

UNIVERSITI PUTRA MALAYSIA

EFFECTS OF DIETARY FATTY ACID SATURATION ON BROHLER CHICKENS SUBJECTED TO HIGH AMBIENT TEMPERATURES

NWE NWE HTIN.

FP 2005 4



EFFECTS OF DIETARY FATTY ACID SATURATION ON BROILER CHICKENS SUBJECTED TO HIGH AMBIENT TEMPERATURES

By

NWE NWE HTIN

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

January 2006



Dedicated to my beloved parents, devoted husband and dearest only one son



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

EFFECTS OF DIETARY FATTY ACID SATURATION ON BROILER CHICKENS SUBJECTED TO HIGH AMBIENT TEMPERATURES

By

NWE NWE HTIN

January 2006

Chairman: Professor Zulkifli Idrus, PhD

Faculty:

Agriculture

The effects of dietary fat with various fatty acid saturations on physiological response, performance, carcass fatty acid deposition, and immune response and disease resistance in heat stressed broiler chicks were studied. Day old male broilers chicks (Cobb) were brooded and consequently maintained at 24 ± 1 °C in an environmentally controlled house. All the chicks were fed a starter ration without added fat. On day 21 onwards, equal numbers of chicks were provided isocaloric and isonitrogenous finisher diets containing different oil sources namely 8% menhaden fish oil (FO), 8% soybean oil (SO), 8% coconut oil (CO), 8% palm oil (PO) or no added fat (control). From day 28 to 41, all birds were exposed to $36 \pm 1^{\circ}$ C for 2 h/day. Following 14 days of the heat challenge, the PO birds had greater body weights than the other three groups. The control and PO birds less hyperthermic and had smaller increases heterophil/lymphocyte ratio than those provided FO, SO and CO diets. Although the mortality rate of PO birds was higher than the control, it was lower than their



FO, SO and CO counterparts. Diets rich in saturated fatty acids (CO) increased abdominal fat and crude fat per cent of thigh meat as compared to diets rich in polyunsaturated fatty acids (SO and FO). Tissue fatty acid deposition was significantly different according to dietary oil sources, specific to tissue type, fatty acid structure, and the amount of deposition was not proportional to its intake. Broilers fed 8% fish oil showed higher concentration of long-chain n-3 PUFA (EPA and DHA) in the meat tissue than other counterparts. High inclusion levels of dietary PUFA could provide the recommended polyunsaturated to saturated fatty acid ratio in meat tissue of broilers under high ambient temperatures.

Broiler chicks (Cobb) were used to study dietary self-selection of fat under high ambient temperatures. Commencing from day 21, chicks were assigned to one of four dietary treatments: (1) diet with 8% palm oil (PO); (2) diet with 8% soybean oil (SO); (3) diet without added fat (control); and (4) a choice of PO, SO and control (CH). From day 28 to 41, all birds were exposed to 34 ± 1°C continuously. High addition of palm oil but not soybean oil improved survivability and reduced serum creatine kinase levels of broiler chickens during heat exposure. On day 41, the body weights of PO, SO and CH birds were greater than controls. Although the intake of control, PO and SO diets was similar during heat exposure, the CH birds had a lower creatine kinase activity and mortality rate than those provided SO diet but not significantly different from those fed control and PO diets. It was concluded that a high addition of palm oil but not soybean oil is beneficial to heat-stressed broiler chickens. Self-



selection of high fat diet can allow birds to match their physiological requirement under heat stress conditions.

The effects of dietary α-linolenic and linoleic fatty acid on disease resistance and immune response of heat-stressed broiler chicks (Cobb) were investigated. From day 21 onwards, broiler chicks were fed isocaloric and isonitrogenous finisher diets containing either 8% palm oil (neither rich in linolenic or linoleic acid), 8% soybean oil (rich in linoleic acid) and 8% flaxseed oil (rich in linolenic acid). All birds were vaccinated against Newcastle disease on day 7 and 21. From day 36 to 50, equal numbers of birds from each dietary group were exposed to 38 \pm 1°C and 80% relative humidity for 2 h/day. The remaining birds were maintained under 24 ± 1 °C. Feed and water were not provided throughout the heat challenge period. On day 37, all chicks were intranasally challenged with an infectious bursal disease vaccine, V877 strain (Malaysia Vaccine and Pharmaceuticals Sdn Bhd, Kuala Lumpur, Malaysia). Bursal samples were taken for histopathological examination, determination of viral RNA and fatty acid analysis. Significantly less viral replications were detected in both heated and non-heated broiler chicks fed diet containing 8% flaxseed oil on day 7 post infection. Broiler chicks fed 8% palm oil showed significantly higher viral replications on day 7 post infection under both lower and higher ambient temperatures. Mortality, heterophil/lymphocyte ratio, antibody production and bursal lesion scores were not significantly affected which suggests that palm oil may enhance tolerance to infectious bursal disease under both ambient temperatures.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN KETEPUAN ASID LEMAK PERMAKANAN KE ATAS AYAM PEDAGING YANG DIKENAKAN SUHU PERSEKITARAN YANG TINGGI

Oleh

NWE NWE HTIN

January 2006

Pengerusi: Profesor Zulkifli Idrus, PhD

Fakulti:

Pertanian

Kesan lemak pemakanan dengan berbagai ketepuan asid lemak keatas respon fisiologi, prestasi, pengumpulan asid lemak karkas, dan respon imun dan ketahanan penyakit dalam anak ayam bertekanan haba telah dikaji. Anak ayam (cobb) berumur sehari telah diternak pada suhu 24 ± 1°C dalam rumah persekitaran terkawal. Kesemua anak ayam diberi makanan permulaan tanpa tambahan lemak. Selepas hari ke 21, sebilangan yang sama anak ayam telah diberikan diet rangsum yang isokalorik dan isonitrogenus yang mengandungi sumber minyak yang berbeza iaitu 8% minyak ikan 'menhaden', (FO), 8% minyak kacang soya (SO), 8% minyak kelapa (CO), 8% minyak kelapa sawit (PO) atau tiada tambahan lemak (kawalan). Daripada hari 28 hingga 41, kesemua ayam telah didedahkan kepada 36 ± 1 °C selama 2 jam/hari. Selapas 14 hari cabaran haba, ayam PO mempunyai berat badan yang lebih berbanding tiga kumpulan lain. Ayam kawalan dan PO adalah kurang hypertermik dan



mempunyai peningkatan nisbah heterofil/limfosit yang kecil daripada diberikan oleh rangsum FO, SO dan CO. Walaupun kadar mortality ayam PO lebih tinggi dari kawalan, ia adalah lebih rendah dari FO, SO dan CO. Rangsum yang kaya dalam asid lemak tepu telah meningkatkan lemak abdominal dan peratus lemak kasar daging peha lebih daripada rangsum yang kaya dalam asid lemak poli tak tepu. Pemendapan asid lemak pada tisu adalah berbeza dengan bererti mengikut kepada sumber minyak pemakanan; khusus kepada jenis tisu, struktur asid lemak, dan jumlah pemendapan adalah tidak berkadaran dengan pengambilan makanan. Ayam pedaging yang diberi makan 8% minyak ikan menunjukkan kepekatan n-3 PUFA berantaian panjang (EPA dan DHA) yang lebih tinggi dalam tisu daging berbanding dengan kumpulam. Paras kemasukan PUFA yang tinggi mungkin boleh mencadangkan nisbah asid lemak poli tak tepu kepada asid lemak tepu dalam tisu daging ayam pedaging dibawah suhu persekitaran yang tinggi.

Anak ayam pedaging telah digunakan untuk mengkaji pemilihan sendiri lemak pemakanan dibawah suhu persekitaran yang tinggi. Bermula dari hari ke 21, anak ayam telah diberikan salah satu dari rawatan makanan berikut: (1) rangsum dengan 8% minyak kelapa sawit (PO); (2) rangsum dengan 8% minyak kacang soya (SO); (3) rangsum tanpa tambahan lemak (kawalan); dan (4) pilihan PO, SO dan kawalan (CH). Daripada hari ke 28 hingga 41, kesemua ayam telah didedahkan kepada suhu 34 ± 1 °C secara berterusan. Penambahan minyak kelapa sawit yang tinggi telah memperbaiki kelangsunganhidup dan mengurangkan paras kreatinkinase serum ayam semasa pendedahan haba, tetapi



tidak pada minyak kacang soya. Pada hari ke 41, berat badan ayam PO, SO dan CH adalah lebih berbanding kawalan. Walaupun pengambilan makanan bagi rangsum kawalan, PO dan SO adalah sama semasa pendedahan haba, ayam CH mempunyai aktiviti kreatine kinase dan kadar mortality yang rendah dari ayam yang diberikan rangsum SO tetapi tidak berbeza dengan bererti dengan ayam yang diberi makan rangsum kawalan dan PO. Adalah disimpulkan bahawa penambahan tinggi minyak kelapa sawit tetapi tidak minyak kacang soya adalah bermanfaat kepada ayam pedaging bertekanan haba. Pemilihan sendiri rangsum berlemak tinggi membolehkan ayam menyesuaikan keperluan fisiologi di bawah keadaan tekanan haba.

Kesan asid lemak α-linolenik dan linoleik keatas respon imun ayam pedaging terhadap IBD dibawah tekanan haba dan keadaan termoneutral telah dikaji. Daripada hari 21 keatas, anak ayam pedaging telah diberi makanann pengakhir yang isokalorik dan isonitrogenus mengandungi samada 8% minyak kelapa sawit (PO, samada kaya dalam asid linolenik atau linoleik), 8% minyak kacang soya (SO, kaya dalan asid linoleik) dan 8% minyak biji flax (FXO, kaya dalan asid linolenik) telah disediakan kepada ayam pedaging selapas hari ke 22. Kesemua ayam telah diberi vaksin penyakit Newcastle pada hari ke 7 dan hari ke 21. Profil asid lemak pemakanan telah dianalisa dengan kromatografi gas. Daripada hari ke 36 hingga 50, sebilangan yang sama ayam dari setiap kumpulan rawatan telah didedahkan kepada suhu 38 ± 1 °C dan kelembapan bandingan 80% selama 2 jam/hari. Ayam yang selebihnya diletakkan dalam keadaan termoneutral (24 ± 1 °C). Makanan dan minuman tidak diberikan sepanjang tempoh cabaran haba.



Pada hari ke 37, semua ayam telah dicabar secara intranasal dengan vaksin IBD, strain V877 (Malaysia Vaccine & Pharmaceuticals SDN BHD, Kuala Lumpur, Malaysia). Sampel bursal telah diambil untuk analisa histopatologi, penentuan RNA virus dan analisis asid lemak. Kurang replikasi virus telah dikesan dengan bererti dalam kedua anak ayam yang bertekanan haba dan tidak yang diberi rangsum mengandungi 8% minyak biji flax pada hari ke 7 selepas infeksi. Anak ayam pedaging diberi makan 8% minyak kelapa sawit menunjukkan replikasi virus yang lebih tinggi dengan bererti, pada hari ke 7 selepas infeksi dibawah suhu persekitaran yang rendah dan tinggi. Mortriliti, nisbah heterofil/limfosit, pengeluaran antibody dan skor lesi bursal adalah tidak memberi kesan dengan bererti, ia mencadangkan bahawa minyak kelapa sawit mungkin memperbaiki ketahanan kepada IBD dibawah kedua-dua suhu persekitaran.



ACKNOWLEDGEMENTS

The author would like to express deep appreciation and authentic gratitude to esteemed Professor Dr. Zulkifli Idrus, chairman of the supervisory committee for his invaluable advice and guidance, fruitful criticism, creditable suggestions, and patience in reading this dissertation through the labyrinth of study.

The author would like to describe the utmost gratitude to the esteemed members of supervisory committee, Professor, Dr. Abd. Razak Alimon, Associate Professor, Dr. Mohd. Hair-Bejo and Associate Professor Dr. Loh Teck Chwen for their creditable guidance, constructive suggestions and critical reviewing of this dissertation.

The contributions of Professor Dr. Mohd. Ali Rajion and Dr. Goh Yong Meng are highly appreciated. Deep appreciation and heartfelt gratitude are extended to Associate Professor, Dr. Abdul. Rahman Omar, post graduate students from the Biologic Lab, Faculty of Veterinary Medicine, especially to Ms. Kong Lih Ling, Ms. Tan Sheau Wei, Mr. Wang Keng Fei, Dr. Rooservien and Ms. Nurulfiza for guidance and enthusiastic support while studying and conducting RT-PCR and etc. Special thanks are also extended to Puan Rodiah Husin, staff from the Biologic Lab, Faculty of Veterinary Medicine, for guidance in ELISA approach.

The author gratefully acknowledges the Malaysian Technical Cooperation Programme, Department of Public Services (JPA) for sponsorship. Sincere



heartfelt gratitude is extended to esteemed authorities from Universiti Putra Malaysia for supportive encouragement to be able to continue the study here. The author owed with deep appreciation and genuine gratitude to honourable Minister, all the admirable authorities from the Ministry of Livestock Breeding and Fisheries, Myanmar. The esteemed Director General U Maung Maung Nyunt and all those respectful authorities from Livestock Breeding and Veterinary Department, Myanmar are highly appreciated for fruitful encouragement through the labyrinth of study.

The author would like to extend the utmost gratitude with respect to Professor/Rector Dr. Myint Thein, Professor/Pro-rector Dr. Ni Ni Maw and Professor/Pro-rector Dr. Khin Ma Ma from University of Veterinary Science, Yezin, Myanmar for invaluable supportive encouragement. The respectful Professor Dr. Sheikh Omar from the Faculty of Veterinary Medicine, Universiti Putra Malaysia and the esteemed former Rectors, Professor Dr. Min Soe, Professor Dr. Tun Sein, Professor Dr. Aung Than, Professor Dr. Maung Maung san and Pro-rector, Professor Dr. Tin Htwe from University of Veterinary Science, Yezin are gratefully acknowledged with genuine appreciation and heartfelt gratitude for supporting the author to be able to study in Universiti Putra Malaysia, Malaysia.

The help from staffs at the Poultry Research Unit of Universiti Putra Malaysia, Mr. Ponnusamy Muniandy, Mr. Mazlan Hamzah and Mr. Hailunizam Mohd. Sam are gratefully acknowledged. The author would like to express appreciation

to Mr. Saparin Demin, Mr. Ibrahim Mohsin, Mr. Abdul Halin Isa, and Mr. Bakari Abd. Rahman (staffs from the Nutrition Lab, Animal Science Department, Faculty of Agriculture) and Mr. Saipuzaman Ali and Mr. Mohd. Halmi Othman (staffs from the Faculty of Veterinary Medicine) for their enthusiastic help and technical skills while conducting laboratory work.

Special thanks are due to all those friends for their fine co-operations and moral support by all means during the study. The author owed to those, who have made things run smoothly throughout the study.

Ultimately, the author deeply is indebted to her late father U Htin Aung, beloved mother Daw Kyin Yi, devoted husband Professor Dr. Aung Tun Khaing, sister Nyunt Nyunt Htin and brother Pe Thet Htin, dearest only one son, Htin Kyaw Aung for their endurance and precious supportive encouragement in various aspects during her study in Malaysia.



TABLE OF CONTENTS

		Page
DEI	DICATION	ii
ABS	STRACT	iii
ABS	STRAK	vi
DEDICATION ABSTRACT ABSTRAK ACKNOWLEDGEMENTS APPROVAL DECLARATION LIST OF TABLES LIST OF FIGURES LIST OF PLATES LIST OF PLATES LIST OF ABBREVIATIONS CHAPTER I INTRODUCTION II LITERATURE REVIEW General Responses to Stress Heat Stress in Poultry Physiological Response to Heat Stress Behavioural Response to Heat Stress Hormonal Response to Heat Stress Corticosterone Thyroid hormone Heat Shock Proteins Effects of Ambient Temperature on Growth Performance Effects of Ambient Temperature on Dietary Self-Selection Alleviating Heat Stress Problems Dietary Fat in Poultry Essential Fatty Acids in Poultry Diet Chicken Immune System Effects of Dietary Fatty Acids on Chicken Immune System Effects of Dietary Fatty Acids on Chicken Immune System Effects of Dietary Fatty Acids on Chicken Immune System Effects OF VARIOUS SOURCES OF DIETARY FATS ON GROWTH AND HEAT TOLERANCE IN HEAT-STRESSED BROILER CHICKENS Introduction Materials and Methods	x	
	xiii	
	ABSTRACT ABSTRAK ACKNOWLEDGEMENTS APPROVAL DECLARATION LIST OF TABLES LIST OF FIGURES LIST OF PLATES LIST OF ABBREVIATIONS CHAPTER I INTRODUCTION II LITERATURE REVIEW General Responses to Stress Heat Stress in Poultry Physiological Response to Heat Stress Behavioural Response to Heat Stress Hormonal Response to Heat Stress Corticosterone Thyroid hormone Heat Shock Proteins Effects of Ambient Temperature on Growth Performance Effects of Ambient Temperature on Dietary Self-Selection Alleviating Heat Stress Problems Dietary Fat in Poultry Essential Fatty Acids in Poultry Diet Chicken Immune System Effects of High Ambient Temperature on Chicken Immune System	XV
		XX
		xxiii
		xxiv
LIS'	T OF ABBREVIATIONS	XXV
CH	APTER	
I	INTRODUCTION	1
II	LITERATURE REVIEW	7
	General Responses to Stress	7
	Heat Stress in Poultry	9
	Physiological Response to Heat Stress	11
	Behavioural Response to Heat Stress	13
	Hormonal Response to Heat Stress	14
	Corticosterone	14
		15
		16
		16
	<u>-</u>	17
		19
	-	21
		25
		29
		32
	•	34
	Effects of Dietary Fatty Acids on Chicken Immune System	36
Ш		
	· · · · · · · · · · · · · · · · · · ·	48
		48
		51
	Birds, Husbandry and Experimental Procedure	51
	Heat Challenge	54



	Growth Performance	54
	Body Temperature	54
	Blood Sample Collection	55
	Fatty Acid Analysis	55
	Extraction of fatty acid from feed or tissues samples	55
	Preparation of fatty acid methyl esters (FAME)	56
	Gas liquid chromatography	57
	Statistical Analysis	58
	Results	59
	Fatty Acid Profiles of Finisher Diets	59
	Body Weight	59
	Feed Consumption	62
	Feed Conversion Ratio	62
	Mortality Rate (%)	63
	Body Temperature	63
	Heterophil/Lymphocyte Ratio (HLR)	63
	Discussion	66
	Conclusion	71
IV	EFFECTS OF VARIOUS SOURCES OF DIETARY FATS ON RELATIVE ABDOMINAL FAT, FATTY ACID PROFILES AND CRUDE FAT CONTENT OF MEAT TISSUES IN HEAT-	
	STRESSED BROILER CHICKENS	72
	Introduction	72
	Materials and Methods	75
	Birds, Husbandry, Experimental Procedure and Heat Challenge	75
	Abdominal Fat Pad Collection	75
	Meat Sample Collection	75
	Analysis of Ether Extract in Breast and Thigh Meat	76
	Sample preparation	76
	Fatty Acid Analysis	77
	Statistical Analysis	77
	Results	78
	Fatty Acid Profiles of Finisher Diets	78
	Relative Abdominal Fat Pad Weight	78
	Ether Extract Per Cent in Breast and Thigh Meat	78
	Fatty Acid Profiles of Meat Samples	80
	Discussion	88
	Conclusion	89
V	SELF-SELECTION OF DIETARY FAT BY HEAT-STRESSED	
	BROILER CHICKENS	100
	Introduction	100
	Materials and Methods	102
	Birds, Husbandry and Experimental Procedure	102
	Growth Performance	104
	Heat Challenge	104



Body Temperature	105
Blood Sample Collection	105
Enzyme-linked Immunosorbent Assays for Newcastle	
Disease Vaccination-Specific Antibody Titers	106
Analysis of Ether Extract in Breast Meat	107
Abdominal Fat Pad Collection	107
Statistical Analysis	108
Results	109
Growth Performance	109
Mortality Rate (%)	110
Diet Selection	110
Body temperature	110
Heterophil/Lymphocyte Ratio (HLR)	113
Serum Creatine Kinase	113
Levels of Cholesterol, Protein, Sodium, Potassium and Chlorid	đe
in the serum	114
Antibody Titers against Newcastle Disease Virus	115
Relative Abdominal Fat Pad Weight	116
Ether Extract Per Cent in Breast Meat	117
Discussion	118
Conclusion	124
TO INFECTIOUS BURSAL DISEASE IN BROILER CHICKEN UNDER HEAT STRESS CONDITION	125
Introduction	125
Materials and Methods	128
Birds, Husbandry and Experimental Procedure	128
Heat Challenge	
	130
Infectious Bursal Disease Challenge and Traits Measured	130
Tissue sample collection	130 131
Tissue sample collection Determination of bursa to body weight ratio	130 131 131
Tissue sample collection Determination of bursa to body weight ratio Determination of heterophil/lymphocyte ratios (HLR)	130 131 131 131
Tissue sample collection Determination of bursa to body weight ratio Determination of heterophil/lymphocyte ratios (HLR) Determination of infectious bursal disease and Newcas	130 131 131 131 tle
Tissue sample collection Determination of bursa to body weight ratio Determination of heterophil/lymphocyte ratios (HLR) Determination of infectious bursal disease and Newcas Disease vaccination-specific antibody titres	130 131 131 131 tle
Tissue sample collection Determination of bursa to body weight ratio Determination of heterophil/lymphocyte ratios (HLR) Determination of infectious bursal disease and Newcas Disease vaccination-specific antibody titres Lesion Scoring of bursa of Fabricius	130 131 131 131 tle 132
Tissue sample collection Determination of bursa to body weight ratio Determination of heterophil/lymphocyte ratios (HLR) Determination of infectious bursal disease and Newcas Disease vaccination-specific antibody titres Lesion Scoring of bursa of Fabricius Sample preparation	130 131 131 131 ttle 132 132
Tissue sample collection Determination of bursa to body weight ratio Determination of heterophil/lymphocyte ratios (HLR) Determination of infectious bursal disease and Newcas Disease vaccination-specific antibody titres Lesion Scoring of bursa of Fabricius Sample preparation Histopathology	130 131 131 131 ttle 132 132 133
Tissue sample collection Determination of bursa to body weight ratio Determination of heterophil/lymphocyte ratios (HLR) Determination of infectious bursal disease and Newcas Disease vaccination-specific antibody titres Lesion Scoring of bursa of Fabricius Sample preparation Histopathology Determination of Viral RNA	130 131 131 131 ttle 132 132 133 133
Tissue sample collection Determination of bursa to body weight ratio Determination of heterophil/lymphocyte ratios (HLR) Determination of infectious bursal disease and Newcas Disease vaccination-specific antibody titres Lesion Scoring of bursa of Fabricius Sample preparation Histopathology Determination of Viral RNA Virus extraction	130 131 131 131 ttle 132 132 133 133
Tissue sample collection Determination of bursa to body weight ratio Determination of heterophil/lymphocyte ratios (HLR) Determination of infectious bursal disease and Newcas Disease vaccination-specific antibody titres Lesion Scoring of bursa of Fabricius Sample preparation Histopathology Determination of Viral RNA Virus extraction Extraction of RNA	130 131 131 131 tle 132 132 133 133 133
Tissue sample collection Determination of bursa to body weight ratio Determination of heterophil/lymphocyte ratios (HLR) Determination of infectious bursal disease and Newcas Disease vaccination-specific antibody titres Lesion Scoring of bursa of Fabricius Sample preparation Histopathology Determination of Viral RNA Virus extraction Extraction of RNA Determination of RNA concentration and purity	130 131 131 131 ttle 132 133 133 133 134 135
Tissue sample collection Determination of bursa to body weight ratio Determination of heterophil/lymphocyte ratios (HLR) Determination of infectious bursal disease and Newcas Disease vaccination-specific antibody titres Lesion Scoring of bursa of Fabricius Sample preparation Histopathology Determination of Viral RNA Virus extraction Extraction of RNA Determination of RNA concentration and purity Primer design	130 131 131 131 ttle 132 132 133 133 134 135 135
Tissue sample collection Determination of bursa to body weight ratio Determination of heterophil/lymphocyte ratios (HLR) Determination of infectious bursal disease and Newcas Disease vaccination-specific antibody titres Lesion Scoring of bursa of Fabricius Sample preparation Histopathology Determination of Viral RNA Virus extraction Extraction Extraction of RNA Determination of RNA concentration and purity Primer design Real-time quantitative RT-PCR	130 131 131 131 ttle 132 132 133 133 134 135 135
Tissue sample collection Determination of bursa to body weight ratio Determination of heterophil/lymphocyte ratios (HLR) Determination of infectious bursal disease and Newcas Disease vaccination-specific antibody titres Lesion Scoring of bursa of Fabricius Sample preparation Histopathology Determination of Viral RNA Virus extraction Extraction of RNA Determination of RNA concentration and purity Primer design	130 131 131 131 ttle 132 133 133 133 134 135



	Fatty Acid Profiles of Finisher Diets	138
	Morbidity and Mortality	138
	Fatty Acid Profiles of Bursal Tissues	138
	Heterophil/Lymphocyte Ratio (HLR)	144
	Antibody Titers against Newcastle Disease Virus	144
	Antibody Titers against Infectious Bural Disease Virus	144
	Bursa to Body Weight Ratio	141
	Lesion Scoring of Bursa of Fabricius	147
	Determination of Viral RNA	148
	Standard curve	153
	PCR amplification	153
	Discussion	160
	Conclusion	171
VII	GENERAL DISCUSSION AND CONCLUSION	172
BIBL	LIOGRAPHY	177
APPENDICES BIODATA OF THE AUTHOR		208
		213
PUBLICATIONS		215



LIST OF TABLES

Table		Page
3.1	Compositions of starter diet (% unless otherwise stated) (day 1-20)	52
3.2	Compositions of finisher diets (% unless otherwise stated) (day 21-41)	53
3.3	Major fatty acid compositions of finisher diets (% of total fatty acid methyl esters)	60
3.4	Mean (± SEM) body weights, feed consumptions, feed conversion ratios (FCR) and mortality rate (%) of broiler chicks by diet	61
3.5	Mean (± SEM) body temperatures of broiler chicks on day 28, 35 and 41 where diet by day interactions were significant	64
3.6	Mean (± SEM) heterophil/lymphocyte ratios of broiler chicks where diet by day interactions were significant	64
3.7	Mean (± S.D) correlations between body temperatures and heterophil/lymphocyte ratios in heat stressed broiler chicks by diet throughout heat exposure	65
4.1	Mean (± SEM) ether extract (%) of breast and thigh meat in broiler chicks where diet by meat type interactions were significant	79
4.2	Mean (\pm SEM) meat tissue fatty acid deposition (% of total fatty acids methyl esters) in broiler chicks by diet	83
4.3	Mean (± SEM) meat tissue fatty acid deposition (% of total fatty acid methyl esters) in broiler chicks by meat type	84
4.4	Mean (± SEM) fatty acid (% of total fatty acid methyl esters) in broiler chicks where diet by day interactions were significant	85
4.5	Mean (± SEM) total n-6 fatty acid (% of total fatty acid methyl esters) where diet by meat type interactions were significant	86
4.6	Mean (± SEM) total n-3 fatty acid (% of total fatty acid methyl esters) in broiler chicks where day by meat type interactions were significant	86
4.7	Mean (± SEM) fatty acids (% of total fatty acid methyl esters) in broiler chicks by day	87
5.1	Compositions of finisher diets (% unless otherwise stated) (day 21-41)	103



5.2	Mean (± SEM) body weights, feed consumptions, feed conversion ratios (FCR) and mortality rate (%) of broiler chicks by diet	111
5.3	Mean (± SEM) proportional intake of each diet by choice fed broiler chicks by day	
- 4		112
5.4	Mean (± SEM) body temperatures of broiler chicks where diet by day interactions were significant	112
5.5	Mean (± SEM) heterolphil/lymphocyte ratios in broiler chicks where diet by day interactions were significant	113
5.6	Mean (\pm SEM) levels of serum creatine kinase in broiler chicks where diet by age interactions were significant (U/L)	114
5.7	Mean (± SEM) serum levels of cholesterol, protein, sodium, potassium and chloride in broiler chicks by diet and by day (m mol/L)	115
5.8	Mean (± SEM) antibody titres against Newcastle disease virus (NDV) in broiler chicks by diet and by day	116
5.9	Mean (\pm SEM) relative abdominal fat pad weight and crude fat (%) of breast meat by diet on day 41	117
6.1	Compositions of finisher diets (% unless otherwise stated) (day 21-50)	129
6.2	Mean (± SEM) fatty acid compositions (% of total fatty acid methyl esters) of diets	139
6.3	Mean mortality rate (%) of broiler chicks by diet and temperature regimen	140
6.4	Mean (± SEM) fatty acid compositions (% of total fatty acid methyl esters) of bursal tissue in broiler chicks by diet	140
6.5	Mean (± SEM) fatty acid compositions (% of total fatty acid methyl esters) of bursal tissue in broiler chicks by temperature regimen	142
6.6	Mean (± SEM) fatty acid compositions (% of total fatty acid methyl esters) of bursal tissue in broiler chicks where diet by day post infection by IBDV interactions were significant	143
6.7	Mean (± SEM) heterophil/lymphocyte ratios (HLR) by diet, temperature regimen and day post infection by IBDV in broiler chicks	145
6.8	Mean (± SEM) antibody titers against Newcastle disease virus and infectious bursal disease virus by diet, temperature regimen and	



	day post infection by IBDV in broiler chicks.	146
6.9	Mean (\pm SEM) bursa to body weight ratios ($\times 10^{-3}$) of broiler chicks where diets by day post infection by IBDV interactions were significant	147
6.10	Mean (± SEM) histological lesion scores of bursa tissue by diet, temperature regimen and day post infection by IBDV in broiler chicks	149
6.11	Mean (± SEM) Cycle Threshold value for viral RNA amplification by diet and temperature regimen in broiler chicks	159



LIST OF FIGURES

Figure		Page
2.1	The main pathways of desaturation and elongation of the essential fatty acids	31
2.2	Effects of dietary lipid manipulation on immune system functions	39
2.3	Oxidative metabolism of arachidonic acid and eicosapentaenoic acid by the cyclooxygenase and 5-lipoxygenase pathways	45
4.1	Mean (± SEM) relative abdominal fat weight in broiler chicks by diet	7 9
6.1	Mean (\pm SEM) bursa to body weight ratio (\times 10 ⁻³) of broiler chicks by temperature regimen	148
6.2	Standard curve of serial 10-fold dilutions from 10 ⁻¹ to 10 ⁻⁵ of V877 vaccine virus	154
6.3	Viral RNA amplification curve and melting curve of vaccine IBDV V877	155
6.4	Viral RNA amplification curve and melting curve of bursal lymphoid cells from broilers infected with vaccine IBDV V877 on day 0 post infection	156
6.5	Viral RNA amplification curve and melting curve of bursal lymphoid cells from broilers infected with vaccine IBDV V877 on day 7 post infection	157
6.6	Viral RNA amplification curve and melting curve of bursal lymphoid cells from broilers infected with vaccine IBDV V877 on day 14 post infection	158



LIST OF PLATES

Plate		Page
6.1	Light microscopic observation of bursa of Fabricius from broilers fed with various dietary oil sources on day 0 post infection. Under heated $(38 \pm 1^{\circ}\text{C})$ and non-heated $(24 \pm 1^{\circ}\text{C})$ conditions. H&E, ×4×10	150
6.2	Light microscopic observation of bursa of Fabricius from broilers fed with various dietary oil sources on day 7 post infection. Under heated $(38 \pm 1^{\circ}\text{C})$ and non-heated $(24 \pm 1^{\circ}\text{C})$ conditions. H&E, ×4×10	151
6.3	Light microscopic observation of bursa of Fabricius from broilers fed with various dietary oil sources on day 14 post infection. Under heated $(38 \pm 1^{\circ}\text{C})$ and non-heated $(24 \pm 1^{\circ}\text{C})$ conditions, H&E, ×4×10	152

