisyen 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 signifikasi 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 dicabar 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-) 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 daripdada 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% daripada 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- daripada 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 (2.5mmo/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 menghasikan daging diperkaya PUFA *n-3* dengan nisbah PUFA *n-6:n-3* yang optima demi kesihatan manusia.

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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).

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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

	page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	Viii
ACKNOWLEDGEMENTS	Xiii
APPROVAL	Xv
DECLARATION	Xvii
TABLE OF CONTENTS	Xviii
LIST OF TABLE	Xxiii
LIST OF FIGURES	Xxvii
LIST OF ABBRIVATION	Xxviii

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

2.7 Significance of Study 51

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 52
3.2. Materials and Methods
3.2.1. Birds and housing environment 54
3.2.2. Experimental design 54
3.2.3. Chemical analysis 57
3.2.4. Chicken vaccination 59
3.2.5. Challenge protocol 59
3.2.6. Performance parameters 59
3.2.7. Lymphoid Organ weight 59
3.2.8. Serum metabolites 60
3.2.9. Serology and immune responses 60
3.2.10. Histopathological changes and bursal lesion score 61
3.2.11. Statistical analysis 62
3.3. Results
3.3.1. Body weight 64
3.3.2. Feed intake 64
3.3.3. Feed conversion rate 64
3.3.4. Mortality 65
3.3.5. ND and IBD antibody titer levels 66
3.3.6. Lymphoid organ weight 68
3.3.7. Lesion score of bursa Fabricious 70
3.3.8. Serum proteins 72
3.4. Discussion
3.4.1. Growth performance traits 75
3.4.2. Serum antibody responses 78
3.4.3. Histopathology of IBDV-induced lesions in bursa 79
3.4.4. Serum biochemical parameters 80
3.2. Conclusion 82

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 83
4.2 Materials and Methods
4.2.1 Birds and housing environment 85
4.2.2 Experimental design 85
4.2.3 Challenge protocol 85
4.2.4 Performance parameters 88
4.2.5 IL2 and INF- 88
4.2.6 Fatty acid composition analysis 89
4.2.7 Blood parameters, antibody titers, organ weight and histopathological examination 90
4.2.8 Statistical analysis 92

4.3 Results
4.3.1 Growth performance 93
4.3.2 Mortality rate 93
4.3.3 IL-2 and INF- serum levels 95
4.3.4 Serum antibody titer 97
4.3.5 Blood parameters 99
4.3.6 Lymphoid organ weight 101
4.3.7 Histopathological lesion scores 103
4.3.8 Fatty acid composition of oil and diets 105
4.3.9 Plasma fatty acid 108
4.3.10 Fatty acid profile of breast meat 111

4.4 Discussion
4.4.1 Comparison of growth, feed intake, and feed efficiency 113
4.4.2 Cell mediate immunity (CMI) response 114
4.4.3 Antibody responses 117
4.4.4 Changes in the fatty acid composition 118

4.5 Conclusion 121

5 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 122

5.2 Materials and Methods
- 5.2.1 Birds and housing environment 124
- 5.2.2 Experimental design 124
- 5.2.3 Chemical analysis 127
  - 5.2.3.1 Determination of dry matter 127
  - 5.2.3.2 Determination of ash content 127
  - 5.2.3.3 Determination of crude protein content 127
  - 5.2.3.4 Determination of crude fiber 128
  - 5.2.3.5 Determination of crude fat 128
  - 5.2.3.6 Amino acid composition of diets 130
- 5.2.4 Challenge protocol 132
- 5.2.5 Performance parameters 132
- 5.2.6 Lymphoid Organ weight 132
- 5.2.7 Serum metabolites, liver function test, heterophils to lymphocytes ratio and total white blood cell count 132
- 5.2.8 Serological analysis 135
- 5.2.9 IL2 and INF- assay 135
- 5.2.10 Fatty acid analysis 135
- 5.2.11 Peroxide value (PV) of oil 135
- 5.2.12 Histopathological changes and bursal lesion score 135
- 5.2.13 Statistical analysis 136

5.3 Results
- 5.3.1 Growth characteristics 137
- 5.3.2 Mortality rate 137
- 5.3.3 Serum antibody titer to IBDV 139
- 5.3.4 Cytokine production 142
- 5.3.5 Lesion scores 145
- 5.3.6 Liver Function Test (LFT) 147
- 5.3.7 Serum metabolites 148
- 5.3.8 Serum Cholesterol and triglyceride concentration 151
- 5.3.9 Lymphoid organ weight 154
- 5.3.10 Fatty acid composition of dietary feeds, oils and peroxide value 156
- 5.3.11 Plasma fatty acid profile 161
- 5.3.12 Breast meat fatty acid profile 161

5.4 Discussion
- 5.4.1 Performance parameters 163
- 5.4.2 Humoral responses to IBD 165
- 5.4.3 Chickens IL-2 and INF- ELIZA assay 156
- 5.4.4 Histopathological changes in bursa of Fabricious 158
- 5.4.5 Assessment of biochemical functions in serum 170
- 5.4.6 Feed and plasma, breast meat fatty acids determination 171

5.5 Conclusion 173

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